The Impact of Smoking Status on Antioxidant Enzyme Activity and Malondialdehyde Levels in Chronic Periodontitis

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Background: The aim of this study is to investigate the impact of smoking status on the systemic and local superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT) activities and malondialdehyde (MDA) levels in subjects with chronic periodontitis (CP).

Methods: Sixty-five CP patients (23 smokers [CP-S], 23 former smokers [CP-FS], and 19 non-smokers [CP-NS]) and 20 periodontally healthy non-smoker controls (PH-NS) were included in the study. After the clinical measurements, serum and gingival tissue samples were collected. SOD, GSH-Px, and CAT activities and MDA levels in hemolysates and gingival tissue samples were spectrophotometrically assayed.

Results: Blood MDA levels in all the periodontitis groups were higher than in the PH-NS group but only the difference between CP-FS and PH-NS groups was significant (P <0.01). Gingival tissue MDA levels in the periodontitis groups were significantly higher than that in the control group (P <0.01). However, the control group had the highest gingival SOD, GSH-Px, and CAT activities compared with all the periodontitis groups (P <0.01). The CP-S group had the highest gingival MDA levels and SOD, GSH-Px, and CAT activities among the periodontitis groups, whereas the lowest values were observed in the CP-NS group (P <0.01). The blood and gingival MDA levels in the CP-FS group were similar in the CP-NS group, whereas they were lower than in the CP-S group.

Conclusions: Systemic and local MDA levels are increased by smoking in addition to the impact of periodontitis. The decreased local SOD, GSH-Px, and CAT activities observed in periodontitis patients may increase with smoking. *J Periodontol 2011;82:1320-1328.*

KEY WORDS

Antioxidants; oxidative stress; periodontitis; smoking.

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Smoking causes the development of various chronic diseases.[1] In addition, cigarette smoking is a well-established risk factor for periodontitis and is associated with an increased risk for periodontal attachment loss (AL) and bone loss.[2] Smoking has several detrimental effects on periodontal tissues. These effects include chronic reduction of blood flow, altered neutrophil function and cytokine and growth factor production, inhibition of fibroblast growth and attachment, and decreased collagen production and vascularity.[2] Furthermore, smoking stimulates the oxidative burst of neutrophils; increases reactive oxygen species (ROS) production; and leads to lipid peroxidation (LPO), oxidation of protein thiols, and alterations in protein carbonyls in plasma.[3-5] The compulsory use of the body's reserves of antioxidants (AO) to detoxify the excess free radicals in smokers results in the alteration of the level of the AO.[6]

ROS are toxic substances that attack and damage biologic molecules. ROS are also involved in the pathogenesis of several inflammatory disorders, such as type 2 diabetes[7] and vascular diseases.[8] The human body has an array of nonenzymatic and enzymatic AO defense mechanisms to remove harmful ROS to prevent their deleterious effects. The

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non-enzymatic AOs include vitamins E, A, and C; uric acid; bilirubin; reduced glutathione; albumin; transferrin; lactoferrin; ceruloplasmin; and haptoglobin. The enzymatic AOs include superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT).[9] There is a balance between the production of ROS and tissue concentration of AOs in the body.

Periodontal disease is associated with reduced total AO capacity and increased oxidative damage within the oral cavity.[10-14] Furthermore, it has been shown that patients with periodontitis have elevated levels of local and systemic LPO.[15-21] Recently, it was demonstrated that smoking increases the levels of free radicals[22] and LPO[23] in periodontal tissues. In addition, decreased AO levels in blood, gingival tissue, saliva, and gingival crevicular fluid (GCF) have been shown in patients with periodontitis and gingivitis who smoke.[22,24,25]

Quitting smoking is essential for improving the chances of a favorable outcome before beginning periodontal treatments.[26] It was suggested that the effects of smoking are reversible, because the risk of developing periodontitis was clearly reduced on smoking cessation.[27] In light of these findings, a decrease in LPO and oxidative damage in periodontal tissues can be expected after smoking cessation. There are no data in the literature regarding the systemic and local periodontal SOD, GSH-Px, and CAT activities and LPO levels in former smoker patients with periodontitis. In addition, the mechanism for the negative effects of smoking on the periodontium is still unclear.

