

The Effects of Non Surgical Periodontal Therapy on Total Antioxidant Status, Total Oxidative Status and IL-6 Levels in Gingival Crevicular Fluid and Serum of Hyperlipidemic Smokers

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Introduction

Chronic periodontitis (CP) is a bacterially induced chronic inflammatory disease resulting in periodontal tissue destruction and attachment loss^[1]. Periodontitis has been related to various systemic diseases, including diabetes, coronary heart disease, myocardial infarction and stroke^[2]. Atherogenic alterations are also associated with lipoprotein metabolism. Hyperlipidemia is associated with an abnormal lipid profile, which is characterized by elevated blood concentrations of triglycerides, elevated levels of total cholesterol and lowdensity lipoprotein (LDL) and decreased levels of high-density lipoprotein (HDL) cholesterol^[3]. Recent studies suggested that hyperlipidemia could be associated with periodontitis^[3,4]. Researchers demonstrated the capacity of periodontal pathogens to alter the lipid profile, increasing LDL, very low-density lipoprotein (VLDL), and total cholesterol levels (TC) and reducing HDL levels. The pathogenesis of periodontal disease is determined by the nature and control of both innate and adaptive immune responses^[5,6]. Inflammatory cytokines play a key role in periodontal inflammation.

Interleukin-6 (IL-6) is a key cytokine in the initiation and maintenance of systemic inflammation, which has been implicated in the progression and severity of periodontitis^[7]. In vitro and animal experiments suggest that IL-6 plays an important role in the regulation of the synthesis of other acute phase proteins which are established risk factors for atherosclerosis, such as fibrinogen. Increased level of IL-6 have been found in plasma and gingival crevicular fluid in periodontitis and also plays a key role in promoting lipid metabolism and atherogenesis^[8,9]. Recently studies have demonstrated that periodontal treatment improves the lipid profile^[10]. Periodontal pathogens affect the oxidative processes of different lipoproteins and increase the expression of the LDL receptor^[11,12]. Conversely, an altered lipid profile has been associated with the expression of systemic proinflammatory cytokines that affect the pathogenesis of periodontitis^[11,12]. The association between altered lipid profile and periodontitis has been investigated in several studies and the results of these studies remain controversial. Katz et al. and Machado et al. reported no significant differences between triglycerides and periodontitis and no significant differences between the serum lipid levels of periodontitis cases and healthy controls^[13,14]. On

Abstract

The aim of this study is to investigate the effect of periodontal therapy on clinical parameters, IL-6, TAS (Total Antioxidant Status), TOS (Total Oxidative Status) in serum, GCF and serum lipid parameters and to evaluate the effect of smoking on the interaction between periodontal disease and hyperlipidemia.

Materials and Methods: This study included 28 patient, aged between 42-64 years (mean 52.5±10 years), with non-systemic disease and without hyperlipidemia. Patients were divided into two groups: smoking (n = 14) and non-smoking (n = 14). Clinical parameters were recorded at baseline. GCF and blood samples were obtained from patients for IL-6, TAS, TOS and serum lipid parameters. Following baseline measurements and sampling, non-surgical periodontal treatment was performed on all participants. Serum sampling, clinical measurements, and GCF were repeated at the 3rd month. GCF and serum IL-6, TAS and TOS levels were determined by the ELISA method and analyzed using the t-test and univariate variance analysis.

Results: There were statistically significant differences between S(+) and S(-) groups at the 1st and 3rd months (p < 0.05). GCF and serum IL-6 and TOS levels decreased while the TAS level increased after therapy in the S(+) and S(-) groups. TC and LDL levels were decreased significantly but HDL level was not increased significantly between groups.

Conclusion : There were no significant differences between S(+) and S(-) groups after non-surgical periodontal treatment of serum lipid parameters. Smoking had limited effects on changes of TAS and TOS values after the periodontal treatment of individuals with hyperlipidemia.

