Relationship Between Periodontal Condition and Plasma Reactive Oxygen Metabolites in Patients in the Maintenance Phase of Periodontal Treatment

Naofumi Tamaki,* Takaaki Tomofuji,* Takayuki Maruyama,* Daisuke Ekuni,* Reiko Yamanaka,* Noriko Takeuchi,* and Tatsuo Yamamoto*

Background: The relationship between systemic antioxidative status and periodontal condition has been investigated in epidemiologic studies. However, little literature is available with regard to the correlation between systemic reactive oxygen species and periodontal condition. The purpose of this cross-sectional study was to investigate the relationship between plasma reactive oxygen metabolites (ROM) and periodontal condition in patients in the maintenance phase of periodontal treatment.

Methods: Eighty-one subjects (mean age: 57.4 years) who had entered a periodontal maintenance program were examined for probing depth (PD) and clinical attachment level (CAL). Plasma levels of ROM and biologic antioxidant potential (BAP) were determined with a free radical electric evaluator.

Results: The plasma level of ROM was positively correlated to mean CAL (r = 0.281; $P = 0.011$) and percentage of teeth with CAL ≥4 mm (r = 0.236; $P = 0.034$), but not mean PD (r = 0.196; $P = 0.080$). Logistic regression analysis showed that subjects with ROM levels >400 Carratelli units (CARR U) had significantly higher mean CAL compared to subjects with ROM ≤400 CARR U after adjusting for age, gender, and the number of teeth present ($P = 0.011$). However, the plasma level of BAP was not significantly correlated with the periodontal parameters.

Conclusions: A positive association was found between plasma oxidative status and CAL in patients in the maintenance phase of periodontal therapy. A systemic increase in oxidative stress may influence the rate of progression of periodontal disease. *J Periodontol 2008;79:2136-2142.*

KEY WORDS

Antioxidant; oxidative stress; periodontitis; plasma.

* Department of Oral Health, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan.

In aerobic organisms, the imbalance between reactive oxygen species (ROS) generation and the antioxidant defense system leads to increased oxidative stress.[1,2] Epidemiologic studies have investigated the relationship between systemic antioxidative status and periodontal conditions. For instance, a negative association was reported between plasma vitamin C levels and periodontal attachment loss.[3,4] It was also shown that plasma levels of vitamin E and glutathione were lower in chronic periodontitis patients than in periodontally healthy individuals.[5] The changes in systemic antioxidative status affect periodontal health. However, little literature is available with regard to the relationship between blood ROS and periodontal condition; direct measurement of ROS is difficult because of its biochemical instability.

Recently, a method for measuring reactive oxygen metabolites (ROM) in blood samples has been developed, which was recognized to be useful for the evaluation of oxidative stress in the body.[6-8] This analysis measures the whole oxidant capacity of blood against N, N-diethylparaphenylendiamine in acidic buffer. The main components of ROM are hydroperoxides,

doi: 10.1902/jop.2008.080082

which are intermediate oxidative products of lipids, peptides, and amino acids. Despite fair oxidant power, hydroperoxides in the plasma are relatively stable compared to its parent free radicals; therefore, the level can be detected. Elevated levels of blood ROM have been associated with obstructive sleep apnea[7] and diets with less vegetables or fruit.[8]

Smoking and diabetes mellitus, well-known risk factors for periodontal disease,[9] can result in a systemic oxidant–antioxidant imbalance as reflected by increased products of lipid peroxidation.[10,11] A recent epidemiologic study[12] showed that ethanol consumption is an independent modifiable risk factor for periodontal disease, and an animal study[13] revealed that circulating oxidative stress might be involved in the mechanism of periodontal breakdown by ethanol consumption. These findings suggested that a systemic increase in oxidative stress may have a detrimental effect on the periodontal condition. The purpose of the present study was to investigate the relationship between the periodontal condition and plasma ROM levels. Because periodontal inflammation may influence systemic oxidative status,[14,15] patients in the maintenance phase of periodontal treatment who had little inflammation were included in this study. In addition, the plasma level of the biologic antioxidant potential (BAP) was measured to evaluate the corresponding antioxidative status.[16-18] This test could be conveniently performed at chairside within 10 minutes with the same machine used to analyze the plasma ROM level.

