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Araştırma/ Research Article

EVALUATION OF THE EFFECT OF CHRONIC PERIODONTITIS AND ADDITIONAL TOBACCO ABUSE ON OXIDATIVE STATUS : A CROSS-SECTIONAL STUDY[≠]

KRONİK PERİODONTİTİS VE SİGARA KULLANIMININ OKSİDATİF DURUM ÜZERİNE OLAN ETKİSİNİN DEĞERLENDİRİLMESİ : KESİTSEL ÇALIŞMA[≠]

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ABSTRACT

Aim: The aim of the survey was to evaluate the 8-hydroxy-2' -deoxyguanosine (8-OHdG) and melatonin (MLT) levels in saliva as well as myeloperoxidase (MPO) levels in gingival clevicular fluid (GCF) in smoker and nonsmoker chronic periodontitis (CP) patients and periodontally healthy individuals.
Methods: 4 groups of 15 applicants were formed in accordance with the research protocole as follows; periodontally healthy nonsmokers (CP-S-), periodontally healthy smokers (CP-S+), nonsmoker periodontitis patients (CP+S-) and smoker periodontitis patients (CP+S+). Clinical periodontal status were measured before taking the saliva and GCF samples. 8-OHdG, MLT and MPO levels were measured biochemically.
Results: The lowest level of 8-OHdG and MPO were recorded in (CP-S-) group ($p<0.05$). MLT levels in saliva were statistically increased in periodontally healthy (CP-) groups than CP groups ($p<0.05$).
Conclusions: CP causes significant changes in parameters connected with oxidative stress. For periodontally healthy applicants, smoking leads to statistically significant changes on the parameters in general; while the effect of smoking to the parameters was found insignificant in the CP groups. Findings of this study reveal the role of smoking on the development of periodontitis, due to negatively affecting the parameters linked to oxidative stress, that are crucial in the patogenesis of periodontal diseases.
Key Words: 8-hydroxy-2' -deoxyguanosine (8-OHdG), chronic periodontitis (CP), melatonin (MLT), myeloperoxidase (MPO), oxidative stres (OS), smoking, tobacco.

ÖZ

Amaç: Bu çalışmada amaçlanan; kronik periodontitis (KP) tanısı konan bireyler vile periodontal açıdan sağlıklı bireylerin dişeti oluğu sıvısı (DOS) ve tükürük numunelerinde sigaranın MLT, MPO ve 8-OHdG düzeyleri üzerine muhtemel etkilerinin değerlendirilmesidir.
Gereç ve yöntem : Çalışma protokolü gereği her biri 15 katılımcıdan oluşan 4 grup oluşturuldu; periodontal olarak sağlıklı ve sigara içemeyen grup (KP-S-), periodontal olarak sağlıklı ve sigara içen grup (KP-S+), kronik periodontitisli ve sigara içmeyen grup (KP+S-) ile kronik periodontitisli ve sigara içen grup (KP+S+). DOS ve tükürük örnekleri alınmadan önce klinik periodontal parametreler kaydedildi. MLT, MPO ve 8-OHdG düzeyleri biyokimyasal olarak ölçüldü.
Bulgular : En düşük 8-OHdG ve MPO düzeyleri (KP-S-) grubunda tespit edildi ($p<0.05$). Tükürük MLT düzeyi periodontal olarak sağlıklı (KP-) gruplarda, periodontitisli gruplara nispeten (KP+) daha düşük bulundu ($p<0.05$).
Sonuç : KP'nin oksidatif stres ile ilişkili parametrelerde anlamlı düzeyde farklılık oluşturduğu gözlendi. Sigara kullanımının genel olarak periodontal sağlıklı bireylerde parametrelerde belirgin bir değişime yol açtığı, periodontitisli gruplarda ise bu değişimin istatistiksel olarak anlamlı olmadığı görüldü. Bulgularımız, sigara içmenin periodontal hastalıkların patogenezinde rol alan oksitatif parametreleri olumsuz yönde etkileyerek periodontitis gelişiminde rol oynayabileceğini göstermektedir.
Anahtar Kelimeler: 8-hydroxy-2'-deoxyguanosine (8-OHdG), kronik periodontitis (KP), melatonin (MLT), myeloperoksidaz (MPO), oksitatif stres (OS), sigara.