the contrary, Losche et al. reported significantly higher levels of total cholesterol, LDL cholesterol and triglycerides in periodontitis subjects as compared with controls^[15]. Nibali et al. reported a fairly strong association between periodontal infection and unfavorable lipid composition^[16]. There is an abundance of evidence that smoking is an important risk factor for periodontitis and also may contribute to a less favorable response to periodontal treatment^[17]. Studies evaluating gingival crevicular fluid of individuals with chronic periodontitis (CP) verified that smoking increased the production of cytokines, such as IL-1 α , IL-1 β , and IL-6^[18,19]. Smoking has shown both indirectly affect the vascular system by increasing blood lipid levels and directly influence it by increasing serum cytokine levels^[19,20]. The most important exogenous source of free oxygen radicals is smoking. Clinical studies have shown that smoking damage leads to free radical proteins and lipid metabolism. Smoking accelerates the initiation and progress of oxidative stress in many disease processes^[21]. Oxidative stress is affected in the pathogenesis of several chronic inflammatory diseases associated with periodontitis, such as vascular disease^[22]. The aim of this study was to investigate the effect of non-surgical periodontal therapy on clinical parameters including IL-6, TAS, TOS in serum, GCF and serum lipid parameters and to evaluate the effect of smoking on the interaction between periodontal disease and hyperlipidemia.

Materials and Methods

Subjects Twenty-eight patients (14 female and 14 male) were included in this study at the department of Periodontology, School of Dentistry, University of Selçuk Konya, Turkey. The age of participants was between 42–64 years (mean 52.5 \pm 10 years), with non-systemic disease and without hyperlipidemia. Patients were divided into two groups; smoking (n = 14) and nonsmoking (n= 14). Hyperlipidemia was defined as the presence of one or more altered values of the lipid profile (total cholesterol > 200 mg/dL, triglycerides (TGs) > 150 mg/dL, LDL cholesterol >130 mg/dL, HDL >35 mg/dL)^[23]. The diagnosis of hyperlipidemia had to be made at least 6 months before the study.

Inclusion criteria were as follows: 1) age >18 years; 2) presence of at least 16 teeth (excluding third molars); and 3) no receipt of periodontal treatment during the previous 1 year, 4) smoking >20 cigarettes for test group, 5) no smoking for control group. **Exclusion criteria** were as follows: 1) pregnancy; 2) breastfeeding; 3) the presence of systemic disease without hyperlipidemia 4) antibiotic treatment during the previous 6 months; 5) treatment with drugs that affect bone metabolism; and 6) receipt of radiation or hormone therapy. Informed consent was signed by all participants in the study.

The study protocol was approved by the Ethics Committee of the Medical Faculty of Selçuk University (Ethics Board Decision No: 2010/04) according to the Declaration of Helsinki. **Smoking Habits** Regarding the characterization of smoking, individuals were classified as non-smokers (NS) and smokers (S). Individuals were asked about the duration of their smoking habit in years (SH/years) and how many cigarettes they smoked per day (C/day). **Periodontal Parameters** The CP was defined according to the criteria of the 1999 International World Workshop for a Classification of Periodontal Diseases and Conditions^[24]. All clinical examinations were performed at a periodontology

clinic. The examinations were collected by the same examiner. All participants had at least 16 natural teeth. Clinical periodontal parameters including probing depth (PD), plaque index (PI) and gingival bleeding (GB) score and clinical attachment loss (CAL) were recorded at six sites for each tooth with a Williams probe. Periodontal diagnoses were mainly based on CAL from six index teeth^[25-27].

GCF Sampling Collection and Analyses At least 24 hours after periodontal measurements, GCF samples were collected from the maxillary and mandibular right and left premolar and molar region using standardized paper strips (PerioPaper, Orowflow, Amityville, NY). Samples were collected after isolation of the tooth with cotton rolls and removal of any supragingival deposits on the tooth surface. The samples were collected from the mesiobuccal and disto-buccal region from each of eight teeth. The collection region was air dried gently for 2 seconds to reduce any contamination with plaque and saliva. The strip was left at the top of the gingival sulcus for 30 seconds. After the collection of GCF, the strips were moved to a micro-moisture meter device, for precalibration with a dry, sterile strip. Samples with evidence of bleeding were not included. After volume determination, GCF samples were placed in sterile Eppendorf tubes (Eppendorf, Interlab, Istanbul, Turkey) and stored at -80°C until the day of laboratory analysis. **Blood Sampling and Analysis** Venous blood samples were collected in vacutainer tubes on the day of the GCF sampling. All patients fasted overnight. Samples were centrifuged at 3,000 rpm for 5 minutes, and serum was isolated and placed in sterile Eppendorf tubes that were wrapped securely and stored at -80°C, until the day of laboratory analysis. Quantification of IL-6, TAS and TOS Levels GCF and other samples were analyzed for IL-6, TAS and TOS. IL-6 was analyzed using enzyme-linked immunosorbent assay (ELISA) following the instructions of the manufacturer. The assays were run separately for the IL-6 cytokine using the standard curves. The separated plasma samples were stored at -80°C until use when serum TAS and TOS were determined. TAS and TOS were measured at the Selçuk University Biochemistry laboratories, and the oxidative stress index (OSI) was calculated. **Non-Surgical Periodontal Therapy** All patients were treated non-surgically by plaque control and supra- and sub-gingival debridement manually (Hu-Friedy, USA) or with ultrasonic instruments (Stalec, Suprasson P5 Booster, France) in a split mouth approach. Following baseline measurements and sampling, non-surgical periodontal treatment (including scaling and root planning) was performed on all participants.