MATERIALS AND METHODS

Subject Recruitment

Eighty-one individuals (62 females and 19 males; mean age: 57.4 ± 12.3 years; range: 26 to 84 years) were recruited from the patients under maintenance therapy at the Department of Preventive Dentistry, Okayama University Hospital from June 2007 to November 2007 (Table 1). All subjects had been diagnosed with chronic periodontitis and were receiving comprehensive dental care that included non-surgical periodontal therapy consisting of oral hygiene instructions, supra/subgingival debridement, and scaling and root planing of all pockets (≥4 mm) every 3 to 4 months for 9.5 ± 7.7 years. They had ≥15 teeth, <20% sites with bleeding on probing (BOP), and had shown no features of acute periodontal inflammation or gingival abscess within the previous 6 months. The subjects were systemically healthy, and the exclusion criteria were as follows: pregnancy, previous or current smoker, or user of antioxidant (e.g., vitamin C and coenzyme Q_{10}) supplementation and/or anti-inflammatory drugs within the previous 3 months. The study was approved by the Ethical 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.

Table 1.
Age and Gender Distribution of Subjects

Age Group (years)	Male (n)	Female (n)	Total (n)
20 to 29	2	1	3
30 to 39	2	4	6
40 to 49	3	3	6
50 to 59	5	25	30
60 to 69	5	19	24
70 to 79	1	9	10
80 to 89	1	1	2
Total	19	62	81

Clinical Measurements

Probing depth (PD) and clinical attachment level (CAL) were measured using a color-coded probe† at six sites (mesio-buccal, mid-buccal, disto-buccal, mesio-lingual, mid-lingual, and disto-lingual) on all teeth. Sites that bled upon gentle probing were recorded, and the percentage of bleeding sites was calculated in each subject. Plaque levels were measured after staining with erythrosine and recorded as the presence or absence of plaque at four sites (mesial, distal, buccal, and lingual) around each tooth. The percentage of sites in which plaque was detected was calculated for each subject.[19]

Measurement of Plasma ROM Level

The measurement of plasma ROM level was performed using a free radical electric evaluator,‡ according to the analysis procedures.[6-8] This test evaluates the ability of transition metals to catalyze, in the presence of peroxides, the formation of free radicals, which are trapped by an alkylamine. First, a 20-μl plasma sample (from the fingertip) and 1 ml acetate buffer (pH 4.8) were gently mixed in a cuvette, and then 20 μl chromogenic substrate (N, N-diethylparaphenylendiamine) was added to it. After mixing, the cuvette was immediately incubated in the thermostatic block of the analyzer for 5 minutes at 37°C. An absorbance of 505 nm was recorded. The measurement unit was expressed as Carratelli units (CARR U). It has been established that 1 CARR U corresponds to 0.08 mg/dl hydrogen peroxide.[8] From the

† CP-11, Hu-Friedy, Chicago, IL.
‡ Diacron International, Grosseto, Italy.

manufacturer's instructions, the following classification was made: normal, 250 to 300 CARR U; borderline, 301 to 320 CARR U; slight oxidative stress, 321 to 340 CARR U; oxidative stress, 341 to 400 CARR U; high oxidative stress, 401 to 500 CARR U; and very high oxidative stress, >500 CARR U.

Measurement of Plasma BAP Level

The plasma levels of BAP were also measured using a free radical electric evaluator, according to the analysis procedures.[16-18] This test is based on the ability of the plasma samples to reduce the ferric (Fe^{3+}) ions in a colored solution to ferrous (Fe^{2+}) ions. In brief, the addition of 10 μl plasma to a colored solution, obtained by mixing solutions of 50 μl Fe^{3+} chloride and a thiocyanate derivative, causes decoloration, the intensity of which is measured photometrically at 505 nm and is proportional to the ability of plasma to bind to ferric ions. The results are expressed as μmol/l. The manufacturer's instructions showed the following classification: optimum range, 2,201 to 4,000 μmol/l; borderline, 2,001 to 2,200 μmol/l; moderate shortage, 1,801 to 2,000 μmol/l; shortage, 1,601 to 1,800 μmol/l; severe shortage, 1,401 to 1,600 μmol/l; and very severe shortage, ≤1,400 μmol/l.

