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The effect of initial periodontal treatment on plasma, gingival crevicular fluid and salivary levels of 8-hydroxy-deoxyguanosine in obesity

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ABSTRACT

Objective: Recent studies have shown adverse effects on the periodontium from the increased production of reactive oxygen species (ROS) in obesity. The purpose of this study was to investigate the effects of obesity on 8-hydroxy-deoxyguanosine (8-OHdG) levels in the bodily fluids of patients with and without periodontal disease and to evaluate changes after initial periodontal treatment.
Design: Forty-five obese individuals and 45 normal-weight individuals were included in this study. Obese and normal-weight groups were classified into three sub-groups: chronic periodontitis (CP), gingivitis (G) and periodontally healthy controls (CTRL). Gingival crevicular fluid (GCF), plasma, saliva samples and clinical measurements were obtained at baseline and a month after initial periodontal treatment. Levels of 8-OHdG were analysed by ELISA.
Results: While plasma 8-OHdG levels were significantly higher at baseline in the obese patients with periodontal disease than in the normal-weight individuals ($P < 0.05$), no significant differences in GCF and saliva 8-OHdG levels were found ($P > 0.05$). GCF and salivary 8-OHdG levels in obese patients with G and CP were significantly higher than in CTRL groups at baseline ($P < 0.05$). After treatment, 8-OHdG levels were decreased in all groups with periodontal disease ($P < 0.01$). Statistically significant positive correlations were observed between GCF 8-OHdG levels and GI in all the groups ($P < 0.001$).
Conclusions: The significant increase of plasma 8-OHdG levels in obese patients did not correlate with saliva and GCF 8-OHdG levels when compared to normal-weight individuals. Periodontal treatment had a positive effect on the periodontal parameters and 8-OHdG levels of both obese and normal-weight individuals.

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1. Introduction

Obesity is a medical condition characterised by abnormal or excessive deposits of fat in the adipose tissue, which may have an adverse effect on health (Bullon, Newman, & Battino, 2014). Several studies have reported a significant positive relationship between obesity and periodontal disease and suggested that obesity is a major risk factor for periodontal disease (Ritchie & Kinane, 2003; Boesing, Patino, Da Silva, & Moreira, 2009; Chaffee & Weston, 2010; Shimazaki et al., 2010; Zuza et al., 2011; Dahiya, Kamal, & Gupta, 2012). The underlying biological mechanisms of the way in which obesity affects the periodontium are currently poorly understood. However, it is known that adipocytes act as an active organ by secreting various proinflammatory cytokines and hormones, contributing to the pathogenesis of periodontal diseases (Jagannathachary & Kamaraj, 2010; Zimmermann, Bastos, Gonçalves, Chambrone, & Duarte, 2013). Adipose tissue secretes several bioactive substances, such as reactive oxygen species (ROS) (Matsuzawa-Nagata et al., 2008; Suresh & Mahendra, 2014). It has been stated that obesity leads to excessive ROS in adipose tissue and, as a result, increases the amount of circulating ROS (Mohora et al., 2006). Recent studies have shown that increased oxidative stress and decreased anti-oxidative status play critical roles in the progression of periodontal disease (Chapple & Matthews, 2007; Tamaki et al., 2009). Obesity is a disease process that is defined as 'increased chronic oxidative stress status'

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(Higdon & Frei, 2003). Tomofuji et al. (2009) emphasised that obesity may affect periodontal health by inducing oxidative damage to the gingiva through the increased production of circulating ROS.

The most common stable product of oxidative DNA damage caused by ROS is 8-hydroxy-deoxyguanosine (8-OHdG) (Chapple & Matthews, 2007). Several studies have demonstrated that 8-OHdG in bodily fluids can act as a biomarker of oxidative DNA damage in periodontal diseases as well as a measure for evaluating the effect of periodontal treatment (Sezer, Ciçek, & Canakçi, 2012; Chandra et al., 2013). Also, the levels of 8-OHdG showed that body mass index (BMI) can affect DNA damage, which is associated with oxidative stress in overweight individuals (Hofer, Karlsson, & Möller, 2006).

Tomofuji et al. (2009) showed that gingival 8-OHdG levels were higher in obese rats than in lean rats. Similar results were reported in obese rats that were fed a high-fat diet and received no exercise (Azuma et al., 2011). Results of animal studies have indicated that the increase of ROS in obesity causes acute inflammation in the periodontal tissue, though this inflammation is not a direct equivalent of the chronic disease in humans (Tomofuji et al., 2009; Azuma et al., 2011). However, no studies have investigated the effect of obesity-induced oxidative stress in patients with periodontal disease.