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INTRODUCTION

Periodontal disease is considered as an inffammatory disorder affecting 10-15 % of the population worldwide that damages hard and soft tissues through the complex interactions between host defense systems and periodontopathogenic bacteria.[1-3] Oxidative stress (OS) is defined as the result of imbalance amongst oxidant factors and protective antioxidant factors. The reason for destruction of periodontal tissues is the formation of inflammatory and immune response due to periodontopathogenic factors and release of and neutrophil enzymes and reactive oxygen species (ROS).[4,5]

Additional tobacco abuse is one of the main risk factor related to chronic periodontal disease. Smoking aggravates pocket formation, bone loss, attachment loss, eventually tooth loss and causes failure of periodontal treatment and dental implant treatment or relapse of disease.[6-9] Cigarette smoke is rich in ROS and keeping in approximately 10^{15} free radicals in the gas and tar phase.[10] Use of tobacco derivatives elevates the OS parameters in patients with periodontitis.[11-13]

Free radicals in cigarette smoke lead to oxidative damage by Deoxyribose Nucleic Acid-Ribo Nucleic Acid (DNA-RNA) damage, lipid peroxidation, protein oxidation, enzyme oxidation and even cell apoptosis.[14] Clinical trials have shown that free radical-mediated damage to DNA, proteins and lipids is increased by smoking.[15] 8-OHdG is the most known parameter of ROS-induced detrimental effect on DNA.[16,17] The level of 8-OHDG in body fluids also increases due to increased OS by smoking.[18] MLT is an immunomodulator hormone that stimulates proliferation and synthesis of type 1 collagen and bone formation.[19,20] MLT has strong antioxidant effects that counteract the inflammatory condition and oxidative damage.[21,22] Neutrophils is an significant cell type in host defense. Neutrophil granules includes hydrolytic enzymes such as myeloproxidase.[16] Oxidative burden and tissue damage was increased in the diseased areas as a result of local production of MPO, hypochlorous acid (HOCl) and other ROS, resulting in neutrophil infiltration in tissues.[23,24]

Smoking has an important place among the acquired risk factors for CP. Studies have shown that smokers are 5-7 times more likely to occur periodontitis than non-smokers, and that the vast majority of individuals with severe periodontal disease are at the same time smokers.[25,26] The purpose of the survey was; assessment of possible influence of smoking and CP on salivary 8-OHDG, MLT and MPO levels in GCF and evaluate the relationship between CP and smoking in individuals.

MATERIALS AND METHODS

Patient Selection

60 individuals who applied to the periodontology department of Atatürk University Faculty of Dentistry were included in the study on a voluntary basis. The approved study protocol was taken from Institute of Health Sciences Ethics Committee, Ataturk University, Erzurum, Turkey. An information and consent form was given to all participants. All participants were informed about the purpose and method of the study, and written consent forms prepared in accordance with the Helsinki Declaration were given. Afterwards, clinical periodontal examinations, saliva and DOS sampling were performed.

4 groups of 15 applicants were formed in accordance with the research protocole as follows; periodontally healthy nonsmokers (CP-S-), periodontally healthy smokers (CP-S+), nonsmoker periodontitis patients (CP+S-) and smoker periodontitis patients (CP+S+).

Individuals who did not have a history of pregnancy, systemic disease and who did not take any medication within the last three months were included in the study.[6] Individuals with a history of periodontal treatment in the previous six months were not included in the study. Smokers are smoking ≥ 10 cigarettes per day for at least the last 5 years and non-smoker participants have never smoked in their life or have quit smoking at least 1 year ago.

Smoking status was determined by the method of asking the individual. Another method is biochemical analysis such as "measurement of serum cotinine level" in which the amount of actual cigarette that an individual is exposed to can be determined more objectively. Studies have shown that the data obtained by the individual questioning method is compatible with the level of serum cotinine. It was stated that it is appropriate for the individuals who left smoking at least 1 year before the study to be evaluated as 'non-smokers' in the same group with no smokers[27]

Dental Examination

Periodontal clinical parameters were recorded via gingival index (GI)[28], plaque index (PI)[29], probing pocket depth (PPD), clinical attachment loss (CAL). PPD is the distance between the free gingival marjin and the base of the pocket. CAL is the distance from the semento-enamel junction to the base of the pocket. Measure- ments were obtained from 6 sites (mesio- mid- disto buccal, mesio- mid- disto lingual) for each tooth. During the measurements it was noted that the tooth was parallel to the long axis and that no excessive force was applied. CP was defined as one or more interproximal sites in different teeth with a PPD ≥5 mm and CAL ≥ 6 mm.[30] Periodontal health was stated as a PPD of ≤3mm.[31] All individuals had ≥20 natural teeth, CAL and BOP in ≤25% of teeth.