The aims of this study are to investigate the impact of smoking status on the blood and gingival tissue SOD, GSH-Px, and CAT activities and malondialdehyde (MDA) levels in patients with periodontitis who are current, former, or non-smokers and to explore the relationships between the periodontal parameters and SOD, GSH-Px, and CAT activities and MDA levels.

MATERIALS AND METHODS

A total of 65 otherwise healthy patients with chronic periodontitis (CP) (32 males and 33 females; age range: 20 to 50 years) and 20 periodontally healthy non-smoker (PH-NS) volunteers (11 males and 9 females; age range: 25 to 49 years) were recruited for the study. The patients with chronic periodontitis were chosen from patients admitted to the Suleyman Demirel University, Faculty of Dentistry, Department of Periodontology, Isparta, Turkey, from October 2006 to July 2008. None of the patients had received periodontal therapy or used antibiotics, non-steroidal analgesics, sympathomimetics, or immunosuppressive agents in the last 6 months. Patients with systemic disease, those using regular supplementary vitamins, and pregnant or lactating females were excluded from the study. Patients with moderate generalized chronic periodontitis (i.e., ≤3 mm AL, <5 mm throughout ≤ 30% of the mouth)[28] were recruited for the study to make a clear distinction among the participants in the groups of smokers, non-smokers, and former smokers.

Participants with chronic periodontitis were classified into one of three groups as follows: 1) the current smoker chronic periodontitis (CP-S) group consisted of 23 smoker patients with chronic periodontitis who have smoked ≤10 cigarettes per day for >5 years;[29] 2) the former smoker chronic periodontitis (CP-FS) group consisted of 23 former smoker subjects with chronic periodontitis who had quit smoking for >6 months; and 3) the non-smoker chronic periodontitis (CP-NS) group consisted of 19 non-smoker patients with chronic periodontitis who never smoked.

The control group (PH-NS) consisted of 20 non-smoker volunteers who were admitted to Suleyman Demirel University, Faculty of Dentistry, and Department of Oral Surgery for tooth extraction, during the same time period as the periodontitis patients, who had neither history of periodontal disease nor tooth loss caused by periodontitis and had no clinical signs of periodontitis (clinical attachment level [CAL] <1 mm, probing depth [PD] ≤3 mm, gingival index [GI] <1).

Written informed consent was obtained from all patients. Ethical approval for the study was obtained from the ethics committee of Suleyman Demirel University, Faculty of Medicine (decree number: 02.06.2006 to 01/11).

All periodontal patients underwent supragingival scaling to prepare tissues for biopsy. One week after periodontal clinical measurements, gingival tissue, and blood samplings were performed in the morning hours after overnight fasting.

Clinical Measurements

Plaque index (PI),[30] GI,[31] PD, CAL, and presence of bleeding on probing (BOP)[32] were measured at six sites and recorded on each tooth, except third molars. All clinical periodontal measurements were performed by the same examiner (MÖT).

Blood Sampling

Venous blood samples were collected from the antecubital vein in tubes with K_3EDTA. The anticoagulated blood was separated into plasma and erythrocytes by centrifugation at 1,500 × g for 10 minutes at 4°C. The erythrocyte samples were washed three times in cold isotonic saline (0.9%, vol/wt) and then hemolyzed with 2 mL of bidistilled water. All samples were kept in –80°C conditions until the date of analysis. The SOD, GSH-Px, and CAT activities and MDA

levels of the blood were measured in the hemolysates. Butylated hydroxytoluene and EDTA were added to the sample and reaction mixture to minimize the oxidation of lipids that contributes artifactually during sample processing and the thiobarbituric acid reaction.

Gingival Tissue Sampling

Tissues were collected from patients before any periodontal surgical procedure including scaling and root planing under asepsis and local anesthesia, avoiding local anesthetic infiltration into the biopsy site.[33] Gingival tissue samples were harvested from the sulcular margins of the gingival papillae between premolar or molar teeth with moderate periodontal destruction (2 mm <PD <4 mm or 2 mm <CAL <5 mm) to standardize the gingival sampling.

The gingival tissue samples of the control group were harvested from the gingival margins of the premolar or molar teeth undergoing extraction because of profound occlusal caries or orthodontic reasons. All gingival samples were washed in saline solution immediately after excision and dried with gauze at 4°C. Tissue samples were stored in firmly wrapped sterile Eppendorf tubes at –80°C until analysis.