In the present study, we hypothesised that high circulating ROS levels may increase oxidative stress levels in the GCF and saliva in obese patients with periodontal disease, and thus, that periodontal therapy could have positive effects on ROS levels. The aim of this study was to evaluate clinical periodontal parameters and to analyse 8-OHdG levels in saliva, GCF and plasma in obese and normal-weight patients with periodontal disease at baseline and after initial periodontal treatment.

2. Materials and methods

2.1. Study population

The protocol for the study was approved by the Ethics Committee of the Faculty of Medicine (2013-25-12/02), Bulent Ecevit University, Turkey, in accordance with the Helsinki Declaration of 1975, as revised in 2002. Individuals were informed about the protocol of the study and gave their written consent for the described procedures. Individuals were selected from a population of people who had received periodontal treatment at the Periodontology Department of the Faculty of Dentistry, Bulent Ecevit University, from March 2013 to January 2014. Selected patients were directed to the Endocrinology Department of the Faculty of Medicine, Bulent Ecevit University. Obesity was diagnosed by using body mass index (BMI) (Dahiya et al., 2012). BMI was categorised using the World Health Organization (WHO) classification: normal weight was identified as BMI = 18.50–24.99 kg/m^2; obese $\geq$ 30 kg/m^2 (Linden, Patterson, Evans, & Kee, 2007)

Ninety individuals (43 males and 47 females, aged 25–60 years; mean age: 0.30 $\pm$ 8.66 years) fulfilled the inclusion criteria (45 obese and 45 normal-weight). Both the obese and the normal-weight groups were classified into three sub-groups: (1) periodontal healthy, (2) gingivitis and (3) chronic periodontitis. The diagnoses were based on their periodontal conditions as outlined in the criteria proposed by the 1999 International World Workshop for a Classification of Periodontal Disease and Conditions (Armitage, 1999). The selected individuals had a minimum of 20 natural teeth, excluding third molars. The study included 15 individuals with obese-chronic periodontitis (O-CP; 8 males and 7 females, aged 34–60 years; mean age: 47.13 $\pm$ 7.17 years), 15 individuals with normal-weight CP (CP; 8 males and 7 females, aged 26–51 years; mean age: 38.47 $\pm$ 7.50 years), 15 individuals with obese-gingivitis (O-G; 7 males and 8 females, aged 25–55 years; mean age: 35.73 $\pm$ 7.23 years), 15 individuals with normal-weight gingivitis (G; 8 males and 7 females, aged 25–53 years; mean age: 31.53 $\pm$ 6.80 years), 15 individuals with obese-periodontally healthy controls (O-CTRL; 7 males and 8 females, aged 25–50 years; mean age: 41.33 $\pm$ 6.47 years) and 15 individuals with normal-weight periodontally healthy controls (CTRL; 8 males and 7 females, aged 25 to 33 years; mean age: 29.60 $\pm$ 2.30 years).

2.2. Clinical measurements and intra-examiner reproducibility

The periodontal status of patients was determined by measuring the probing depth (PD), clinical attachment level (CAL), gingival index (GI) (Löe & Silness, 1963), bleeding on probing (BOP) (Ainamo & Bay, 1975), and plaque index (PI) (Silness & Löe, 1964). The level of periodontal bone loss was determined by taking full-mouth periapical radiographs. All the clinical parameters were measured on six sites per tooth (mesiobuccal, distobuccal, midbuccal, mesiolingual, distolingual, and midlingual) using a William's periodontal probe (Hu-Friedy, Chicago, IL, USA) calibrated in millimetres by the same examiner (FÖD). Prior to the actual measurement, 10 individuals were randomly selected and used to calibrate the investigator. The investigator evaluated the individuals on two separate occasions, 48 h apart. Calibration was accepted, if measurements at baseline and at 48 h did not differ more than 10% at the millimetre level (Schwarz, Bieling, Latz, Nuesry, & Becker, 2006).

