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Influence of smoking on interleukin-1beta level, oxidant status and antioxidant status in gingival crevicular fluid from chronic periodontitis patients before and after periodontal treatment

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Background and Objective: The aim of this study was to evaluate the impact of smoking on the relationship between interleukin-1 (IL-1β) and oxidation in patients with periodontitis and response to nonsurgical periodontal therapy.

Material and Methods: Data were obtained from 30 patients with generalized chronic periodontitis (15 smokers and 15 nonsmokers) and from 10 periodontally healthy controls. IL-1β level, total oxidant status (TOS) and total antioxidant status (TAS) were recorded in gingival crevicular fluid. Probing depth, clinical attachment level, gingival and plaque indices and bleeding on probing were also measured. The gingival crevicular fluid and clinical parameters were recorded at baseline and 6 wk after periodontal treatment.

Results: The study showed statistically significant improvement of clinical parameters in both smokers and nonsmokers after periodontal treatment. Moreover, the baseline IL-1β levels were significantly higher in smokers compared with nonsmokers ($p < 0.05$). After periodontal treatment, the IL-1β levels were significantly reduced in both smokers and nonsmokers ($p < 0.05$). There were no significant differences in TOS and TAS between periodontitis patients and healthy controls at baseline and 6 wk after periodontal treatment. The level of IL-1β in gingival crevicular fluid was positively correlated with TOS in both smokers and nonsmokers.

Conclusions: Periodontal treatment improved the clinical parameters in both smokers and nonsmokers. The results confirm that periodontal therapy has an effect on IL-1β levels in gingival crevicular fluid, but not on TOS and TAS.

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Periodontitis is a chronic inflammatory disease in adults. The progression of the disease is dependent on the host response to pathogens that colonize the tooth surface (1). In addition to the direct etiological impact of bacteria, cigarette smoking is considered to be one of the most important environmental risk factors for periodontitis. Significantly higher clinical attachment loss and bone loss have been observed in smokers (2). Studies on the mechanism of how smoking modifies the host response and eventually results in the progression of periodontal tissue destruction have suggested that smoking alters vascular function, neutrophil/monocyte activities, adhesion molecule expression, antibody production and cytokine and inflammatory mediator release (3), implying that phagocytes could be the key cells through which the effect of smoking is mediated. In addition to cytokines, reactive oxygen species (ROS) produced by activated phagocytes are potentially deleterious to tissues (4). The imbalance between ROS and antioxidants in the human body, which leads to oxidative stress, contributes significantly to many inflammatory diseases, such as atherosclerosis (5), diabetes mellitus (6,7), rheumatoid arthritis (8) and periodontal diseases. ROS can cause DNA and protein damage, initiate lipid peroxidation, oxidize important enzymes such as protease inhibitors, and stimulate pro-inflammatory cytokine [interleukin (IL)-1 and IL-6]) release through depleting intracellular thiol compounds and activating nuclear factor-κB (NF-κB) (4). Smoking has been reported to attenuate (9), have no effect on (10) or increase (11) the FcγR-stimulated respiratory burst of peripheral blood neutrophils observed in smokers. While the data are conflicting, we have hypothesized that there is a link between cigarette smoking and ROS-mediated tissue destruction in periodontitis. IL-1 is capable of stimulating ROS production in some cell types. As the release of cytokines by phagocytes is closely associated with the generation of ROS, and IL-1 may contribute to the pathologic process of the inflammatory response in periodontitis (12), we have further hypothesized that there is a link between ROS and IL-1 in periodontitis and that this axis could be the target for smoking.

Of the 11 members of the IL-1 family, IL-1β appears to be the most potent, having a catabolic effect on bone approximately 10-fold greater than that of IL-1α (13). Some studies have suggested that smoking increases the level of IL-1β in gingival crevicular fluid (3,14), although other studies have shown no effect of smoking on the amount of IL-1β (15,16). IL-1β activates, and is activated by, NF-κB, contributing to the positive feedback loop for NF-κB activation (4), and this may be mediated through glutathione peroxidase (17) where the inhibition of glutathione peroxidase was correlated with over-activation of NF-κB.

