Clin Oral Invest (2011) 15:953–958
DOI 10.1007/s00784-010-0458-y

ORIGINAL ARTICLE

Periodontal treatment decreases plasma oxidized LDL level and oxidative stress

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Received: 18 February 2010 / Accepted: 29 July 2010 / Published online: 18 August 2010

Abstract Periodontitis induces excessive production of reactive oxygen species in periodontal lesions. This may impair circulating pro-oxidant/anti-oxidant balance and induce the oxidation of low-density lipoprotein (LDL) in blood. The purpose of this study was to monitor circulating oxidized LDL and oxidative stress in subjects with chronic periodontitis following non-surgical periodontal treatment. Plasma levels of oxidized LDL and oxidative stress in 22 otherwise healthy non-smokers with chronic periodontitis (mean age 44.0 years) were measured at baseline and at 1 and 2 months after non-surgical periodontal treatment. At baseline, chronic periodontitis patients had higher plasma levels of oxidized LDL and oxidative stress than healthy subjects ($p<0.001$). Periodontal treatment was associated with a significant reduction in plasma levels of oxidized LDL (oxLDL) ($p<0.001$) and oxidative stress ($p<0.001$). At 2 months after periodontal treatment, the degree of change in the oxLDL was positively correlated with that in the oxidative stress ($r=0.593$, $p=0.004$). These observations indicate that periodontitis patients showed higher levels of circulating oxLDL and oxidative stress than healthy subjects. In addition, improved oral hygiene and non-surgical periodontal treatment were effective in decreasing oxLDL, which was positively associated with a reduction in circulating oxidative stress.

Keywords Periodontitis · Oxidative stress · Oxidized LDL · Non-surgical therapy

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Introduction

Periodontitis is a chronic infectious disease of the tooth-supporting tissues, characterized by gingival bleeding, periodontal pocket formation, destruction of connective tissue attachment, and alveolar bone loss [1]. Recently, periodontitis has been implicated in the onset and development of cardiovascular disorders [2, 3]. Although the mechanisms by which periodontitis influences cardiovascular disorders are not fully understood, there is a close relationship between periodontitis and cardiovascular disorders, with chronic inflammation being the common denominator [4, 5].

In periodontal lesions, excessive production of reactive oxygen species (ROS) appears as a result of inflammatory responses [6–9]. This may induce the oxidation of various molecules in the blood. In fact, studies have reported that lipid peroxidation [10] and protein carbonyl levels (a marker of protein oxidation) [11] in serum or plasma are significantly higher in chronic periodontitis patients than in periodontally healthy controls. Furthermore, a case–control study revealed that antibodies against oxidized low-density lipoprotein (oxLDL) in blood were significantly higher in periodontitis patients than in controls [12]. This indicates that systemic increases in oxidative stress induced by periodontitis might also promote the oxidation of LDL, the major carrier of plasma cholesterol. Because oxLDL has been shown to have proatherogenic and proinflammatory properties [13], periodontitis may have detrimental effects on cardiovascular disorders by increasing circulating oxLDL.

Clinical investigations have indicated that periodontal treatment offers clinical benefits by decreasing cardiovascular risk factors. For instance, it is known that periodontal treatment reduces serum inflammatory markers such as C-reactive protein (CRP) [14, 15], a predictor of cardiovascular disorders [16]. Removal of oxLDL is also a promising

therapeutic strategy against cardiovascular disorders [17]. A previous study revealed that periodontal treatment decreases blood levels of oxLDL in subjects with coronary heart disease [18]. In the study by Montebugnoli et al. [18], periodontal treatment might influence coronary heart disease, which subsequently led to decrease circulating oxLDL levels. It remains unclear whether improvement of periodontal inflammation by periodontal treatment can directly affect circulating oxLDL levels.

Our previous study demonstrated that periodontal treatment was effective in decreasing circulating ROS in subjects with chronic periodontitis [19]. Therefore, we hypothesized that improving circulating oxidative stress by periodontal treatment results in decreased plasma levels of oxLDL. The purpose of this study was to assess the plasma levels of oxidative stress and oxidized LDL in subjects with chronic periodontitis before and after non-surgical periodontal treatment. To evaluate the plasma levels of oxidative stress, a simple oxidative stress index (Oxidative-INDEX), which reflects both the pro-oxidative and anti-oxidant counterparts, was calculated after automated determination of reactive oxygen metabolites (ROM) and total anti-oxidant capacity [20]. In addition, plasma levels of high-sensitive CRP (hs-CRP) were measured as an inflammatory parameter related to cardiovascular disorders.