2.3. Inclusion criteria

Inclusion criteria for the selection of patients were: (1) non-smokers who had never smoked; (2) no history of systemic disease; (3) had not undergone periodontal treatment or taken medicine for at least 6 months before the study; (4) no pregnancy or lactation; (5) no alcohol or antioxidant vitamin consumption; (6) GI = 0, PD and CAL $\leq$ 3 mm, with no signs of attachment and bone loss as demonstrated by clinical and radiographic examination for the periodontally healthy groups; (7) the presence of BOP in at least 50% of the total gingiva, no clinical signs of periodontitis, and no radiographic evidence of alveolar bone loss for the gingivitis groups; and (8) clinical signs of inflammation (red colour and swelling of the gingival margin) GI $\geq$ 2, PD and CAL $\geq$ 5 mm, and bone loss affecting >30% of the existing teeth on clinical and radiographic examination for the chronic periodontitis groups.

2.4. Collection of samples

All the samples were obtained in the morning following an overnight fast, during which patients were requested not to eat or drink anything except water. Before sample collection, the individuals were checked for their adherence to protocol.

Whole saliva samples were collected prior to gingival crevicular fluid samples and before clinical periodontal measurements were taken. Patients' mouths were rinsed with distilled water, and unstimulated salivary samples were collected by patients expectorating into disposable tubes (Navazesh, 1993). About 2 ml of whole saliva were immediately centrifuged to remove cell debris (10,000 $\times$ g for 10 min). The supernatants (50 μl each) were stored at $-40\,^{\circ}$C until they were analysed.

In order to avoid irritation, GCF samples were collected two days after the clinical measurements were taken, in the morning between 8:00 and 10:00. GCF samples were collected from a mesiobuccal and disto-palatal site on each tooth (molars, premolars, canines/incisors). In the CP group, the samples were

obtained from patients that were found to have ≥5 mm CAL, ≥6 mm PD and ≥30% bone loss. In the gingivitis group, GCF samples were obtained from teeth with BOP and without CAL. In the healthy group, GCF samples were collected from teeth exhibiting PD < 3 mm without CAL or BOP. Six GCF samples were collected from each patient. The sample area was isolated with cotton rolls, the prevention of saliva contamination was ensured, and then the area was slightly air dried. GCF was sampled using paper strips (Periopaper; Ora Flow Inc., Amityville, NY, USA). Paper strips were placed into the crevice until mild resistance was met (intracrevicular method) and were left in place for 30 s (Griffiths, 2003). Strips contaminated with blood or saliva were discarded. Each sample strip was placed into a disposable tube and stored at −40 °C until analysed. For laboratory analysis, 200 μl phosphate buffered saline (pH 7.4) was added into each tube containing a sample strip. Centrifugation was carried out at 10,000 × *g* for 5 min, and then supernatant was used to determine 8-OHdG levels with a commercially available enzyme immunoassay.

Five millilitres of venous blood were taken from the antecubital vein by using a standard venipuncture method. Obtained blood samples were collected in vacutainer tubes and anticoagulated with EDTA. The blood samples were then stored at −40 °C until they were required for use in ELISAs.

2.5. Biochemical analysis

A Genomic DNA Mini Kit (Geneaid Biotech Ltd., Cat. No. GB 300, New Taipei City, Taiwan) was used for the DNA isolation of blood samples. The absorbance was measured at 260 nm in a spectrophotometer. The data were expressed as μg/ml DNA in blood. GCF, saliva and isolated DNA samples were used to determine 8-OHdG levels with a DNA/RNA Oxidation Damage enzyme immunoassay (EIA) Kit (Cayman Chemical Company, Item No. 589320 Ann Arbor, MI, USA). The 8-OHdG levels were calculated according to standard curves. GCF and saliva concentrations of 8-OHdG were expressed as picograms per millilitre (pg/ml). 8-OHdG levels in the isolated DNA samples were rated according to DNA concentrations. The data were expressed as pg 8-OHdG/μg DNA.

2.6. Periodontal treatment

All the patients were instructed and encouraged to maintain daily plaque control. Non-surgical periodontal treatment was performed on patients with CP and G. Periodontal treatments for patients were performed at different periods according to their periodontal condition with a mean frequency of four recall visits for four weeks. Treatment included an intensive hygiene phase, full-mouth scaling and root planing (SRP; performed in all four quadrants), and the maintenance and monitoring of oral hygiene. Clinical data and samples were obtained at baseline and one month after the final SRP appointment.