Gingival crevicular fluid provides an avenue to evaluate the multiple effects of smoking on the host response in the periodontium (3). Also, it seems logical that plasma will provide the basic oxidant–antioxidant profile of gingival tissue and crevicular fluids, as there is a constant flow of fluid from the blood into the gingival crevice (18). Therefore, the aim of this study was to assess the level of the pro-inflammatory cytokine IL-1β and the oxidant/antioxidant status in gingival crevicular fluid in smokers and nonsmokers with periodontitis and to study the effects of periodontal treatment on these parameters.

Material and methods

Study population

Forty subjects – 30 periodontally diseased subjects and 10 periodontally healthy nonsmoker individuals – were selected for the study. Complete medical and dental histories were taken from all subjects. None of the subjects had a history of systemic disease, or had received antibiotics or other medications or periodontal treatment within the past 6 mo. Informed consent was obtained from all the subjects, and the study protocol was approved by the Medical Ethics Committee of Cumhuriyet University. Periodontally diseased subjects were diagnosed with generalized chronic periodontitis ($> 30\%$ of sites with a clinical attachment level and probing depth of ≥ 5 mm). Cigarette consumption was determined by verbal questioning. Smokers were enrolled if they regularly smoked ≥ 20 cigarettes/d, and nonsmokers were characterized as not having smoked cigarettes in their lifetime. The same number of smokers and nonsmokers were present in the periodontitis group. Control subjects were designated as healthy if the full-mouth probing pocket depth was < 3 mm, gingival index scores were 0 and they had no radiographic evidence of alveolar bone loss. These individuals were systemically and periodontally healthy volunteers.

Clinical measurements and nonsurgical periodontal therapy

Before collection of crevicular fluid, supragingival plaque was scored using the plaque index (19). After collection of crevicular fluid, gingival inflammation was scored using the gingival index (19). Bleeding on probing was also recorded. Probing depth and clinical attachment level measures were obtained using a Williams' periodontal probe. In all subjects, individual acrylic stents were fabricated with grooves as reference points for the clinical measurements. One researcher (H.T.), who was calibrated for use of a pressure-sensitive probe, performed all the clinical measurements. After recording the baseline measurements, nonsurgical periodontal therapy consisting of oral hygiene instructions and scaling and root planing (SRP) was performed, quadrant by quadrant, under local anesthesia in four visits using specific curettes (Hu-Friedy, Chicago, IL, USA) by one examiner (A.A.) (20). Treatment was completed within 14 d. No antibiotics were prescribed during the treatment. The clinical measurements and gingival crevicular fluid samples taken from the sampling site were recorded at baseline and 6 wk after the periodontal treatment.

Crevicular fluid sampling

Generalized chronic periodontitis sampling was done during the same session

as the clinical measurements. Only the upper anterior teeth were included in the study to improve access and to reduce the risk of salivary contamination during these processes. Gingival crevicular fluid was collected during the same session that clinical measurements were made. Before gingival crevicular fluid sampling, the sample site was carefully isolated using cotton rolls to avoid contamination with saliva. A standard paper strip (Pro Flow, Amityville, NY, USA) was placed in the pocket until mild resistance was felt and then left in place for 30 s. The strip was then placed in an Eppendorf tube and immediately frozen at −80°C until the day of analysis. Strips that were visibly contaminated with blood were discarded.

Analysis of IL-1β levels and determination of total oxidant status and total antioxidant status in gingival crevicular fluid

All laboratory analyses were performed on the same day. On the day of the assay, 500 μL of phosphate-buffered saline (pH 7) was added to the tubes containing the sample strips. The tubes were gently shaken for 1 min then centrifuged at 2000 *g* for 5 min. The supernatants were then divided into three aliquots to be used for the determination of IL-1β content, total oxidant status (TOS) and total antioxidant status (TAS).

The amount of IL-1β was determined by ELISA (D*İ*Asource, Rue de l'Industrie, Nivelles, Belgium), in accordance with the manufacturer's instructions. After halting color development, the optical density was measured using a computerized microtiter plate reader set to a wavelength of 450 nm. The cytokine levels were calculated from standard curves and defined as pg/site for the total amount of IL-1β. The sensitivity of the IL-1β ELISA was 0.35 pg/mL. Sites with cytokine levels below the detection limit of the assay were scored as 0.

TOS and TAS levels were measured using commercially available kits (Rel Assay, Mega Tıp, Gaziantep, Turkey) by Erel's colorimetric method (21,22) at an absorbance of 520 nm, as previously described for gingival crevicular fluid by Çanakçı *et al*. (23).