Statistical Analyses

All the analyses were performed with SPSS for Windows (Version 13.0, SPSS Inc., Chicago, IL, USA). For the statistical analyses, normality was tested with the Kolmogorov Smirnov test. For normally distributed groups, parametric (Paired, Independent) t-tests were used to compare group differences among groups. One-way analysis of variance (ANOVA) was used to compare differences among groups. The Tukey test was used in conjunction with ANOVA to find which means are significantly different from one another. A significance level of $p < 0.05$ was used for assigning statistical significance.

Results

Clinical Findings The distribution of age and gender and periodontal parameters at the beginning of treatment are shown in Table 1 and Table 2. Age and gender parameters were not significantly different between the groups (Phigher in the test group compared with the control groups (P< 0.05) (Table 2). IL-6, TAS, TOS Values in GCF and Serum Pre-treatment value of IL-6, TAS, and TOS in GCF and serum are shown in Table 3 and

Table 4, respectively. The total amount of IL-6, TOS and GCF values were significantly greater in the smoking group (p≤0.05). Serum Lipid Values Pre-treatment and post-treatment values of serum lipid levels are shown in Table 5. TC levels and LDL were decreased significantly but the HDL level was not increased significantly in the S(+) and S(-) groups after periodontal therapy (Table 5). There were no significant differences between S(+) and S(-) groups after non-surgical periodontal treatment on serum lipid parameters.

Table 1: The distribution of age and gender and periodontal parameters at the beginning of treatment

Groups	Age	Gender	Diagnosis Age of Hyperlipidemia	Brushing habits
	average	(n)	average	%
Test Groups	52.9	9Male, 5 Female	5,5	1-%63 2-%27 3-%10
Control Groups	52.1	5 Male, 9 Female	6, 5	1-%61 2-%25 3-%14

Table 2: Phigher in the test group compared with the control groups

	Pre-treatment		First months		Third months		P Values	
	Test Groups	Control Groups	Test Groups	Control Groups	Test Groups	Control Groups	Pre-treatment first months	Pre-treatment third months
	average	average	average	average	average	average		
PI	2,44±0,12	2,30±0,13	1,18±0,09	1,10±0,07	1,32±0,21	1,27±0,22	0,0477*	0,557
GB	2,10±0,10	2,13± 0,13	1,11 ± 0,12	0,96 ± 0,04	1,12 ± 0,10	1,10 ± 0,09	0,001*	0,002*
PD (mm)	5,04 ± 0,30	4,65 ± 0,38	3,17 ± 0,17	2,46 ± 0,18	3,51 ± 0,25	2,69 ± 0,37	0,001*	0,001*
CAL (mm)	3,57 ± 0,38	3,27 ± 0,43	1,57 ± 0,38	1,05 ± 0,16	1,77 ± 0,24	1,45 ± 0,36	0,335	0,168
GCF (ml)	0,43 ± 0,12	0,39 ± 0,09	0,29 ± 0,06	0,23 ± 0,04	0,28 ± 0,03	0,26 ± 0,03	0,020*	0,058

Table 3: IL-6, TAS, TOS Values in Serum Concentrations

Serum Concentration						P Values	
	Pre-treatment		Third months		pre-treatment-third months		
	Test Groups	Control Groups	Test Groups	Control Groups			
	average	average	average	average			
IL-6 Serum	5,75 ± 3,19	6,23 ± 2,81	5,34 ± 3,13	3,64 ± 2,14	0,018*		
TAS Serum	1,67 ± 0,23	1,71 ± 0,21	1,69 ± 0,24	1,85 ± 0,27	0,101		
TOS Serum	16,76 ± 6,11	27,10 ± 21,36	13,13 ± 5,79	15,77 ± 8,42	0,852		