2.7. Statistical analyses

Statistical analysis was conducted using a commercially available software program (SPSS 15.0; SPSS Inc., Chicago, IL). The Shapiro Wilk test was used to investigate whether the data were normally distributed. Continuous variables with unequal variances were compared by means of the Welch and Tamhane's T2 post hoc test for BMI, PD, CAL and the levels of 8-OHdG. Age, GI, PI and BOP were analysed using the Kruskal–Wallis non-parametric test and were followed by post hoc group comparisons with the Bonferroni-adjusted Mann–Whitney *U* test. A paired Student's *t*-test or the Wilcoxon Rank-Sum test were used to compare the measurements at two points (baseline and after SRP). The Spearman's rank correlation coefficient was also used to detect the relationship between biochemical and clinical findings. A power analysis indicated that 12 individuals from each group would be sufficient to achieve 80% power to detect a difference of 0.05 between the alternative and the null hypotheses.

3. Results

3.1. Clinical findings

The clinical findings are summarised in Table 1. BMI was statistically higher in individuals with obesity than in normal-weight individuals ($P < 0.05$). The full-mouth PD, CAL, BOP, PI and GI were statistically higher in the CP groups than in the G and CTRL groups ($P < 0.05$). The mean PD and CAL decreased in the CP groups after SRP; the decrease was statistically significant ($P < 0.05$; Table 1).

3.2. Biochemical findings

The presence of 8-OHdG was observed in all the samples. Plasma levels of 8-OHdG were significantly higher at baseline in

Table 1
Clinic parameters in the study population.

		O-CP (*n* = 15)	O-G (*n* = 15)	O-CTRL (*n* = 15)	CP (*n* = 15)	G (*n* = 15)	CTRL (*n* = 15)
BMI (kg/m^2)		35.80 ± 3.89	38.63 ± 6.22	36.95 ± 5.18	22.87 ± 1.03	22.55 ± 1.62	22.26 ± 1.67
PI	Baseline	2.40 ± 0.40	1.68 ± 0.58	0.68 ± 0.20	2.44 ± 0.30	1.66 ± 0.32	0.20 ± 0.19
	After SRP	0.89 ± 0.31	0.48 ± 0.18	NA	0.62 ± 0.29	0.42 ± 0.22	NA
GI	Baseline	2.39 ± 0.41	1.69 ± 0.51	0.00 ± 0.00	2.12 ± 0.21	1.83 ± 0.32	0.00 ± 0.00
	After SRP	0.84 ± 0.38	0.43 ± 0.24	NA	0.92 ± 0.28	0.41 ± 0.15	NA
BOP (%)	Baseline	90.26 ± 7.23	69.26 ± 11.28	0.00 ± 0.00	90.84 ± 6.62	65.39 ± 18.51	0.00 ± 0.00
	After SRP	38.41 ± 18.20	13.35 ± 3.35	NA	27.03 ± 13.20	10.18 ± 5.50	NA
PD (mm)	Baseline	4.34 ± 1.08	1.54 ± 0.23	1.46 ± 0.16	3.82 ± 1.23	1.69 ± 0.25	1.48 ± 0.14
	After SRP	3.82 ± 1.05	1.50 ± 0.23	NA	3.22 ± 1.07	1.69 ± 0.25	NA
CAL (mm)	Baseline	5.02 ± 1.16	1.54 ± 0.23	1.46 ± 0.16	4.65 ± 1.33	1.69 ± 0.25	1.48 ± 0.14
	After SRP	4.23 ± 0.92	1.50 ± 0.23	NA	4.01 ± 1.05	1.69 ± 0.25	NA

Data are expressed as the mean ± standard deviation.
NA, not applicable; BMI, body mass index; PI, plaque index; GI, gingival index; BOP, bleeding on probing; PD, probing depth; CAL, clinical attachment level; O-CP, obese individuals with chronic periodontitis; O-G, obese individuals with gingivitis; O-CTRL, obese-periodontally healthy controls; CP, normal-weight individuals with chronic periodontitis; G, normal-weight individuals with gingivitis; CTRL, normal-weight periodontally healthy controls; SRP, scaling and root planing.

Table 2
Plasma (pg 8-OHdG/μg DNA), salivary (pg/ml) and GCF 8-OHdG levels (pg/ml) in the study population.