Laboratory Analyses

Preparation of the tissue homogenates. The gingival tissue samples were weighed by an ocular sensitive scale and homogenized[¶] (1:5, wt/vol) in 100 mmol/L phosphate buffer (pH 7.4) in an ice bath. The homogenate was sonicated[#] for 30 seconds and centrifuged at 10,000 × g for 10 minutes at 4°C to remove debris. The clear upper supernatant was taken and assays were carried out on this part. The preparation procedures of tissue homogenates were performed in an anaerobic environment. Protein concentrations of homogenates were determined by the method of Lowry et al.[34]

Measurements of MDA levels in hemolysates and gingival tissue homogenates. To evaluate the LPO levels of hemolysates and gingival tissues, levels of MDA were determined by the double heating method of Draper and Hadley.[35] The principle of this method is spectrophotometric measurement of the color developed during reaction to thiobarbituric acid with MDA. The concentration of MDA is expressed as nanomole per milligram hemoglobin and nanomole per milligram protein in hemolysates and tissues.

Measurements of SOD, GSH-Px, and CAT activities in hemolysates and gingival tissue homogenates. SOD, GSH-Px, and CAT activities in the hemolysates and the gingival tissues were determined. The measurement of SOD was based on the principle that xanthine reacts with xanthine oxidase to generate superoxide radicals, which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride to form a red formazan dye. SOD activity is then measured by the degree of inhibition of this reaction.[36] The determination of GSH-Px activity was based on the method of Paglia and Valentine.[37] The activities of SOD and GSH-Px are expressed as unit per milligram hemoglobin and unit per milligram protein in hemolysates and tissues. CAT activity was measured according to the method of Aebi.[38] The principle of the assay is based on the determination of the rate constant (k, s-1) of decomposition of hydrogen peroxide by the enzyme CAT. The activity of CAT is expressed as k/mg hemoglobin and milligram protein in hemolysates and tissues.

Hemoglobin concentration was determined by the cyanomethemoglobin method from the hemolyzed erythrocytes.[39] An autoanalyzer** was used to determine the activities of SOD and GSH-Px, and a spectrophotometer†† was used to estimate the activities of the enzyme CAT.

Statistical Analyses

The sex differences of the groups were investigated by χ^2 test. The normality of the data distribution was examined by Kolmogorov-Smirnov test. The only normally distributed data were age. Age was expressed as mean ± SD and the group comparisons regarding age were made by using an independent samples t test. The other parameters were presented as median values (min-max). The Kruskal-Wallis test followed by the Mann-Whitney U test with Bonferroni correction was used for the group comparisons for the non-normally distributed data. Pearson correlations were used to look at the relationships among smoking status, blood and tissue AO enzyme, and MDA levels and clinical parameters. A significance level of P <0.05 was used for the Kruskal-Wallis test and P <0.01 was accepted as the significance level for the Mann-Whitney U test with Bonferroni correction. The statistical analyses were performed by a package program.[‡‡]

RESULTS

Clinical Findings

Demographic variables and median (min-max) values of clinical periodontal measurements are given in Table 1. There were no statistically significant differences among the study groups regarding age and sex (matching variables).

There were no statistically significant differences among the periodontitis groups regarding periodontal clinical parameters except PI. The PI value of the CP-S

¶ Ultra Turrax T25, Janke & Kunkel, Staufen, Germany.
# Bandelin Sonoplus UW 2070, Berlin, Germany.
** Aeroset, Abbott, Abbott Park, IL.
†† UV-1601, Shimadzu, Kyoto, Japan.
‡‡ SPSS 15, IBM, Chicago, IL.

Table 1.

Intergroup Comparisons of Demographic Variables and Clinical Periodontal Parameters