Collection of Saliva and GCF Samples

Saliva sampling. participants were asked not to drink, eat or chew to take unstimulated saliva before clinical periodontal measurements were taken at 9-12 a.m and then to kept their mouth open for 5 minutes and approximately 5 mL of whole saliva was collected and centrifuged for 10 minutes at 1,000 x rpm at $+4^0$ C to remove cell debris. The supernatant was stored in 500 µL aliquots at -80^0 C until the biochemical analyses were performed.

GCF sampling. In order not to affect the current periodontal status of the patients, no periodontal procedures were performed in patients before the intake of GCF samples. GCF obtained from both healthy and diseased regions in gingiva of periodontitis patients. The area was isolated with cotton rolls and dried for GCF sampling. The filter paper strips were kept into the gingival sulcus for 30 seconds. Uncontaminated paper strips were measured for fluid volume with a cilibrated periotron 8000, then placed into eppendorf tubes containing 125µl PBS (Phosphate Buffered Saline-0.1% Tween 20, pH 7.4). Then samples were stored -80^0 C until analyzed.

Measurement of 8-OHdG and MLT Levels in Saliva and MPO Levels in GCF

To determine 8-OHdG levels in saliva, high sensitivity 8-OHdG enzyme-linked immune-sorbent assay (ELISA) kits were used.

To determine MLT in saliva, competitive enzyme-linked immune-sorbent assay (Non-Extraction Melatonin Saliva ELISA Kit) kits were used.

Measurement of MPO activity was performed using commercially available Myeloperoxidase Activity Colorimetric Assay Kit $^{\mu}$.The method of measurement is based on colorimetric analysis technique.

8-OHdG and MLT Levels in Saliva and MPO Levels in GCF analysis was performed according to the measurement method recommended by the manufacturer's firm.

Statistical Analysis

All these statistical evaluations were performed with SPSS® 18.0 Windows® program. Independent data analyzes were used in our study because the samples we obtained from the groups were samples taken from different individuals. In addition, dependent data analysis was applied to the comparison of different data taken from the same individual. P <0.05 was regarded as statistically significant. Arithmetic values given according to groups were shown with mean ± standard deviation.

RESULTS

Demographic and Clinical Findings

The demographic features and periodontal clinical measurements of the individuals are summarised in Table 1 and 2. There was no statistically significant difference between the groups in terms of age averages (p> 0.05). All clinical periodontal parameters were found to be significantly higher in CP groups, when CP groups and periodontally healthy individuals were compared (p> 0.05).

Table 1. The age ranges of the groups were given as Mean ± Standard Deviation (SD).

	CP-S-	CP-S+	CP+S-	CP+S+
MAN	2	12	7	10
WOMAN	13	3	8	5
AGE	37.35 ± 4.84	35.69 ± 4.22	36.70± 4.52	37.12 ± 4.61

Table 2. Values are presented mean±standart deviation.

	CP-S-	CP-S+	CP+S-	CP+S+
GI	0.47 ± 0.01 ^{a}	0.31 ± 0.02 ^{a}	2.20 ± 0.50 ^{b}	2.01± 0.40 ^{b}
PI	0.27 ± 0.01 ^{a}	0.33 ± 0.01 ^{a}	1.85 ± 0.75 ^{b}	1.93 ± 0.36 ^{b}
PPD	1.47 ± 0.19 ^{a}	1.53 ± 0.18 ^{a}	3.72 ± 0.32 ^{b}	4.11 ± 0.48 ^{b}
CAL	0.20 ± 0.01 ^{a}	0.27 ± 0.01 ^{a}	3.39 ± 0.30 ^{b}	3.52 ± 0.41 ^{b}

^{a,b} values means; different letter statistically different from each other (p<0.05).

Biochemical Findings

Mean values of 8-OHdG, MLT in the saliva and MPO in GCF of the four groups has shown in Table 3.

Table 3. Values are presented mean±standart deviation.

		CP-S-	CP-S+	CP+S-	CP+S+
SALIVA	8-OHDG	1.00 ±0.09 [a]	2.11 ±0.59 [b]	2.56 ±0.61 [b]	2.58 ±0.52 [b]
	MLT	5.83 ±1.12 [a]	4.41 ±1.22 [a]	1.65 ±0.42 [b]	1.05 ±0.27 [b]
GCF	MPO	16.39 ±3.51 [a]	46.15 ±7.69 [b]	48.67 ±8.23 [b]	49.59 ±7,94 [b]

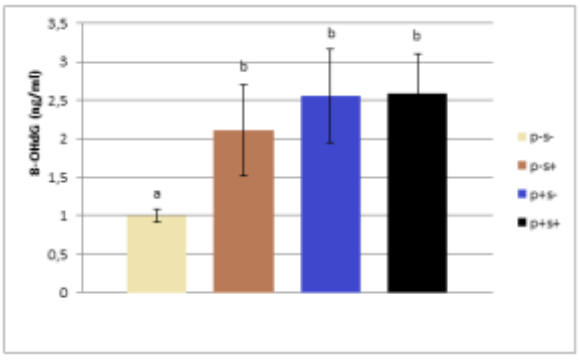
[a,b] values means; different letter statistically different from each other (p<0.05).