Materials and methods

Subject recruitment

The study was approved by the Ethics Committee of Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences. After obtaining written informed consent, a detailed medical questionnaire was completed by the dentists, and subjects who fulfilled the study requirements were enrolled. A total of 44 subjects were recruited at the Clinic of Preventive Dentistry, Okayama University Hospital from April 2008 to August 2009. Exclusion criteria included hypertension, past cardiovascular events, diabetes, cancer, gastrointestinal disorders, skin conditions, pregnancy, lactation, previous or current smoking, and use of anti-oxidant supplements, anti-inflammatory medications, or antibiotics within the previous 3 months. Individuals with fewer than 15 teeth were also excluded.

Twenty-two patients with chronic periodontitis [10 men and 12 women; age, 44.0±19.2 years (mean ± SD)] and 22 age- and gender-matched controls (10 men and 12 women; age, 43.9±20.0 years) were enrolled in this study, according to previously described criteria [19]. In brief, periodontitis patients had at least four teeth exhibiting probing pocket depth (PPD) ≥4 mm at baseline. The patients in this study partly consisted of the same subjects in our previous study [19]; i.e., there are four men and five women extra in both patients and controls groups. Therefore, the values of clinical parameters and plasma ROM in the present study partly included the same data in our previous study [19].

Non-surgical periodontal treatment and measurements of clinical parameters

All periodontitis patients received oral hygiene instructions and scaling and root planing by two dentists (D. E. and R. Y.), as previously reported in detail elsewhere [19]. A formal oral hygiene instruction was given to the periodontitis patients at each visit. Clinical measurements made included PPD, clinical attachment level (CAL), BOP, and plaque index (Plaque Control Record [21]) at baseline (prior to scaling and root planing) and at 2 months after periodontal treatment. The intra- and inter-examiner agreement, evaluated with kappa statistics, of the PPD and CAL was more than 0.9.

Blood sampling

Plasma samples in periodontitis patients were obtained at baseline and at 1 and 2 months after periodontal treatment. Plasma samples in control subjects were also obtained at baseline. After 2 months, plasma samples in 17 consenting control subjects were further gathered to confirm whether plasma levels of oxidative stress and oxLDL remained stable during the experimental period.

Blood samples were taken from the fingertip at chairside within a few minutes between 10:00 am and 12:00 pm and were immediately kept on ice and centrifuged at 3,000×*g* for 5 min. The obtained plasma samples (approximately 100 μl per individual) were used to determine plasma levels of oxLDL (15 μl per individual), ROM (20 μl per individual), total anti-oxidant capacity (1 μl per individual), and CRP (3 μl per individual). The measurements of ROM and total anti-oxidant capacity were performed at the same day of blood sampling. The remaining plasma aliquots were stored at −80°C until subsequent analysis. The determination of oxLDL and CRP were done at the same time later after thawing of all blood samples.

Measurement of plasma levels of oxLDL

Measurement of plasma oxLDL was performed using an ELISA method (Immundiagnostik AG, Bensheim, Germany) [22]. The assay is based on a direct sandwich technique in which monoclonal antibodies are directed against epitopes on the malondialdehyde-modified apolipoprotein 100 molecule. OxLDL measurements were performed in duplicate, and both intra- and inter-assay coefficients of variation were <5%.

Table 1 Clinical and plasma parameters for control subjects and periodontitis patients at baseline

Variable	Control subjects	Periodontitis patients	*p* value
N (males; females)	22 (10; 12)	22 (10; 12)	–
Age (years)	48.0 (26.0, 62.5)	48.0 (27.5, 61.5)	NS
Clinical parameters			
Probing pocket depth (mm)	1.8 (1.6, 1.9)	2.1 (1.7, 2.9)	0.012
Percent of teeth with 4–6 mm probing pocket depth	–	20.4 (14.3, 26.5)	–
Percent of teeth with 6 mm≤probing pocket depth	–	3.6 (0, 16.8)	–
Clinical attachment level (mm)	1.8 (1.6, 1.9)	2.3 (1.8, 3.0)	0.002
Percent of sites with bleeding on probing	4.2 (2.6, 8.8)	29.8 (19.7, 37.0)	<0.0001
Percent of sites with dental plaque	17.3 (6.3, 29.5)	41.4 (31.0, 54.1)	<0.0001
Plasma parameters			
Oxidized low-density lipoprotein (ng/ml)	268 (249, 278)	424 (392, 536)	<0.0001
Oxidative-INDEX	−0.63 (−1.00, −0.19)	1.59 (0.99, 2.28)	<0.0001
C-reactive protein (ng/ml)	0.5 (0.4, 0.9)	1.0 (0.7, 1.1)	0.01

Data are expressed as the median (25%, 75%).