Parameters	CP-S (n = 23)	CP-FS (n = 23)	CP-NS (n = 19)	PH-NS (n = 20)
Sex (male/female)[a]	11/12	12/11	9/10	11/9
Age median (min-max)	20 to 47	22 to 49	20 to 50	25 to 49
Age (mean ± SD)[b]	(36.08 ± 8.33)	(36.65 ± 8.14)	(36.00 ± 9.32)	(35.21 ± 9.07)
Packs/year (SD)[c]	15.82 ± 6.16	15.38 ± 12.25†	0	0
Smoking duration (year) (SD)[c]	16.95 ± 8.26	17.47 ± 7.58†	0	0
Time since smoking cessation (year) (SD)[c]	0	3.43 ± 4.04	0	0
PI median (min-max)[c]	1.28 (0.47 to 2.79)	0.87 (0.38 to 2.29)*	0.98 (0.47 to 2.56)	0.44 (0.29 to 0.68)‡§‖
GI median (min-max)[c]	0.96 (0.05 to 1.29)	0.64 (0.03 to 1.93)	0.76 (0.27 to 1.57)	0.27 (0.07 to 0.65)‡§‖
PD (mm) median (min-max)[c]	2.78 (1.18 to 4.24)	2.56 (1.06 to 4.32)	2.41 (1.57 to 4.59)	2.05 (1.67 to 2.32)‡§‖
PD gingival tissue sampling site (mm) median (min-max)[c]	3 (2 to 4)	3 (2 to 4)	3 (2 to 3)	1 (1 to 2)‡§‖
CAL (mm) median (min-max)[c]	3.24 (1.96 to 5.08)	3.24 (1.06 to 5.04)	2.55 (1.57 to 5.06)	2.05 (1.67 to 2.32)‡§‖
BOP (%) median (min-max)[c]	62 (0 to 100)	64 (0 to 100)	78 (0 to 100)	28 (10 to 31)‡§‖

* Significant difference between CP-S and CP-FS groups (P <0.01).
† Significant difference between CP-FS and CP-NS groups (P <0.01).
‡ Significant difference between CP-S and PH-NS groups (P <0.01).
§ Significant difference between CP-FS and PH-NS groups (P <0.01).
‖ Significant difference between CP-NS and PH-NS groups (P <0.01).
a = χ^2 test; b = Independent samples t test; c = Mann-Whitney U test with Bonferroni correction.

group was significantly higher than that of the CP-FS group (P <0.01). There was no significant difference between the periodontitis groups regarding the mean PD of the gingival tissue sampling site (P>0.05). However, there were significant differences between the PH-NS group and all of the periodontitis groups regarding the PD of the sampling region (P <0.01).

Laboratory Findings

The median (min-max) MDA levels and SOD, GSH-Px, and CAT activities in hemolysates and gingival tissues of the groups are given in Table 2. Blood MDA levels in all the periodontitis groups were higher than in the PH-NS group but only the difference between CP-FS and PH-NS groups was significant (P <0.01). Although blood MDA levels and SOD and CAT activities were close to each other in all periodontitis groups (P >0.05), the GSH-Px activity in the CP-S group was significantly lower compared to that of the CP-NS group (P <0.01). The lowest blood MDA levels and SOD and GSH-Px activities were observed in the PH-NS group, whereas the highest blood CAT activity was detected in this group, compared to the periodontitis groups.

Gingival tissue MDA levels in the periodontitis groups were significantly higher than that in the PH-NS group (P <0.01). There was a progressive reduction in the gingival tissue MDA levels and SOD, GSH-Px, and CAT activities from the CP-S to the CP-FS to the CP-NS patients. The highest AO enzyme activity and the lowest MDA levels were observed in the gingival tissues of the PH-NS compared to the other groups (P <0.01). The gingival MDA levels detected between the groups were statistically significantly different (P <0.01), except for the difference between the CP-NS and the CP-FS groups.

Gingival tissue SOD activity in the CP-NS group was significantly lower than in the other groups (P <0.01). However, there was no significant difference in tissue SOD levels between the CP-S and CP-FS groups.

Tissue GSH-Px activity levels of the CP-S and CP-FS groups were close, but that of the CP-NS group was significantly lower than those of the other groups (P <0.01). The tissue CAT activities were significantly different between the groups (P <0.01). The lowest tissue CAT activity was observed in the CP-NS group, whereas the same and the highest CAT activities were observed in the CP-S and PH-NS groups.

Correlations

There were significant correlations between periodontal parameters, smoking-related parameters, and AO enzyme activity and MDA levels in the blood and gingiva. The correlations between the parameters are presented in Table 3.

Table 2.