Table 4: IL-6, TAS, TOS Values in GCF Concentrations

GCF Concentrations								
	Pre-treatment		First month		Third month		P Values	
	Test Groups	Control Groups	Test Groups	Control Groups	Test Groups	Control Groups	Test Groups	
	average	average	average	average	average	average	average	average
IL-6 GCF	2,13 ± 0,40	1,63 ± 0,59	1,47 ± 0,41	1,14 ± 0,18	1,65 ± 0,56	1,28 ± 0,30	0,113	0,147
TAS GCF	9,344 ± 13,53	9,272 ± 11,68	11,342 ± 11,81	11,211 ± 6,77	10,11 ± 12,43	10,814 ± 6,67	0,737	0,564
TOS GCF	14,72 ± 4,81	19,83 ± 1,35	9,98 v 1,66	10,3 ± 1,64	10,22 ± 1,55	11,09 ± 1,37	0,807	0,193

Table 5: Serum Lipid Values Pre-treatment and post-treatment values of serum lipid levels

Serum Concentrations						P Values	
	Pre-treatment		Third months		pre-treatment-third months		
	Test Groups	Control Groups	Test Groups	Control Groups			
	average	average	average	average			
TC Serum	251,78 ± 24,75	247,64 v 25,82	206,07 ± 6,75	194 ± 7,98	0,91		
HDL Serum	44,42 ± 5,07	44,37 ± 5,97	44,5 ± 4,79	44,64 ± 5,62	0,86		
LDL Serum	166,07 ± 13,87	164,23 ± 17,39	127,28 ± 9,21	124,52 ± 13,71	0,57		
VLDL Serum	41,28 ± 20,19	39,02 ± 18,08	43,28 ± 12,72	27,11 ± 10,49	0,11		
TG Serum	178,92 ± 25,50	181,92 ± 31,31	177,78 ± 24,71	180,57 ± 30,75	0,98		

Discussion

It is known that smoking is a common risk factor for periodontal disease as well as cardiovascular illnesses. Thus, our study aimed to specify the impact of smoking upon clinical and biochemical parameters of patients who are administered periodontal treatment without surgery with hyperlipidemia. Our study included patients who are smokers and non-smokers with hyperlipidemia. This study was performed to identify the effect of non-surgical periodontal treatment on several clinical parameters, GCF and serum IL-6, TAS and TOS values as well as the serum lipid values (TC, LDL, HDL, VLDL, TG) were statistically analyzed in the GCF and serum and the results were interpreted. This study found that at 3 months after periodontal treatment, the non-smoker control group had statistically significant improvements regarding recovery based on clinic periodontal parameters in comparison with smokers. However, we could not determine a meaningful differentiation between serum lipid parameters and TAS and TOS parameters that were evaluated in the GCF and serum. Smoking is one of the major reasons for periodontal diseases. Smoking reduces perfusion in periodontal tissues because of initial vasodilatation followed by a vasoconstrictor effect; as a result of this, it is observed that early symptoms of periodontal problems are inhibited by a decrease in gingival inflammation, bleeding and hemorrhage. Although in the study of Bergström et.al., the plaque level was much greater than non-smokers, it was stated that probing hemorrhage is less pronounced in smokers with periodontitis^[28]. Kibayashi et al. suggested that smoking affects the volume of periodontal illnesses by repressing the host defense system^[29,30]. In comparison with non-smokers, it was determined that clinical symptoms of periodontitis are more severe and more common in smokers. Moreover, it has been stated that a greater pocket depth and clinical attachment loss are seen in smokers. Most of the studies which have inspected the relationship between smoking and respond to recovery after different periodontal treatments have stated that smoking has a negative effect on the recovery response^[31]. Buduneli et al^[32], stated that after the non-surgical periodontal treatment, there is recovery for both smokers and non-smokers; however, the amount of recovery is significantly high for the non-smoker group. In accordance with the general literature and within this study, it was found that the amount of plaque that is observed before treatment for smokers was significantly greater than non-smokers. After the treatment, recovery was observed for both groups but recovery on clinical parameters after the treatment was found to be significantly higher for non-smokers. Fentoğlu et al, who investigated the correlation between periodontal diseases and hyperlipidemia, reported that individuals with hyperlipidemia systematically show a worse periodontal situation than healthy individuals^[33] Furthermore they stated that the degree of periodontal destruction showed a positive correlation with plasma cholesterol levels. Ebersole et al constituted a monkey model (n=51) to evaluate the relationship between periodontitis and the atherosclerotic process^[34]. They analyzed the relationship between periodontitis, serum lipid levels and systemic inflammatory indicators and they reported that there was a significant relationship between volume of periodontal illnesses and total cholesterol, triglyceride and LDL cholesterol levels. We determined that clinical findings at the end of this study parallel with studies that were performed with the patients who do