The data were compared by Mann–Whitney *U* test

Measurement of plasma levels of ROM

Measurement of plasma levels of ROM (whole oxidant capacity of serum against *N*, *N*-diethylparaphenylenediamine in acidic buffer) was performed using a spectrophotometer (Free Radical Elective Evaluator, Diacron International, Grosseto, Italy), according to previously reported methods [19]. Measurement data are given in terms of Carratelli units (CARR U); it has been established that 1 CARR U corresponds to 0.08 mg/dl hydrogen peroxide.

Measurement of plasma total anti-oxidant capacity

Plasma levels of total anti-oxidant capacity were measured by OXY-adsorbent test (Diacron International) [20]. This test evaluates the capacity of plasma to oppose the massive oxidative action of a hypochlorous acid (HClO) solution. Briefly, 10 μl of standards or samples, previously diluted 1:100 with distilled water, were added and mixed with 1 ml of the HClO solution. After 10 min of incubation, at 37°C, 10 μl of chromogenic mixture (solution provided in the kit) was added. Absorbance was measured immediately at 505 nm by a spectrophotometer (Diacron International), and the total anti-oxidant capacity was expressed as micromole of HClO consumed by 1 ml of sample (μmol HClO/ml).

Calculation of the Oxidative-INDEX

In order to compare parameters with different measurement units and variability, standardized values of the ROM and OXY-adsorbent tests were used to represent the Oxidative-INDEX. Standardization of values for these tests was performed as described previously [20]. The Oxidative-INDEX was calculated by subtracting the OXY standardized variable from the ROM standardized variable [20]. High Oxidative-INDEX values indicate high oxidative stress in the blood.

Measurement of plasma levels of hs-CRP

Concentrations of hs-CRP were measured by a sensitive ELISA kit according to the manufacturer's instructions [23]. The lower limit of detection was 0.10 ng/ml.

Table 2 Changes in clinical parameters after periodontal treatment in periodontitis patients (n=22)

Variable	Baseline	2months	*p* value
Probing pocket depth (mm)	2.1 (1.7, 2.9)	1.8 (1.6, 2.0)	<0.0001
Percent of teeth with 4–6 mm probing pocket depth	20.4 (14.3, 26.5)	11.1 (7.1, 21.9)	<0.0001
Percent of teeth with 6 mm ≤ probing pocket depth	3.6 (0, 16.8)	0 (0, 9.4)	<0.0001
Clinical attachment level (mm)	2.3 (1.8, 3.0)	2.0 (1.7, 2.3)	0.002
Percent of sites with bleeding on probing	29.8 (19.7, 37.0)	5.1 (3.2, 8.5)	<0.0001
Percent of sites with dental plaque	41.4 (31.0, 54.1)	17.9 (12.2, 25.0)	<0.0001

Data are expressed as the median (25%, 75%).

The data were compared by Wilcoxon signed-rank test

Table 3 Changes in plasma parameters after periodontal treatment in periodontitis patients (n=22)

Variable	Baseline	1 month	2 months
Oxidized low-density lipoprotein (ng/ml)	424 (392, 536)	286 (277, 302)*	285 (277, 304)**
Oxidative-INDEX	1.59 (0.99, 2.28)	0.12 (−0.38, 0.72)**	−0.77 (−1.34, −0.16)**
C-reactive protein (ng/ml)	1.0 (0.7, 1.1)	0.7 (0.5, 0.8)*	0.5 (0.5, 0.9)*

Data are expressed as the median (25%, 75%)

*p<0.01, compared with the baseline, using the Wilcoxon signed-rank test; **p<0.0001, compared with the baseline, using the Wilcoxon signed-rank test

Statistical analysis

Clinical and biochemical parameters were analyzed statistically using Wilcoxon signed-rank test (for intra-group comparisons) and Mann–Whitney U test (for inter-group comparisons). Spearman rank correlation coefficients among variables were determined. All analyses were performed using a software program (SPSS 15.0 J for Windows, SPSS Japan, Tokyo, Japan). A p value of <0.05 was considered to be statistically significant. In addition, sample size was calculated using statistical software (nQuery Advisor, Statistical Solutions, Sangus, MA, USA), based on the results of plasma levels of ROM between baseline and 2 months after periodontal treatment in a previous study [19]. A sample size of 15 per group was required for detection of a significant difference in plasma levels of ROM (80% power, two-sided 5% significance level).