Statistical Analysis

Means ± SD were calculated for the following study variables: subject age, number of teeth present, PD, CAL, percentage of teeth with CAL ≥4 mm, percentage of sites with BOP, percentage of sites with plaque, plasma ROM level, and plasma BAP level. The statistical significance of associations among variables was determined by using the Spearman rank correlation coefficient. The Mann-Whitney U test was used to compare periodontal parameters between females and males, between the subjects with plasma ROM >400 CARR U and those with plasma ROM ≤400 CARR U, and between the subjects with plasma BAP >2,200 μmol/l and those with plasma BAP ≤2,200 μmol/l. Logistic regression analysis was used to determine the characteristics of subjects with high and low levels of plasma ROM. Using the data from all subjects, the high or low level of plasma ROM was used as the dependent variable, and age, gender, number of teeth present, and mean CAL were regarded as the independent variables. All analyses were performed using a software program.§ A *P* value <0.05 was considered significant.

Table 2.

Summary of Each Parameter (mean ± SD)

	Male (n = 19)	Female (n = 62)	Total (n = 81)
Age (years)	52.1 ± 14.8	59.1 ± 11.0	57.4 ± 12.3
Teeth present (n)	26.6 ± 2.7	25.1 ± 3.9	25.5 ± 3.7
Periodontal parameters			
PD (mm)	1.9 ± 0.3	1.8 ± 0.4	1.8 ± 0.4
CAL (mm)	2.3 ± 0.8	2.5 ± 1.0	2.5 ± 1.0
Teeth with CAL ≥4 mm (%)	13 ± 19	17 ± 22	16 ± 22
Sites with BOP (%)	5.5 ± 4.8	4.8 ± 4.3	4.9 ± 4.4
Sites with plaque (%)	26 ± 20	18 ± 15	20 ± 17
Plasma parameters			
ROM (CARR U)	353 ± 62	390 ± 61*	381 ± 63
BAP (μmol/l)	2,454 ± 300	2,369 ± 251	2,389 ± 264

* Significantly different from males (P <0.05).

RESULTS

Clinical Parameters

With the exception of plasma ROM level, there was no significant difference in any of the parameters between males and females (Table 2). Mean plasma levels of ROM and BAP were 381 ± 63 CARR U and 2,389 ± 264 μmol/l, respectively. Subjects with oxidative stress (plasma ROM level from 341 to 400 CARR U) and with an optimum level of plasma BAP (from 2,201 to 4,000 μmol/l) were the most prevalent (Table 3).

Correlations Between Plasma ROM Level and Clinical Parameters

The plasma ROM level was negatively correlated with the number of teeth present (P = 0.039) and positively correlated with mean CAL (P = 0.011) and the percentage of teeth with CAL ≥4 mm (P = 0.034) (Table 4; Fig. 1). Subjects with plasma ROM >400 CARR U had a significantly greater mean CAL (P = 0.005) and percentage of teeth with CAL ≥4 mm (P = 0.005) compared to those with plasma ROM ≤400 CARR U (Table 5). In the logistic regression model, only mean CAL was significantly associated with high or low levels of plasma ROM (P = 0.011; Table 6).

Correlations Between Plasma BAP Level and Clinical Parameters

There was no significant correlation between the plasma BAP level and other parameters, including age, number of teeth present, and periodontal condition (Table 4). No significant differences were observed in age, number of teeth present, mean PD, mean

§ SPSS 15.0 J for Windows, SPSS Japan, Tokyo, Japan.

Table 3.

Distribution of Plasma Levels of ROM and BAP in the Study Subjects

ROM			BAP		
Classification	Range (CARR U)	Subjects (n [%])	Classification	Range (μmol/l)	Subjects (n [%])
Normal	250 to 300	4 (4.9)	Optimum	2,201 to 4,000	61 (75.3)
Borderline	301 to 320	5 (6.2)	Borderline	2,001 to 2,200	15 (18.5)
Slight oxidative stress	321 to 340	15 (18.5)	Moderate shortage	1,801 to 2,000	4 (4.9)
Oxidative stress	341 to 400	29 (35.8)	Shortage	1,601 to 1,800	1 (1.2)
High oxidative stress	401 to 500	24 (29.6)	Severe shortage	1,401 to 1,600	0 (0)
Very high oxidative stress	>500	4 (4.9)	Very severe shortage	≤1,400	0 (0)

Table 4.