	Plasma		*P* value	Salivary		*P* value	GCF		*P* value
	Baseline	After SRP		Baseline	After SRP		Baseline	After SRP	
O-CP	1.91 ± 0.35	0.54 ± 0.23	0.000	927.94 ± 116.66	652.58 ± 139.51	0.000	1178.44 ± 97.34	1003.66 ± 157.85	0.000
O-G	1.03 ± 0.15[a]	0.40 ± 0.13	0.000	824.32 ± 115.65	716.60 ± 76.62	0.000	961.86 ± 153.70[a]	775.45 ± 250.66	0.000
O-CTRL	0.52 ± 0.16[a,b]	NA	NA	630.76 ± 74.89[a,b]	NA	NA	506.21 ± 61.33[a,b]	NA	NA
CP	0.78 ± 0.12[a]	0.54 ± 0.10	0.000	1023.48 ± 87.35	888.13 ± 113.13[a]	<0.01	1232.70 ± 44.70	856.95 ± 191.84	0.000
G	0.63 ± 0.16[b]	0.49 ± 0.09	<0.01	864.06 ± 139.60[d]	667.56 ± 161.22[d]	0.000	866.71 ± 90.92[d]	668.06 ± 75.89[d]	0.000
CTRL	0.34 ± 0.14[c,d,e]	NA	NA	4.61 ± 50.40[c,d,e]	NA	NA	434.61 ± 95.45[d,e]	NA	NA

Data are expressed as the mean ± standard deviation.
NA, not applicable; 8-OHdG, 8-hydroxy-deoxyguanosine; GCF, gingival crevicular fluid; O-CP, obese individuals with chronic periodontitis; O-G, obese individuals with gingivitis; O-CTRL, obese-periodontally healthy controls; CP, normal-weight individuals with chronic periodontitis; G, normal-weight individuals with gingivitis; CTRL, normal-weight periodontally healthy controls; SRP, scaling and root planing.
[a] Statistically significant difference from group O-CP ($P = 0.000$).
[b] Statistically significant difference from group O-G ($P = 0.000$).
[c] Statistically significant difference from group O-CTRL ($P < 0.05$).
[d] Statistically significant difference from group CP ($P < 0.05$).
[e] Statistically significant difference from group G ($P = 0.000$).

obese individuals than in normal-weight individuals in the CP, G and CTRL groups ($P < 0.05$; Table 2).

Salivary levels of 8-OHdG were significantly higher in the O-CP and O-G groups than in the O-CTRL group ($P < 0.05$), whereas the salivary levels were not significantly different between the O-CP and O-G groups ($P > 0.05$). GCF levels of 8-OHdG were significantly higher in the O-CP group than in the O-G and O-CTRL groups, and they were also significantly higher in the O-G group than in the O-CTRL group ($P < 0.05$). Salivary and GCF levels of 8-OHdG were significantly higher in the CP group than in the G and CTRL groups, and they were also significantly higher in the G group than in the CTRL group ($P < 0.05$). There were no significant differences in saliva and GCF 8-OHdG levels between the O-CP and CP groups and between the O-G and G groups ($P > 0.05$).

Levels of 8-OHdG in plasma, saliva and GCF significantly decreased in the CP, O-CP, G and O-G groups after SRP ($P < 0.01$). Significant differences were observed in GCF and salivary 8-OHdG levels between the CP and G groups after SRP ($P < 0.05$).

3.3. Correlations

There were statistically significant positive correlations between GCF 8-OHdG levels and GI and between BMI and plasma 8-OHdG levels for all the groups ($P < 0.001$; Table 3). Additionally, statistically significant positive correlations were observed between plasma, GCF and salivary 8-OHdG levels for all the groups ($P < 0.001$; Table 3).

4. Discussion

Obesity may induce oxidative damage and increase inflammation to the gingiva through increased production of circulating ROS (Tomofuji et al., 2009). Our study is the first of its kind to determine the effect of initial periodontal treatment on the levels of plasma, saliva and GCF 8-OHdG in obese individuals with periodontal disease. The findings indicated that plasma levels of 8-OHdG were significantly higher at baseline in obese individuals than in normal-weight individuals in the CP, G and CTRL groups, whereas saliva and GCF levels of 8-OHdG were found to have no significant differences. Additionally, plasma, saliva and GCF levels of 8-OHdG decreased after the treatment in all the groups with periodontal disease.