Results

Comparisons between subjects with and without periodontitis at baseline

At baseline, there were significant differences between the subjects with periodontitis and the control subjects with regard to PPD (p=0.012), CAL (p=0.002), percentage of sites with bleeding on probing (p<0.0001), and percentage of sites with dental plaque (p<0.0001) (Table 1). Subjects with periodontitis had higher levels of plasma oxLDL (p<0.0001), Oxidative-INDEX (p<0.0001), and hs-CRP (p=0.01) when compared to control subjects.

Effects of periodontal treatment on clinical and plasma parameters

Periodontal treatment led to an improvement in all clinical parameters in the subjects with periodontitis, as revealed by significant differences found between data obtained at baseline and at 2 months after treatment (p<0.01) (Table 2).

Plasma levels [median (25%, 75%)] of oxLDL and Oxidative-INDEX in 17 control subjects were 278 (243, 290) ng/ml and −0.60 (−1.05, −0.17) ng/ml at baseline, and 272 (236, 292) ng/ml and −0.64 (−1.08, −0.16) ng/ml at 2 months, respectively. No significant differences in plasma levels of oxLDL and Oxidative-INDEX were detected between baseline and 2 months. Plasma levels of oxLDL and Oxidative-INDEX for subjects with periodontitis

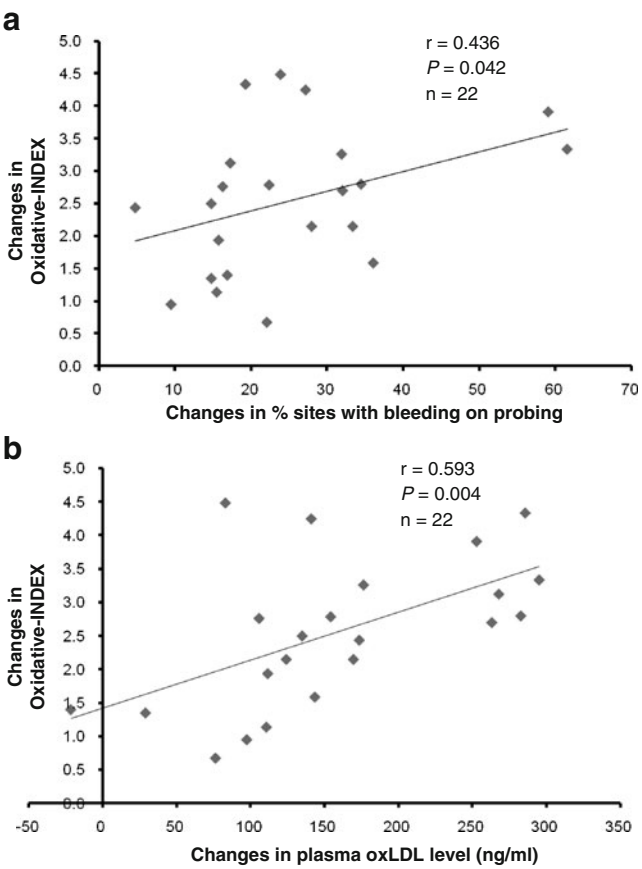

Fig. 1 Correlations between changes in Oxidative-INDEX and those in percentage of sites with bleeding on probing (**a**) or oxidized LDL (**b**) at 2 months after periodontal treatment. Changes in each parameter were calculated by subtracting data at the 2-month assessment from those at baseline

decreased significantly at 1 and 2 months after periodontal treatment ($p<0.0001$) (Table 3). Plasma levels of hs-CRP were also significantly lower at 1 month ($p<0.01$) and 2 months ($p<0.01$) after periodontal treatment. At the 2-month assessment after periodontal treatment, the degree of change in Oxidative-INDEX was positively correlated with that in the percentage of sites with BOP ($r=0.436$, $p=0.042$) (Fig. 1a). Furthermore, the degree of change in oxLDL levels was positively correlated with that in the Oxidative-INDEX ($r=0.593$, $p=0.004$) (Fig. 1b). However, there were no significant correlations between the degree of change in oxLDL levels and that in hs-CRP levels ($r=0.175$, $p=0.436$).