Correlations Between the Plasma Parameters and Other Parameters in All Subjects (N = 81)

	Plasma ROM Level (CARR U)		Plasma BAP Level (μmol/l)	
	r*	*P* Value	r*	*P* Value
Age	0.087	0.439	0.029	0.795
Number of teeth present	–0.230	0.039	–0.029	0.795
Mean PD	0.196	0.080	0.045	0.689
Mean CAL	0.281	0.011	0.123	0.274
Percentage of teeth with CAL ≥4 mm	0.236	0.034	0.065	0.564
Percentage of sites with BOP	0.039	0.727	–0.040	0.725
Percentage of sites with plaque	–0.124	0.269	0.032	0.774

* Spearman rank correlation coefficient.

CAL, percentage of teeth with CAL ≥4 mm, percentage of sites with BOP, or percentage of sites with plaque between the subjects with plasma BAP >2,200 μmol/l and those with plasma BAP ≤2,200 μmol/l (data not shown).

DISCUSSION

To the best of our knowledge, this was the first epidemiologic study to assess the relationship of plasma ROM/BAP levels to periodontal conditions and to show that plasma oxidative status was positively associated with periodontal conditions in patients under periodontal maintenance. Plasma ROM is considered to be a reliable indicator of oxidative status,[6-8] and it correlated positively with mean CAL and the percentage of teeth with CAL ≥4 mm. Logistic regression analysis also showed that the subjects with ROM >400 CARR U had significantly higher mean CAL compared to those with ROM ≤400 CARR U after adjusting for age, gender, and the number of teeth present. Because CAL reflects periodontal breakdown,[20] these results suggested that the high oxidative status of plasma could have affected the rate of progression of periodontal disease in the past. In addition, the subjects in the present study had <20% of sites with BOP, which shows that they had relatively low inflammation in their periodontium. Furthermore, no significant correlation between plasma ROM level and the percentage of sites with BOP was found. It is suggested that the present disease activity in the periodontium had little influence on the plasma level of ROM.

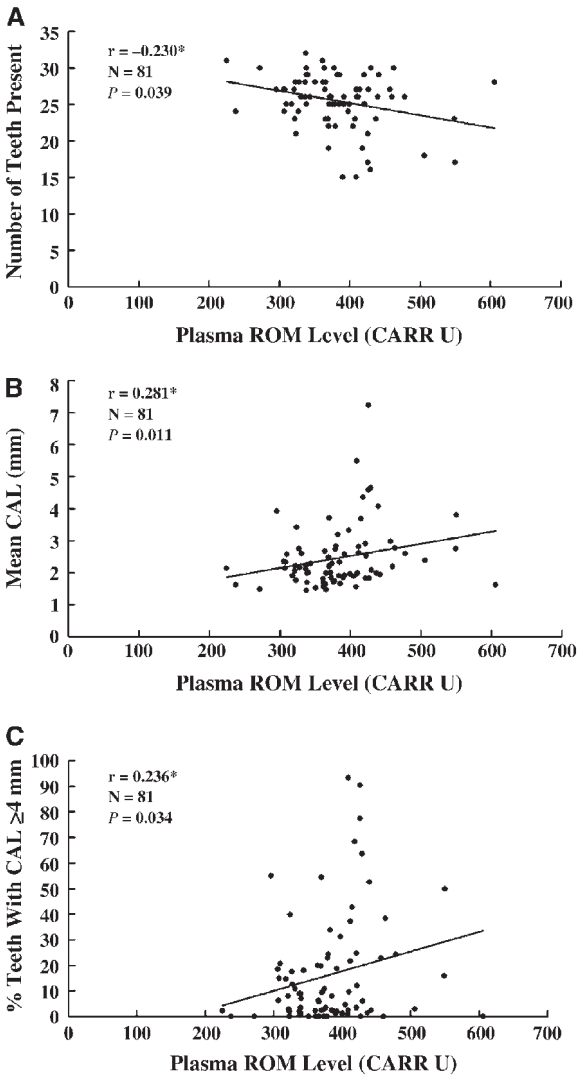

Figure 1.
*Correlation between the plasma ROM level and clinical parameters. The y axis represents the number of teeth present **(A)**, mean CAL **(B)**, and the percentage of teeth with CAL ≥4 mm **(C)**. The solid lines represent regression lines. *Spearman rank correlation coefficient.*