Median (min-max) Values and Intergroup Comparisons of Malondialdehyde and Antioxidant Enzyme Levels in Blood and Gingival Tissue

	Biochemical Parameters	CP-S (n = 23)	CP-FS (n = 23)	CP-NS (n = 19)	PH-NS (n = 20)
Blood	MDA (nmol/mg hemoglobin)	45.9 (27 to 138.2)	44.8 (26.5 to 161.6)	42.5 (27.8 to 180.5)	36.3 (30 to 54.3)*
	SOD (U/mg hemoglobin)	1,500 (991.2 to 2,639.8)	1,393.4 (425 to 3,786.6)	1,426.9 (344.6 to 2,184.5)	1,023.8 (343.8 to 1,596.8)†‡
	GSH-Px (U/mg hemoglobin)	10.1 (2.6 to 29.2)	16.5 (1.1 to 75.7)	17.5 (6.6 to 26.7)§	7.4 (3.4 to 18.6)*‡
	CAT (CAT/mg hemoglobin)	19.7 (5.4 to 46.4)	18.3 (7.8 to 95.8)	19.1 (8 to 39.9)	51 (3.8 to 539.8)*†‡
Gingival Tissue	MDA (nmol/mg protein)	1.21 (1.12 to 2.54)	1.05 (0.86 to 1.31)‖	1.00 (0.84 to 1.40)§	0.63 (0.34 to 1.62)*†‡
	SOD (U/mg protein)	1.37 (1.08 to 1.95)	1.30 (1.25 to 1.67)	1.12 (1.08 to 1.50)§¶	2.42 (1.58 to 2.80)*†‡
	GSH-Px (U/mg protein)	173.7 (156.8 to 257.3)	155.8 (102.3 to 215.4)	135.6 (73.4 to 189.27)§¶	284.4 (237.3 to 421.4)*†‡
	CAT (CAT/mg protein)	2.63 (0.87 to 6.53)	1.60 (0.62 to 2.36)‖	0.88 (0.33 to 1.19)§¶	2.63 (0.85 to 2.89)*‡

* Significant difference between CP-FS and PH-NS groups (P <0.01).
† Significant difference between CP-S and PH-NS groups (P <0.01).
‡ Significant difference between CP-NS and PH-NS groups (P <0.01).
§ Significant difference between CP-S and CP-NS groups (P <0.01).
‖ Significant difference between CP-S and CP-FS groups (P <0.01).
¶ Significant difference between CP-FS and CP-NS groups (P <0.01).

Table 3.

Correlations Among Periodontal Parameters, Smoking-Related Parameters, Blood and Gingival Tissue, Malondialdehyde, and Antioxidant Enzyme Levels in all Participants

Correlations	*r*	*P*	Correlations	*r*	*P*
Age-PD	0.331	0.002	CAL-GT SOD	–0.425	0.000
Age-CAL	0.384	0.000	CAL-GT GSH-Px	–0.348	0.000
PI-packs/year	0.407	0.000	BOP-GT SOD	–0.493	0.000
PI-smoking duration	0.362	0.001	BOP-GT GSH-Px	–0.445	0.000
PI-GT-SOD	–0.467	0.000	B GSH-Px-B MDA	0.337	0.002
PI-GT GSH-Px	–0.423	0.000	GT SOD-packs/year	–0.383	0.000
GI-GT SOD	–0.494	0.000	GT SOD-smoking duration	–0.357	0.001
GI-GT GSH-Px	–0.513	0.000	GT GSH-Px-time since smoking cessation	–0.340	0.000
GI-packs/year	0.344	0.001	GT GSH-Px-GT SOD	0.777	0.000
PD-GT SOD	–0.345	0.001	GT GSH-Px-GT CAT	0.449	0.001
CAL-packs/year	0.461	0.000	GT CAT-GT MDA	0.442	0.000
CAL-smoking duration	0.465	0.000	GT CAT-GT SOD	0.344	0.001

B = blood; GT = gingival tissue; *r* = Pearson correlation coefficient.

DISCUSSION

The findings of this study indicate that blood and gingival tissue MDA levels and SOD and CAT activities and gingival tissue GSH-Px activity are increased in smoker patients with periodontitis compared with former smoker and non-smoker patients with periodontitis. There are studies conducted on smoker and non-smoker patients with periodontitis regarding the oxidative capacity of blood, saliva, gingiva, or GCF.[22-25,40] However, we did not come across any analogous studies conducted on current, former, and non-smoker patients with periodontitis. To our knowledge, this is the first study investigating the blood and gingival tissue MDA levels and SOD, GSH-Px, and CAT activities in current, former, and non-smokers who have the same level of periodontal AL and comparing them to those of periodontally healthy patients who do not smoke.