Discussion

In the present study, subjects with chronic periodontitis showed higher plasma levels of oxLDL than controls at baseline. Furthermore, plasma levels of oxLDL in periodontitis patients decreased after non-surgical periodontal treatment. These results thus confirm increased circulating oxLDL in periodontitis patients, which improved after periodontal treatment. It is known that oxLDL is a strong marker of cardiovascular diseases [24, 25]. Periodontal treatment, including oral hygiene instructions and scaling and root planing thus offers clinical benefits on cardiovascular health by decreasing oxLDL.

In a previous study [18], a mean reduction in oxLDL (of 18%) was reported in periodontitis patients with coronary heart disease after non-surgical periodontal treatment for 3 months. In the present study, periodontal treatment significantly reduced (by 37%) plasma levels of oxLDL in periodontitis patients without any systemic disease at 2 months. Therefore, the degree of improvement in oxLDL after periodontal treatment may differ depending on systemic conditions.

Oxidative-INDEX is reported to be a useful indicator of oxidative stress in the blood [20]. We found a positive association between the degree of change in the Oxidative-INDEX and the percentage of sites with BOP after periodontal treatment. Because BOP reflects disease activity in the periodontium [26], circulating oxidative stress was thought to be modulated by the disease activity in the periodontium [19].

The degree of change in plasma levels of oxLDL was positively correlated with that in the Oxidative-INDEX. Periodontitis induces excessive ROS production in periodontal lesions [6–9]. Therefore, the improvement of excessive ROS production within the periodontium by periodontal treatment may contribute to a reduction in circulating ROS, resulting in a decrease in the oxidation of circulating LDL. In addition, a recent study demonstrated that peripheral blood neutrophils from periodontitis patients showed a hyperactive/reactive phenotype in terms of ROS production [27]. It is also possible that periodontal treatment affects plasma levels of oxLDL by ameliorating the hyperactive/reactive phenotype in blood neutrophils.

Clinical studies have investigated the effects of periodontal treatment on blood levels of inflammatory markers. Serum interleukin-6 in patients with periodontitis and type 2 diabetes mellitus improved (by 48%) after periodontal treatment [28]. In addition, periodontal treatment significantly reduced the serum levels of matrix metalloprotease-8 (by 35%) and matrix metalloprotease-9 (by 39%) in periodontitis patients [29]. These observations are consistent with the results of the current study, which showed significant reductions in plasma levels of hs-CRP (by 29%) following periodontal treatment. On the other hand, we found no significant associations between the degree of change in oxLDL levels and that in hs-CRP levels. In the present subjects, independent control of blood parameters following periodontal treatment may have been responsible for differences between oxidative stress and systemic inflammation. However, more insight is needed into oxLDL ability to provide independent prognostic information in relation to hs-CRP.

This study had some limitations. For instance, the patients in the current study had a few sites with PPD ≥6 mm at baseline (Table 1). As these patients exhibited mild or moderate periodontitis, it is possible that severe periodontitis may have different effects on the plasma levels of oxLDL and oxidative stress than those indicated by our results. In addition, the present subjects were not starved over night; therefore, the contribution of dietary intake to their pro-oxidant/anti-oxidant balance is unknown and variable. Furthermore, during the experimental period, plasma levels of oxLDL and oxidative stress might have been affected by the general conditions associated with habits such as exercise and nutrition intake. The records and/or measurements of exercise habits, dietary intake of anti-oxidants, and nutritional questionnaire would be necessary to increase the reliability of our data.

In conclusion, patients with chronic periodontitis showed higher levels of circulating oxLDL and oxidative stress than healthy subjects. Non-surgical periodontal treatment was effective at improving periodontal health and decreasing oxLDL, which were positively associated with a reduction in circulating oxidative stress. In periodontitis patients, periodontal treatment may be useful for improving periodontal health, as well as maintaining cardiovascular health, by decreasing plasma levels of oxLDL.

Acknowledgements This work was supported by Grants-in-Aid for Scientific Research (20791641 & 22792119) from the Ministry of Education, Culture, Sports, Science and Technology, Tokyo, Japan.

Conflict of interest The authors declare that they have no conflict of interest.

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