In contrast to the plasma ROM level, the plasma BAP level did not correlate with any periodontal parameter. In previous studies,[15,21] there were no significant differences in serum or plasma antioxidant concentration between the group with chronic periodontitis and controls. These findings suggested that the correlation of blood total antioxidant status with periodontal conditions was modest. However, clinical studies also showed that the blood antioxidant status in subjects with periodontitis was lower than in subjects with healthy gingiva by analyzing total antioxidant status,[22] total antioxidant capacity,[23,24] superoxide dismutase concentration,[23,24] and glutathione peroxidase activity.[23,24] Several methodologies are available to evaluate the antioxidant status in blood samples. A previous study[25] suggested that the Fe^{3+} reducing antioxidant power (FRAP) assay has some drawbacks, such as interference and reaction kinetics, compared to the oxygen radical absorption capacity (ORAC) method. Therefore, the reason for not observing any correlation between the blood antioxidative status and the periodontal parameters might be related to the BAP test performed. Additional assays (e.g., ORAC method) might be required to investigate the correlation between systemic antioxidant status and periodontal condition.

Another possible reason for no correlation between plasma BAP level and periodontal parameter may be the distribution of plasma BAP. The majority of the subjects (75%) had normal plasma BAP levels (Table 3).

Although it is known that estrogen (one of the female sex hormones) acts as an antioxidant,[26-28] there was no significant difference in plasma BAP levels between females and males in our findings. In this study, >80% of the female subjects were >50 years old. Because most of the female subjects were postmenopausal, it is possible that the influence of estrogen was minimal in this study.

All subjects in the present study had entered a supportive periodontal care program. Their low plaque scores and the percentage of sites with BOP reflected a well-maintained periodontal patient population. Good oral hygiene and reducing gingival inflammation are important for maintaining periodontal health. However, in our study, the periodontal condition correlated with plasma oxidative stress, even when few local risk factors for periodontal disease were present. Because oxidative stress may be involved in the pathogenesis of periodontal disease,[29-31] therapeutic approaches to systemic oxidative stress may also offer clinical improvements in periodontal health for patients in the maintenance phase of periodontal treatment. Further studies are needed to clarify this issue.

Habitual smoking and systemic diseases such as diabetes mellitus create high oxidative stress.[10,32] In our study, all subjects were systemically healthy and never-smokers. It is conceivable that other factors caused changes in plasma ROM level. It is accepted that nutritional status affects systemic oxidative stress,[33] and it may have played an important role in the current study. Further studies are needed to examine the relationships among nutrition intake, plasma ROM level, and periodontal conditions.

It is not clear whether the increase in plasma ROM level is a cause or result of periodontal disease. It was reported that hydroperoxides, which are the main components of ROM, cause cell death and tissue

Table 5.

Comparison of Each Parameter (mean ± SD) Between Subjects With Plasma ROM >400 and ≤400 CARR U

	Plasma ROM >400 CARR U (n = 28)	Plasma ROM ≤400 CARR U (n = 53)	*P* Value
Age (years)	58.4 ± 13.0	56.9 ± 12.0	0.263
Teeth present (n)	24.1 ± 4.4	26.2 ± 3.1	0.069
PD (mm)	1.9 ± 0.4	1.8 ± 0.4	0.299
CAL (mm)	3.0 ± 1.3	2.2 ± 0.6	0.005
Teeth with CAL ≥4 mm (%)	28 ± 29	10 ± 13	0.005
Sites with BOP (%)	4.9 ± 4.5	4.9 ± 4.4	0.952
Sites with plaque (%)	17 ± 13	21 ± 18	0.450

Table 6.

Logistic Regression Analysis With High (>400 CARR U) or Low (≤400 CARR U) Levels of Plasma ROM as the Dependent Variables

Indicator	Odds Ratio	95% CI	*P* Value
Mean CAL	3.000	1.293 to 6.964	0.011
Gender (male: 0; female: 1)	2.862	0.708 to 11.574	0.140
Age (years)	0.977	0.934 to 1.023	0.321
Number of teeth present	0.966	0.818 to 1.140	0.680

CI = confidence interval.

damage,[34,35] suggesting that an increased level of plasma ROM may cause the progression of periodontal disease. However, an animal study[36] showed that toothbrushing improved the increased plasma level of oxidative DNA damage induced by periodontal inflammation. Longitudinal studies are needed to examine the causal relationship between plasma ROM level and periodontal conditions.