BMI and obesity are directly related to the severity, prevalence and development of periodontal disease (Suvan et al., 2014). Munoz-Torres, Jimenez, Rivas-Tumanyan, and Joshipura (2014) noted that BMI is used as the primary measure of adiposity for evaluating the association between obesity and periodontitis. For the diagnosis of obesity, we used BMI ≥ 30 kg/m^2 as the threshold, in accordance with WHO guidelines. Jagannathachary and Kamaraj (2010) have suggested that BMI reflects the risk of obesity-related diseases in a wide range of populations, but there is a discrepancy when BMI is used in populations over 65 years old due to high body fat composition. Therefore, we excluded individuals over 65 years of age in order to eliminate age-related factors.

Suvan et al. (2014) demonstrated that BMI and obesity are associated with probing pocket depths two months after non-surgical periodontal therapy, and they have suggested that BMI and obesity may be independent predictors of poor response to treatment. However, other studies showed a significant improvement in all clinical periodontal parameters of obese and normal-weight groups after non-surgical periodontal therapy (Zuza et al., 2011; Altay, Gürgan, & Ağbaht, 2013). These studies emphasised that obesity did not have a negative impact on periodontal clinical response after non-surgical periodontal treatment. Our study showed a significant reduction in all clinical periodontal parameters after SRP in the O-CP and CP groups; additionally, there was a difference in the PI, GI and BOP values between the G and O-G groups. However, no differences were observed in the BMI values for any of the patients one month after periodontal treatment. The present study showed the importance of initial periodontal therapy for obese individuals with periodontal disease.

Recent studies indicated that salivary levels of 8-OHdG were higher in patients with CP than in control groups (Sawamoto, Sugano, Tanaka, & Ito, 2005; Rai & Anand, 2008; Sezer et al., 2012; Miricescu et al., 2014). Sawamoto et al. (2005) showed that salivary 8-OHdG levels decreased after periodontal treatment. Our study had results similar to these studies (Miricescu et al., 2014; Sawamoto et al., 2005). Additionally, we examined the levels of

Table 3
The Spearman's rank correlation (*r*) among groups with respect to BMI, PI, GI, 8-OHdG levels in all subjects.

Parameter	*r*	*p*
BMI–PI	0.160	0.131
BMI–GI	0.106	0.321
BMI–P 8OHdG	0.527[a]	0.000[a]
BMI–S 8OHdG	0.069	0.521
BMI–GCF 8OHdG	0.175	0.099
GCF 8OHdG–P 8OHdG	0.775[a]	0.000[a]
GCF 8OHdG–S 8OHdG	0.858[a]	0.000[a]
P 8OHdG–S 8OHdG	0.614[a]	0. 000[a]
GI–GCF 8OHdG	0.879[a]	0.000[a]

BMI, body mass index; PI, plaque index; GI, gingival index; P, plasma; S, salivary; GCF, gingival crevicular fluid; 8-OHdG, 8-hydroxy-deoxyguanosine.
[a] Statistically significant ($P < 0.05$).

salivary 8-OHdG in the G group and compared them to those of other groups. It was concluded that these levels were significantly lower in the G group than in the CP group and higher in the G group than in the CTRL group at baseline. Rai and Anand (2008) reported that salivary levels of 8-OHdG were significantly higher in patients with periodontitis than in patients with gingivitis and that they decreased after SRP. Another study found no statistically significant differences in the salivary levels of 8-OHdG between control and gingivitis groups, whereas the levels were significantly higher in the CP group (Sezer et al., 2012). Contrary to our expectations, our previous study found that there were no significant differences in salivary levels of 8-OHdG between the CP and control groups at baseline or in the follow-up periods (Dede, Özden, & Avcı, 2013).

Recent studies showed that GCF levels of 8-OHdG were significantly higher at baseline and that the levels decrease after periodontal treatment in CP patients (Chandra et al., 2013; Dede et al., 2013). In our study, similar results to these studies were found for both obese and normal weight individuals with periodontal disease. Furthermore, a positive correlation was found between GI and GCF 8-OHdG levels for all the individuals. The current data may reveal the importance of GCF analysis in the determination of oxidative DNA damage, which plays an important role in the pathology of periodontal diseases.