For determination of TOS, the method utilizing oxidation of the ferrous ion-o-dianisidine complex to ferric ion by oxidants present in the sample was used. The oxidation reaction was enhanced by glycerol that was present in abundance in the reaction medium. The ferric ion produced a colored complex with xylenol orange in an acidic medium. The color intensity, measured spectrophotometrically, was related to the total amount of oxidant molecules present in the sample. The assay was calibrated with hydrogen peroxide and the results were expressed in terms of micromolar hydrogen peroxide equivalent per liter (μmol H_2O_2 equivalent/L).

Determination of TAS is based on bleaching of the characteristic color of a more stable 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation by antioxidants. The assay has precision values of < 3%. The results were expressed as mmol Trolox equivalent/L.

Statistical analysis

Data were expressed as means ± standard deviation or percentage, as appropriate. Analysis of normality was conducted, and nonparametric approaches were used based on the distribution of the data. Baseline and 6-wk plaque index, gingival index, probing depth and clinical attachment level values in both smokers and nonsmokers were analyzed using the Wilcoxon rank test. The ratio of bleeding on probing in both smokers and nonsmokers was analyzed using the chi-square test. The levels of IL-1β, TOS and TAS in all groups were analyzed by analysis of variance followed by the post-hoc Tukey test. Spearman correlation analyses in all groups were used for IL-1β, TOS and TAS. A p-value of < 0.05 was considered to be statistically significant. The TOS, TAS and IL-1β levels were determined as the expected primary outcomes of the study. The power of the analysis was completed by utilizing data from a previous publication, which evaluated the changes in gingival crevicular fluid TAS following periodontal treatment (24). An alpha of 0.05 was selected for calculation. The required sample size was 15 in periodontitis groups, giving a statistical power of 85%.

Results

Clinical findings

The demographic data of gender and age in the periodontitis patients and healthy controls are summarized in Table 1. Table 2 presents mean probing depth, clinical attachment level, plaque index and gingival index values at baseline and 6 wk after treatment in all study groups. All baseline clinical parameters were found to be significantly higher in periodontitis patients (both smokers and nonsmokers) compared with the healthy controls ($p < 0.05$). Mean probing depth and clinical attachment level values were significantly reduced after periodontal treatment in all periodontitis patients ($p < 0.05$). There were no statistically significant differences in probing depth and clinical attachment level values between smokers and nonsmokers 6 wk after treatment.

The plaque index and gingival index values of all periodontitis patients 6 wk

Table 1. Demographic characteristics of the study subjects

Characteristic	Periodontitis patients		Healthy controls
	Smokers (n = 15)	Nonsmokers (n = 15)	
Age (years)	38.4 ± 5.5	38.7 ± 5.9	38.0 ± 7.2
Gender			
Female	6	8	4
Male	9	7	6

Data are given as n or as mean ± SD.

Table 2. Clinical parameters of the gingival crevicular fluid sample site at baseline and 6 wk after therapy in patients with periodontitis and periodontally healthy controls

	Periodontitis patients				
	Smokers (n = 15)		Nonsmokers (n = 15)		
Parameter	Baseline	6 wk	Baseline	6 wk	Healthy controls
Probing depth (mm)	4.8 ± 0.5	2.7 ± 0.7[a]	4.8 ± 0.3	2.8 ± 0.6[a]	1.4 ± 0.5
Clinical attachment level (mm)	8.2 ± 1.1	7.0 ± 1.3[a]	8.3 ± 1.0	7.5 ± 0.9[a]	0
Plaque index	2.0 ± 0.6	0.1 ± 0.3[a]	1.6 ± 0.6	0.2 ± 0.4[a]	0.2 ± 0.4
Gingival index	2.0 ± 0.3	0.0 ± 0.2[a]	1.9 ± 0.5	0.2 ± 0.4[a]	0
Percentage of bleeding on probing (%)	100	0	100	0	0

Data are given as mean ± standard deviation or percentage.
[a]Significantly different from baseline ($p < 0.05$).

after treatment were significantly lower than those at baseline ($p < 0.05$). There were no significant differences in the plaque index and gingival index values between smokers and nonsmokers at 6 wk ($p > 0.05$). Likewise, there were no significant differences in the percentage of bleeding on probing between smokers and nonsmokers ($p > 0.05$), and the percentage of bleeding on probing values showed significant decreases in response to periodontal treatment in all patients ($p < 0.05$).