The present study had several limitations. The study population was not balanced with regard to gender and age; more male and younger subjects should be included in future investigations. Also, the subjects with normal plasma ROM levels (from 250 to 300 CARR U) made up only 5% of the study population, suggesting that some subjects might have had systemic diseases of which they were unaware. Therefore, it might be better to conduct biochemical blood tests and to have a detailed medical examination performed by a medical doctor. It will also be important to compare plasma ROM level between patients with active periodontitis and periodontally healthy subjects. Because the FRAP assay has some drawbacks, a future study using the other methods to analyze blood antioxidative status is needed.

CONCLUSIONS

There was a positive relationship between periodontal condition and plasma oxidative status in patients in the maintenance phase of periodontal treatment. The results suggested that the high oxidative status in plasma could have affected the rate of progression of periodontal disease in the past.

ACKNOWLEDGMENTS

This study was supported by Grants-in-Aid for Scientific Research (20791641) from the Ministry of Education, Culture, Sports, Science and Technology, Tokyo, Japan. The authors report no conflicts of interest related to this study.

REFERENCES

1. Halliwell B. The role of oxygen radicals in human disease, with particular reference to the vascular system. *Haemostasis* 1993;23:118-126.
2. Darley-Usmar V, Halliwell B. Blood radicals: Reactive nitrogen species, reactive oxygen species, transition metal ions, and the vascular system. *Pharm Res* 1996; 13:649-662.
3. Amarasena N, Ogawa H, Yoshihara A, Hanada N, Miyazaki H. Serum vitamin C-periodontal relationship in community-dwelling elderly Japanese. *J Clin Periodontol* 2005;32:93-97.
4. Amaliya, Timmerman MF, Abbas F, et al. Java project on periodontal diseases: The relationship between vitamin C and the severity of periodontitis. *J Clin Periodontol* 2007;34:299-304.
5. Panjamurthy K, Manoharan S, Ramachandran CR. Lipid peroxidation and antioxidant status in patients with periodontitis. *Cell Mol Biol Lett* 2005;10:255-264.
6. Iamele L, Fiocchi R, Vernocchi A. Evaluation of an automated spectrophotometric assay for reactive oxygen