Boesing et al. (2009) stated that obesity may contribute to the multifactorial effects of periodontitis through an increase of circulating ROS. Our data demonstrated that plasma levels of 8-OHdG were significantly higher in obese individuals than in normal-weight individuals in the CP, G and CTRL groups and that a decrease occurred after SRP. Research studies evaluating the relationship between periodontitis and obesity found that the levels of circulating proinflammatory cytokines (tumour necrosis factor-alpha (TNF-α), interleukin (IL)-6) were higher in obese individuals than in normal-weight individuals at baseline and that there was a decrease in both groups after periodontal treatment, although obesity status did not change during the study (Zuza et al., 2011; Altay et al., 2013). It is probable that ROS produced by an increase in circulating pro-inflammatory cytokines may lead to increased oxidative DNA damage in circulation; additionally, the benefits of periodontal treatment may reduce inflammatory conditions.

Tomofuji et al. (2009) identified an increase in gingival 8-OHdG levels in obese rats compared to lean rats and suggested that an increase of circulating ROS in obesity could impair gingival inflammatory responses. In the present study, GCF and salivary levels of 8-OHdG in both CP groups were higher than those of the other groups with and without obesity. There were no differences between the obesity and normal-weight groups. Furthermore, GCF and saliva 8-OHdG levels in all the groups were significantly reduced after SRP. A previous study demonstrated that GCF TNF-α levels were higher in obese individuals with or without periodontitis than in obese individuals with a BMI $\geq$ 30 and $<$40 (Zimmermann et al., 2013). Moreover, when GCF TNF-α levels were between BMI $\geq$ 40 and BMI $<$ 40, a positive association was observed with a BMI $\geq$ 40 in individuals with no periodontal pathological pocket (Lundin, Yucel-Lindberg, Dahllöf, Marcus, & Modéer, 2004). The individuals in our study were identified as mostly having a BMI $\leq$ 40; therefore, we concluded that GCF and saliva 8-OHDG levels for all the groups may be influenced by periodontal inflammation rather than by obesity.

Epidemiological studies have consistently shown that diabetes is associated with an increased risk of periodontitis. The magnitude of this increased risk is known to be dependent on the level of glycaemic control, which is also a risk factor for all complications of diabetes. Overall, the increased risk of periodontitis in patients with diabetes is estimated to be between 2 and 3 times higher than for patients without diabetes (World Health Organization, 2013). Insulin resistance has been stated to mediate the relationship between periodontal disease and obesity, which is known to lower insulin sensitivity (Genco, Grossi, Ho, Nishimura, & Murayama, 2005). Altay (2013) showed a significant decrease in HOMA-IR (homeostasis model assessment of the insulin resistance) values after periodontal treatment in obese patients without weight loss or changes in lipid parameters, although these values did not change with periodontal treatment in individuals without obesity. A recent review indicated that mitochondrial ROS increase insulin sensitivity via oxidative modification of the insulin receptor (Bullon et al., 2014). Ohnishi et al. (2009) suggested that oxidative stress increases alveolar bone loss in periodontitis, which occurs as a complication of diabetes. These results may show that the existence of insulin resistance in obesity-related periodontal inflammation is due to the increase of ROS.

This study has some limitations. First, the small number of samples may lead to difficulty in determining significant differences between the groups. Second, our study included individuals with moderate (obese class I) and severe (obese class II) obesity; therefore, our results are not generalisable for more severe cases of obesity (obese class III). Third, if proinflammatory markers are analysed together with oxidative stress markers, obesity-induced oxidative stress would be better observed in patients with periodontal disease. The fourth limitation of our study was that the effect of oxidative stress in obesity-related periodontal inflammation would be better explained by a correlation with insulin resistance since insulin resistance mediates the relationship between periodontal disease and obesity. Therefore, further studies are needed to clarify the different mechanisms involved in the relationship between inflammation, periodontal disease and oxidative stress in obese patients, eliminating these limitations in long-term studies with larger groups of patients.

In conclusion, the present study suggested that increased plasma 8-OHdG levels might not influence GCF and saliva 8-OHDG levels in obesity; that is, these levels might be influenced by periodontal inflammation rather than be a condition of obesity. Furthermore, obesity did not play a negative role in the improvement of the periodontal parameters or in the local and systemic 8-OHdG levels after SRP.

Conflict of interest

The authors certify that there is no conflict of interest concerning the contents of the study. Dr. Kuzu report no conflicts of interest related to this study.

Ethical approval

The protocol for the study was approved by the Ethics Committee of the Faculty of Medicine (2013-25-12/02), Bulent Ecevit University, Turkey.

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