not have systemic disease. For this reason, we think hyperlipidemia does not affect clinical parameters after treatment. In our study both GCF obtained from patients and IL-6 levels in the serum were evaluated. As a cytokine with sophisticated functions, IL-6 plays a central role in host defense mechanism and regulates the immune response. IL-6 is not excreted from cells that are healthy. IL-6 is excreted from cells in the case of infection, viral infection and with excitation of lipopolysaccharides and various cytokines. The serum levels increase in trauma, inflammation, autoimmune diseases and various malignancies^[35]. Monteiro et al found in their study that IL-6 and IL-8 levels are statistically significantly higher for individuals with periodontitis than in healthy patients^[36]. It was recently identified that GCF and total amount of IL-6 was higher for smokers with periodontitis than non-smoker periodontitis patients^[13] Vidal et al demonstrated in their study that after the performing non-surgical periodontal treatment in patients with severe periodontitis, there were significant reductions in the levels of GCF, plasma IL-6, CRP and fibrinogen^[37] Shimada et al. reported that after non-surgical periodontal treatment, there are reductions in IL-6 and CRP levels^[38]. Similar to those studies, our study observed that following periodontal treatment, there are decreases in GCF and plasma values for both groups at all times. For both of the groups, the decrease seen at the 1st month for IL-6 was significantly higher than the 3rd month. The amount of decrease of IL-6 in the smoker group was found to be the same as in the non-smoker control group. Fentoğlu et al stated that there is a meaningful relationship between TC/HDL rate and GCF-serum IL-6 levels and these values increase in individuals with periodontitis^[39]. In some studies, it was shown that there was a decrease in serum lipid levels for individuals with periodontitis after the periodontal treatment; while in some other studies the periodontal treatment did not affect the serum lipid levels. In our study, after implementing the nonsurgical periodontal treatment to patients with hyperlipidemia, a significant decrease is was observed in serum TC and LDL parameters in test and control groups. Increase of HDL level was not significantly different. The results were found to be similar for both groups (p>0.05). Considering these results, it can be said that after the nonsurgical periodontal treatment, the changes in serum lipid parameters were limited and the effect of smoking was not meaningful enough to identify an interaction between periodontal disease and hyperlipidemia. Reactive Oxygen Kinds (ROK) take on an important task for physiological and immuno-inflammatory reactions. Many diseases such as periodontal illness are related to an imbalance between oxidation-reduction or oxidative stress^[25]. It is known that initially the amount of antioxidant activity rises to protect tissues against oxidative stress; however in the case of further progression of disease which means chronic periodontitis, the amount of antioxidant reduces because of an increase in pocket depth. Chapple et al in their study confirmed that the decrease in serum TAS amount occurs after the ROT production based on periodontal illness^[25]. In our study, after the non-surgical periodontal treatment at the 1st and 3rd months, a significant increase in GCF TAS values in the S(+) and S(-) groups were observed, whereas there was a significant decrease for TOS values. For the S(+) group, there was a significant decrease only in the TOS value. On this basis; we can claim that as a conclusion of an increase in the TAS value and decrease of TOS value with the effect of nonsurgical periodontal treatment, oxidative stress

was reduced at periodontal tissues. Considering the Serum TAS and TOS changes, we believe that systematic effect of nonsurgical periodontal treatment is better for the non-smoker group. Similar to our study, Kurtis et al suggested that the rate of natural antioxidants within the blood and tissues of smokers are less than non-smokers^[40,41]. In this study, whether patients had hyperlipidemia did not affect the results of treatment. Taking these results into consideration, we think that smoking has limited effects on changes of TAS and TOS values after the periodontal treatment of individuals with hyperlipidemia. The major limitation of this study is having a small number of patients who participated and having a small working time. Another limitation is that the results are not differentiated according to gender. There is a need for further studies of longer duration and with more participants in order to explain the relationship between smoking, periodontal illness, and hyperlipidemia. These studies should also attempt to explain the changes of GCF-serum IL-6, TAS, TOS and serum lipid parameters in addition to analyzing the effects of smoking on these results.

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