The studies investigating local AO capacity and oxidative stress related to periodontal disease

were mostly conducted with saliva or GCF samples.[14,17,20,21,23-25,41-43] The number of studies investigating LPO and AO levels in gingival tissue was quite small and these gingival samples were obtained during flap surgery followed by initial periodontal treatment.[19,22,44,45] The reduction of oxidative stress and increase in AO capacity after the non-surgical periodontal therapy were reported in the literature.[23,42,46] In contrast D'Aiuto et al.[47] demonstrated that acute increases in reactive oxygen metabolites and systemic inflammation occurred after periodontal therapy. In addition, it was suggested that periodontal therapy could not only alter the local ROS production and AO state, but also the host systemic oxidative state.[47] In the present study, to eliminate the effects of periodontal therapy on the local and systemic SOD, GSH-Px, and CAT activities and MDA levels, and to evaluate the additional effects of smoking on the tissues having moderate periodontitis accurately, the blood and tissue samples were obtained before non-surgical periodontal treatment.

An increase in LPO in the blood and periodontium of patients with periodontitis has been reported.[16,20,21] In agreement with those results, blood and gingival tissue MDA levels in all the periodontitis groups were found to be elevated compared to patients who were periodontally healthy. However, only the gingival tissue MDA levels in the periodontitis groups and the blood MDA level of former smoker patients with periodontitis were significantly different than those of the control group.

Smoking increases the level of free radicals and causes oxidative damage in the tissues. A study investigating the effects of smoking on blood oxidative parameters irrespective of periodontal status demonstrated that blood MDA levels of active smokers were higher than those of non-smokers.[41] In addition, it was reported that MDA levels increased in the gingival biopsy specimen and saliva of the smoker patients with periodontitis.[22,23] The results of our study supported the theory that the MDA levels in smokers were elevated and demonstrated that the MDA levels in the gingival tissues of the former smokers were significantly lower than in current smokers.

It has been reported that total AO capacity and non-enzymatic AO levels are decreased in patients with periodontitis,[13,14,19,23,42,43] but the results related to enzymatic AO activity in patients with periodontitis were conflicting. Some studies investigating the effect of periodontitis on the SOD activity in the literature suggested that the SOD activity in plasma,[19,44] erythrocytes,[19] gingival tissue,[19,44] saliva,[46] and GCF[44] of the patients with periodontitis were higher than those of periodontally healthy patients, whereas others reported a decrease in the SOD activity levels in the gingival tissue,[45] serum,[43,48,49] GCF,[43,48,49] and saliva[49] of the patients with periodontitis. Similar to these results, we observed higher blood and lower gingival SOD activity in the subjects with periodontitis compared to the non-smoker healthy controls. It was reported that the SOD activity in the blood and periodontium of the smoker patients with periodontitis was lower than in the non-smoker patients with periodontitis.[22,23,25] In contrast, we observed an insignificant increase in the CP-S group and an insignificant decrease in the CP-FS group compared with the CP-NS group as regards blood SOD activity, whereas the gingival SOD activities of the CP-S and CP-FS groups were significantly higher than that of the CP-NS group. This difference may be caused by the low levels of periodontal destruction of the sampling region in our study compared to the other studies.[22,23,25] The studies evaluating the effects of smoking on SOD activity regardless of periodontal status suggested that smoking increases the SOD activity in the blood and saliva.[40,41,50-52] Moreover, it was reported that after the smoking cessation, the increased SOD activity had decreased to those found in non-smoker subjects.[51] The results of our study support these findings.

The increased GSH-Px activity in the plasma,[19] saliva,[20,23] GCF,[17,20,53] erythrocytes,[19] and gingival tissues[19] of the patients with periodontitis were reported. Similar with these results, the GSH-Px activity in the blood of the non-smoker patients with periodontitis was significantly higher than that of non-smoker controls, whereas GSH-Px activity in gingival tissue was significantly lower. Guentsch et al.[23] reported that GSH-Px activity in the saliva of the smoker patients with or without periodontitis was higher than the matched non-smoker controls. In the present study, a slight and insignificant increase in blood GSH-Px activity was observed in the CP-S group compared to the PH-NS group. However, the blood GSH-Px activity of CP-S group was lower than that of CP-FS and CP-NS groups. These results were supported by the results of other studies that reported decreased plasma and saliva GSH-Px activity of smoker subjects.[50,52] As different from the other studies, the gingival tissue GSH-Px activity in the CP-S, CP-FS, and CP-NS groups was significantly lower than in the PH-NS groups, and the CP-S group had the highest gingival GSH-Px activity among the periodontitis groups.