Laboratory findings

IL-1β was detected in all gingival crevicular fluid samples. The amounts of IL-1β in the gingival crevicular fluid from periodontitis patients and periodontally healthy controls at baseline and 6 wk after treatment are shown in Table 3. Significantly higher levels of IL-1β were present in the gingival crevicular fluid of both smoker and nonsmoker patients with periodontal disease compared with healthy controls ($p < 0.05$). The highest IL-1β levels in gingival crevicular fluid were found in patients with periodontitis who smoked before and after therapy ($p < 0.05$). At 6 wk after treatment, the IL-1β levels of all periodontitis patients were significantly lower than those at baseline ($p < 0.05$).

Table 3 presents data relating to TOS and TAS in gingival crevicular fluid. There was no significant difference in TOS levels in periodontitis patients at baseline or 6 wk after therapy ($p > 0.05$). There were also no significant differences in TAS levels among the three groups at baseline and 6 wk after therapy.

There was a positive, significant correlation between TOS and IL-1β values in smokers ($r = 0.514$, $p < 0.05$). There were negative, but significant, correlations between TAS and TOS values in smokers ($r = -0.586$, $p < 0.05$) and between TAS and plaque index values in smokers ($r = -0.569$, $p < 0.05$). There were statistically significant negative correlations between TOS and IL-1β levels in nonsmokers ($r = 0.584$, $p < 0.05$).

Discussion

This study is the first to compare the influence of smoking on IL-1β levels, and TOS and TAS, in the gingival crevicular fluid of patients with generalized chronic periodontitis undergoing nonsurgical periodontal therapy. We demonstrated significant improvements in clinical parameters after periodontal treatment in both smokers and nonsmokers. The IL-1β content of the gingival crevicular fluid of periodontitis patients was markedly reduced following periodontal therapy, but no significant changes in oxidant or antioxidant levels were found. There were also significant correlations between TOS and IL-1β levels in the gingival crevicular fluid from both smoker and nonsmoker periodontitis patients.

Oxidative stress has often been determined by measurement of the products of oxidative damage of lipids, proteins and DNA (25,26). However, the measurement of different oxidant molecules is time-consuming, labor-intensive and costly, and requires

Table 3. Interleukin-1beta (IL-1β) level, total oxidant status (TOS) and total antioxidant status (TAS) in gingival crevicular fluid samples at baseline and 6 wk after therapy in patients with periodontitis and in periodontally healthy controls

	Periodontitis patients				
	Smokers (n = 15)		Nonsmokers (n = 15)		
	Baseline	6 wk	Baseline	6 wk	Healthy controls
IL-1β (pg/site)	737.2 ± 242.0[b]	613.6 ± 175.9[a,b]	438.1 ± 294.8[b,c]	377.8 ± 296.1[a,b,c]	299.4 ± 142.7[c]
TOS (μmol H_2O_2 equiv./L)	10.06 ± 0.65	10.12 ± 0.39	10.06 ± 0.22	9.92 ± 0.45	10.4 ± 0.48
TAS (mmol Trolox equiv./L)	0.12 ± 0.31	0.13 ± 0.36	0.12 ± 0.33	0.14 ± 0.41	0.13 ± 0.25

Data are given as mean ± standard deviation.
[a]Significantly different from baseline ($p < 0.05$).
[b]Significantly different from control group ($p < 0.05$).
[c]Significantly different from smokers group ($p < 0.05$).