The statistically lowest salivary 8-OHdG level has found in (CP-S-) group than other groups ($p<0.05$). Salivary 8-OHdG levels in both CP groups were statistically insignificantly higher than (CP-S+)($p > 0.05$).

The salivary MLT levels in both periodontitis groups were statistically lower than in the periodontally healty participants ($p<0.05$). MLT levels were highest (CP-S-), and the lowest value was found in the (CP+S+). Salivary MLT levels in the smoker groups were statistically insignificantly higher than those in the non-smoker groups ($p > 0.05$).

(CP-S-) group showed a statistically significant lower MPO level of GCF compared to the other groups ($p<0.05$). There was no statistically significant difference was found between the other groups.

The comparisons of 8-OHdG, MLT levels in saliva and MPO levels of GCF between groups are shown in Figure 1 A-C.



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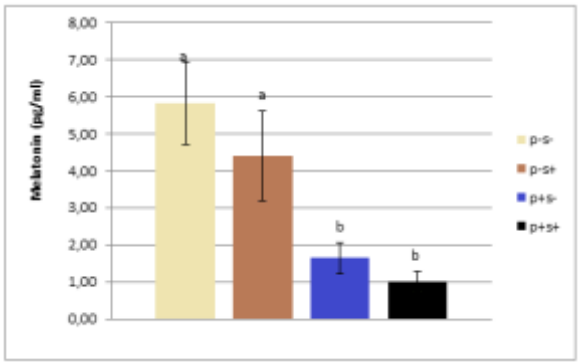

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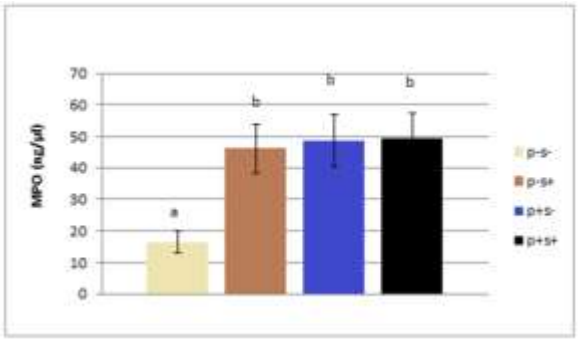

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Figure 1 A-C. The comparisons of 8-OHdG, MLT levels in saliva and MPO levels of GCF between groups. [a,b] values means; different letter statistically different from each other ($p<0.05$).

DISCUSSION

This study analyses the relationship of periodontitis and tobacco using the 8-OHdG, MLT and MPO parameters all together. The results of the study show that both CP and smoking habit can cause an increase in OS. This report also is an evidence of the hypothesise that CP and smoking habit may be linked to OS responses.

Because of large number of oxidants in cigarette smoke, smoking may cause oxidative damage to to many tissues in the body. Damage can be caused by direct effects of cigarette smoke and activation of ROS-producing phagocytic cells.[32-34] A number of cross-sectional studies have demostrated that the risk of periodontal disease existance is greater in smokers.[35-38] It is possible to OS may play an important role in the interaction between periodantitis and smoking.

Saliva and GCF may be used to evaluate the systemic reflection of changes in the antioxidant system or to assess the effectiveness of systemic antioxidant deficiency on periodontal tissue, depen-