metabolites in serum. *Clin Chem Lab Med* 2002;40:673-676.
7. Christou K, Markoulis N, Moulas AN, Pastaka C, Gourgoulianis KI. Reactive oxygen metabolites (ROMs) as an index of oxidative stress in obstructive sleep apnea patients. *Sleep Breath* 2003;7:105-110.
8. Komatsu F, Kagawa Y, Sakuma M, et al. Investigation of oxidative stress and dietary habits in Mongolian people, compared to Japanese people. *Nutr Metab (Lond)* 2006;3:21.
9. Pihlstrom BL, Michalowicz BS, Johnson NW. Periodontal diseases. *Lancet* 2005;366:1809-1820.
10. Yanbaeva DG, Dentener MA, Creutzberg EC, Wesseling G, Wouters EF. Systemic effects of smoking. *Chest* 2007;131:1557-1566.
11. Bonnefont-Rousselot D, Bastard JP, Jaudon MC, Delattre J. Consequences of the diabetic status on the oxidant/antioxidant balance. *Diabetes Metab* 2000;26:163-176.
12. Pitiphat W, Merchant AT, Rimm EB, Joshipura KJ. Alcohol consumption increases periodontitis risk. *J Dent Res* 2003;82:509-513.
13. Irie K, Tomofuji T, Tamaki N, et al. Effects of ethanol consumption on periodontal inflammation in rats. *J Dent Res* 2008;87:456-460.
14. Akalın FA, Baltacıoğlu E, Alver A, Karabulut E. Lipid peroxidation levels and total oxidant status in serum, saliva and gingival crevicular fluid in patients with chronic periodontitis. *J Clin Periodontol* 2007;34:558-565.
15. Brock GR, Butterworth CJ, Matthews JB, Chapple IL. Local and systemic total antioxidant capacity in periodontitis and health. *J Clin Periodontol* 2004;31:515-521.
16. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Anal Biochem* 1996;239:70-76.
17. Nakayama K, Terawaki H, Nakayama M, Iwabuchi M, Sato T, Ito S. Reduction of serum antioxidative capacity during hemodialysis. *Clin Exp Nephrol* 2007;11:218-224.
18. Pasquini A, Luchetti E, Marchetti V, Cardini G, Iorio EL. Analytical performances of d-ROMs test and BAP test in canine plasma. Definition of the normal range in healthy Labrador dogs. *Vet Res Commun* 2008;32:137-143.
19. O'Leary TJ, Drake RB, Naylor JE. The plaque control record. *J Periodontol* 1972;43:38.
20. Klock KS, Gjerdet NR, Haugejorden O. Periodontal attachment loss assessed by linear and area measurements in vitro. *J Clin Periodontol* 1993;20:443-447.
21. Chapple IL, Brock GR, Milward MR, Ling N, Matthews JB. Compromised GCF total antioxidant capacity in periodontitis: Cause or effect? *J Clin Periodontol* 2007;34:103-110.
22. Konopka T, Król K, Kopeć W, Gerber H. Total antioxidant status and 8-hydroxy-2'-deoxyguanosine levels in gingival and peripheral blood of periodontitis patients. *Arch Immunol Ther Exp (Warsz)* 2007;55:417-425.
23. Baltacıoğlu E, Akalın FA, Alver A, Balaban F, Ünsal M, Karabulut E. Total antioxidant capacity and superoxide dismutase activity levels in serum and gingival crevicular fluid in post-menopausal women with chronic periodontitis. *J Clin Periodontol* 2006;33:385-392.
24. Canakci V, Yildirim A, Canakci CF, Eltas A, Cicek Y, Canakci H. Total antioxidant capacity and antioxidant enzymes in serum, saliva, and gingival crevicular fluid of preeclamptic women with and without periodontal disease. *J Periodontol* 2007;78:1602-1611.
25. Ou B, Huang D, Hampsch-Woodill M, Flanagan JA, Deemer EK. Analysis of antioxidant activities of common vegetables employing oxygen radical absorbance capacity (ORAC) and ferric reducing antioxidant power (FRAP) assays: A comparative study. *J Agric Food Chem* 2002;50:3122-3128.
26. Lean JM, Davis JT, Fuller K, et al. A crucial role for thiol antioxidants in estrogen-deficiency bone loss. *J Clin Invest* 2003;112:915-923.
27. Strehlow K, Rotter S, Wassmann S, et al. Modulation of antioxidant enzyme expression and function by estrogen. *Circ Res* 2003;93:170-177.
28. Weitzmann MN, Pacifici R. Estrogen deficiency and bone loss: An inflammatory tale. *J Clin Invest* 2006;116:1186-1194.
29. Chapple IL. Reactive oxygen species and antioxidants in inflammatory diseases. *J Clin Periodontol* 1997;24:287-296.
30. Sculley DV, Langley-Evans SC. Periodontal disease is associated with lower antioxidant capacity in whole saliva and evidence of increased protein oxidation. *Clin Sci (Lond)* 2003;105:167-172.
31. Tsai CC, Chen HS, Chen SL, et al. Lipid peroxidation: A possible role in the induction and progression of chronic periodontitis. *J Periodontal Res* 2005;40:378-384.
32. Graves DT, Kayal RA. Diabetic complications and dysregulated innate immunity. *Front Biosci* 2008;13:1227-1239.
33. Sanbe T, Tomofuji T, Ekuni D, Azuma T, Tamaki N, Yamamoto T. Oral administration of vitamin C prevents alveolar bone resorption induced by high dietary cholesterol in rats. *J Periodontol* 2007;78:2165-2170.
34. Carratelli M, Porcaro L, Ruscica M, De Simone E, Bertelli AA, Corsi MM. Reactive oxygen metabolites and prooxidant status in children with Down's syndrome. *Int J Clin Pharmacol Res* 2001;21:79-84.
35. Yagi K, Ohkawa H, Ohishi N, Yamashita M, Nakashima T. Lesion of aortic intima caused by intravenous administration of linoleic acid hydroperoxide. *J Appl Biochem* 1981;3:58-65.
36. Ekuni D, Tomofuji T, Tamaki N, et al. Mechanical stimulation of gingiva reduces plasma 8-OHdG level in rat periodontitis. *Arch Oral Biol* 2008;53:324-329.

Correspondence: Dr. Tatsuo Yamamoto, Department of Oral Health, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, 2-5-1 Shikata-cho, Okayama 700-8525, Japan. Fax: 81-86-235-6714; e-mail: tatsuo@md.okayama-u.ac.jp.

Submitted February 8, 2008; accepted for publication April 14, 2008.