complicated techniques (27). As the measurement of different oxidant molecules separately is not practical and their oxidant effects are additive, the TOS of a sample (which is based on the oxidation of ferrous ion to ferric ion in the presence of various oxidant species in acidic medium and the measurement of the ferric ion by xylenol orange) (21), was measured in the present study. Assays of total antioxidant capacity have the advantage in that they analyze the combined effectiveness of contributing species, which may be greater than the sum of the effects of the individual antioxidants (24). The assays are more efficient, cheaper and less time-consuming than performing large numbers of individual assays (28). Erel's assay, utilized in the current study, is highly sensitive and reliable (22) and has been used by several investigators when analyzing gingival crevicular fluid (23,27). In a study (28) that investigated the TAS and antioxidants in the serum, saliva and gingival crevicular fluid of women with periodontitis and pre-eclampsia, it was reported that the superoxide dismutase level and the glutathione peroxidase activity in the gingival crevicular fluid and serum, and the TAS in the saliva, gingival crevicular fluid and serum were lower than in the control group. These findings were confirmed in studies (18,29) in which the antioxidant concentration was lower in the gingival crevicular fluid in periodontitis subjects compared with healthy controls. While Brock *et al.* (18) reported that nonsurgical periodontal therapy with improvements in clinical parameters can increase the antioxidant defense in patients with chronic periodontitis, Kim *et al.* (30) found that the TAS in saliva decreased directly after SRP. Over time, the TAS increased slightly and was unchanged relative to the baseline measurement. Moore *et al.* (31) measured the antioxidant capacity of saliva in periodontally diseased and healthy individuals using the Trolox equivalent assay and failed to find any significant difference between the groups. However, in those studies, nonsmoking periodontitis patients were included. In contrast to these studies, Buduneli *et al.* (32) evaluated the possible effects of smoking and gingival inflammation on salivary antioxidants in patients with gingivitis. They reported that no significant difference was found in any of the antioxidant indices between any of the groups. Recently, the potential effects of smoking on the total antioxidant capacity of saliva and plasma samples have been investigated by Charalabopoulos *et al.* (33) in young, healthy men: no significant differences were found in salivary antioxidant defenses of nonsmokers and smokers, either before or after smoking. Furthermore, Guentsch *et al.* (34) found no difference in the total antioxidant activity between smokers and nonsmokers but did find that periodontal therapy had an effect on lipid peroxidation and glutathione peroxidase levels decreased in saliva. In our study, the gingival crevicular fluid TOS and TAS in both smokers and nonsmokers showed a similar profile at baseline and were unchanged after periodontal treatment.

The effect of smoking on IL-1β production in periodontal patients has been extensively investigated and conflicting results have been reported. While Bostrom *et al.* (15) found no influence of smoking on the levels of IL-1β and interleukin-1 receptor antagonist (IL-1ra) in gingival crevicular fluid, Rawlinson *et al.* (35) found that the levels of IL-1β and IL-1ra were lower in diseased sites in smokers vs. nonsmokers. However, similarly to our findings, in a recent study (3) that investigated the expression of 22 chemokines and cytokines in gingival crevicular fluid from smokers and nonsmokers with periodontitis and periodontally healthy patients, higher amounts of IL-1α, IL-1β and IL-3 were observed in diseased sites of smokers when compared with healthy controls. Furthermore, they suggested that production of pro-inflammatory biomarkers is depressed in smokers, but that these mediators are still present at concentrations capable of pathogenesis. In the present study, the IL-1β level in gingival crevicular fluid was higher in smokers than in nonsmokers and was significantly decreased following periodontal treatment in both smokers and nonsmokers, consistent with the literature. Several *in vitro* studies have investigated the relationship between antioxidants and cytokines in many cell types (36–38). Chen *et al.* (38) found that in human peritoneal mesothelial cells, the absence of induction of antioxidant enzymes (such as glutathione peroxidase) by inflammatory cytokines (such as tumor necrosis factor-alpha and IL-1β) may contribute to the susceptibility of these cells to oxidative damage. However, in a study (4) that investigated glutathione peroxidase, lactoferrin and myeloperoxidase, and the pro-inflammatory cytokine, IL-1β, which is important in the regulation of immunological and inflammatory reactions in human periodontal diseases, it was found that the gingival crevicular fluid from periodontitis sites exhibited significantly greater total amounts of glutathione peroxidase, lactoferrin, myeloperoxidase and IL-1β than that from healthy sites, and significantly positive correlations were found between the total amount of glutathione peroxidase and IL-1β. In the present study, while no significant correlations were found between the TAS and IL-1β levels in gingival crevicular fluid in all periodontitis patients, a positive correlation was found between TOS and IL-1β levels in both smokers and nonsmokers.

Within the limitations of this study, we conclude that there was no change in TOS and TAS in the gingival crevicular fluid of patients who smoke and suffer from periodontitis. We also observed an increase in the level of IL-1β in smokers, and found that successful periodontal therapy has an effect on the level of IL-1β, but not on TOS or TAS, and these results suggest a correlation between IL-1β levels and TOS. Future studies are needed to determine the inter-relationships between oxidant–antioxidant status and cytokines in periodontal disease pathogenesis and the impact of smoking on the inflammatory process.

Conflict of interest

The authors declare that they have no conflict of interests.

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