ding on periodontal disease and other factors. Markers of oxidative damage such as 8-OHdG were found higher in saliva of individuals with CP.[39-41] The status of the level of 8-OHDG in the studies performed varies from periodontitis before the onset of the clinical symptoms or clinical improvement indication suggests that it is an important biomarker to assess the effectiveness of early diagnosis and treatment for periodontitis.[42] In the present study, although the 8-OHDG level (CP-S-) group was found to be significantly lower than the other groups, it was found that cigarette smoking among the CP groups increased the level of 8-OHDG, but did not make a statistically significant difference. Studies indicates that 8-OHDG levels were found higher in body tissues or urine in chronic inflammatory diseases such as CP, depending on the OS.[18,42-44] Likewise Çanakçı et al. found that 8-OHDG levels of in saliva of periodontitis patients were increased significantly than healthy individuals.[45,46] Researched data related to smoking and OS, it is stated that OS is increased by smo- king.[47,48] Studies investigating the effect of tobacco abuse on salivary 8-OHDG levels showed that 8-OHDG was increased in the leukocytes of smokers; but did not bring a significant effect on salivary 8-OHDG levels.[39,49] Sorensen et al. stated increased levels of 8-OHDG of healthy smokers.[50,51] In the present research, significant increased 8-OHDG levels in periodontitis groups is consistent with similar studies in the literature. This may be regarded as an indication that increased OS due to inflammatory activation may cause damage to the DNA molecule. Smoking among periodontaly healthy groups caused a significant increase in the level of 8-OHDG. This is an evidence of the oxidatif damage of cigarette smoking on DNA. 8-OHdG levels increased in (CP-S+) group than nonsmoker periodontitiss patients group, however, this difference was statistically insignificant. The statistically insignificant increase in the periodontitis group may be due to the increased DNA damage and oxidative burden because of the chronic inflammatory condition.

MLT performs as an antioxidant and free radical scavenger, immunomodulator, antiinflammatory, antimicrobial, anti-aging agent in oral cavity and supports the bone formation.[52-54] Studies on periodontitis patients found decreased MLT levels in saliva and serum of periodontitis patients.[55-58]. Cutando et al. they stated that as the severity of periodontal disease increases, the salivary MLT level decreases.[52] In addition to clinical trials in the literature, an in-vitro study has shown that MLT inhibits the growth of gram-negative bacteria associated with periodontal disease.[59,60] Burgess et al reported that cigarette use led to a decrease in MLT levels of saliva.[61] Likewise, in the present research, MLT levels in the saliva in both periodontitis patients were statistically lower than both (CP-) individuals. When all the groups were assessed for smoking cessation in the smoking groups, lower MLT levels were measured, although the difference wasn't significant. Periodontal disease and cigarette smoking lead to a sinergistic decline in MLT levels that protect against body bacterial attack by anti-infectious, antiinflammatory and antioxidant properties. This is an indication that periodontal disease and cigarette smoking cause damage to the organism and cigarette consumption increases OS in particular. Also lower levels of MLT in smokers may be considered as a sign of risk at periodontal disease's severity and increased risk for the develop periodontal diseases.

Oxidation-reduction imbalance or OS has an impostant role in the prognosis of CP. Neutrophils, which are very important tasks in host defense, secrete lysosomal enzymes in their bodies as well as their ability to phagocytose. While the MPO in neutrophils is an enzyme for host defense by an antibacterial mechanism.[62,63] In the literature, the increase of MPO levels in DOS is referred to as a marker of OS.[4,64-66] Likewise greater levels of MPO has found in periodontally diseased sites compared to healthy sites.[65] Smith et al. noted that MPO is an important finding in periodontitis, and that there is a significant decrease in MPO levels following perio-dontal treatment.[67,68] When the data in the literature were evaluated, similar findings were obtained in the present study because of the high levels of MPO measured in periodontitis groups. It has been reported that oxidative metabolism is increased in macrophages and PMNLs in smokers. The increase in oxidative metabolism leads to an increase in production of ROS and MPO in smokers.[69] Unlike the literature in general, Bolzan et al. found that antioxidant enzyme activities were not different from non-smokers in smokers.[70] The difference in the results obtained from the studies can be explained by the research methodology or smoking status. In the present study, cigarette use increased the MPO level in GCF significantly between the periodontally healthy groups. MPO was found higher in the smoker group between the periodontitis

patient, however, this increase was statistically insignificant. Both CP and smoking increase MPO levels of GCF. The coexistence of two conditions causes an integrated effect on MPO levels.. According to our results, smoking itself seems to be as effective as CP alone in enhancing MPO. Additionally, smoking has a contributory influence on MPO levels in CP groups, but this effect is not statistically detectable.

Studying large sample sizes to minimize the effects of interactions in such complex diseases will make the study more valuable. Therefore large sampled and varied studies are recommended for explanation of the relationship between smoking, CP and OS .

CONCLUSION

To sum up, periodontitis, which causes higher 8-OHdG levels in saliva and MPO levels in GCF; there withal a decrease in salivary MLT levels caused an additional increase in oxidative damage in tissues. Tobacco use caused an additional increase in levels of 8-OHdG and MPO with an additional decrease in levels of MLT in periodontitis patients and periodontally healty individuals.

Longitudinal and prospective studies to be conducted in different geographies where more individuals are included will bring to the light the relationship of CP and tobacco use, with the mechanisms triggered by OS.

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Conflicts of interest statement
The authors declare no conflict of interest.

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