There was only one study in the literature regarding the effects of periodontitis on CAT activity of plasma, erythrocytes, and gingival tissues of the non-smoker subjects.[19] The increased CAT activity in non-smoker patients with periodontitis was reported.[19] In contrast, we found decreased blood and gingival tissue CAT activity of the non-smoker patients with periodontitis compared to healthy controls. McCusker and Hoidal[51] suggested that the CAT activity in alveolar

macrophages of patients who smoke were twice that found in non-smoker patients. The increased CAT activity in blood and gingival tissues of the smoker patients with periodontitis compared to non-smoker patients with periodontitis were also reported.[22] The findings of our study support these results.

The principal radical in the tar phase of cigarette smoke is a quinine-hydroquinone complex that can reduce molecular oxygen to superoxide radicals.[6] Polymorphonuclear leukocytes and macrophages produce superoxide as an antibacterial agent in case of bacterial challenge to the periodontium.[10] Because periodontal tissues are directly exposed to cigarette smoke, the increase of superoxide level in the periodontal tissues of the smoker patients with periodontitis is an expected result. Superoxide is removed from tissues by dismutation to hydrogen peroxide spontaneously or catalyzed by SOD. The hydrogen peroxide formed is removed by CAT in the intracellular environments or by GSH-Px in the extracellular environments.[10] It was suggested that although smoking induces selective increase of AO enzyme activity in the tissues as self-defense mechanism,[51] this increase is not sufficient to protect the tissues from the harmful effects of smoking.[54] In the present study, increased gingival SOD, GSH-Px, and CAT activities in smokers compared to periodontally matched non-smoker and former smoker patients may be the result of a protective and adaptive mechanism developing in the tissue.

In accordance with the fact that cigarette smoking is one of the important risk factors for periodontal AL, strong positive correlations between CAL and smoking duration and yearly cigarette consumption were observed in this study. In addition, it was determined that there were strong negative correlations between gingival tissue GSH-Px levels and smoking duration and yearly cigarette consumption.

The studies in the literature regarding the effects of quitting smoking on periodontal health reported that preferable results[24] and greater attachment gain[25] were obtained with periodontal treatment and that there was an increase in blood flow to the gingival tissues[27] in patients who quit smoking. Bergström et al.[55] found that former smokers have better periodontal health than current smokers, although worse than that of non-smokers.

The other interesting finding of our study was the reduction in the blood and gingival MDA levels and AO enzyme activity except the blood GSH-Px activity in former smoker patients with periodontitis compared to smoker patients with periodontitis. Moreover, the MDA levels and enzymatic AO activities of former smokers were close to those of the non-smoker patients with periodontitis. It was observed that there was negative correlation between time since smoking cessation and the gingival tissue GSH-Px activity in the former smoker patients with periodontitis. Our findings are consistent with the study suggesting that the filtration of smoke within the tissues induced the increase in AO activities and that after smoking cessation, the increased activities had returned to those found in non-smoker patients.[50]

There were a number of limitations to the study. The non-longitudinal design of the study made it difficult to evaluate the effects of smoking cessation on the MDA levels and AO enzyme activity of the periodontium. The lack of current and former smoker periodontally healthy groups limited the evaluation of the effects of periodontitis and smoking on the MDA levels and AO enzyme activity separately.

CONCLUSIONS

Within the limitation of the study, we conclude that both periodontitis and smoking lead to significant changes in MDA production and AO enzyme activity in blood and gingival tissues. The combination of smoking and periodontitis resulted in significant increases in MDA levels and alterations in SOD, GSH-Px, and CAT activities in periodontium. Quitting smoking can reverse the negative effects of smoking on the AO balance of the periodontium. Smoking cessation programs should be a standard component of periodontal therapy. Further longitudinal and cross-sectional studies are needed for the determination of when the decrease in the oxidative stress begins after smoking cessation and to clarify the role of smoking on the oxidative and AO status in healthy periodontal tissues.

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