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Obesity and oxidative stress in patients with different periodontal status: a case–control study

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Background and Objective: Obesity has become an important global health concern as obesity-associated adiposity is supposedly related to systemic immunologic and inflammatory alterations. The aim of this study was to evaluate the effects of obesity on periodontally healthy and diseased tissue according to the changes in malondialdehyde (MDA), protein carbonyl (PC) and total antioxidant capacity (TAOC) levels in gingival crevicular fluid as biomarkers of oxidative stress (OS).

Material and Methods: The study sample comprised systemically healthy normal-weight ($n = 45$) and obese ($n = 48$) adults. Obesity was diagnosed according to body mass index, waist circumference and waist/hip ratio. Periodontal status was evaluated according to plaque index, gingival index, bleeding on probing, probing depth and clinical attachment level. Participants were distributed among six groups according to obesity and periodontal status, as follows: normal weight+periodontally healthy (NH); normal weight+gingivitis (NG); normal weight+generalized chronic periodontitis (NCP); obese+periodontally healthy (OH); obese+gingivitis (OG); and obese+generalized chronic periodontitis (OCP). MDA, PC and TAOC levels were measured using ELISA.

Results: The MDA and PC levels in gingival crevicular fluid varied among groups, as follows: NCP > NG > NH ($p < 0.01$) and OCP > OG > OH ($p < 0.01$). Conversely, the levels of TAOC in gingival crevicular fluid varied as follows: NCP < NG < NH ($p < 0.01$) and OCP < OG < OH ($p < 0.01$). Paired comparisons conducted according to periodontal status showed MDA and PC levels to be higher, and TAOC levels to be lower, in the OCP group than in the NCP group, in the OG group than in the NG group and in the OH group than in the NH group. However, only the differences between the OCP and NCP groups were significant ($p < 0.01$). In both obese and normal-weight individuals, clinical assessments showed significant, positive correlations with MDA and PC levels and negative correlations with TAOC levels ($p < 0.01$).

Conclusion: Obesity may influence periodontal tissue destruction and disease severity by increasing the level of oxidative stress in the presence of periodontal disease.

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Periodontal disease is not only a local immuno-inflammatory response but also a chronic, low-grade inflammatory process in which systemic inflammatory cytokines are released in response to periodontal colonization of pathogenic bacteria (1). A multifactorial, chronic disease, the initiation and progression of periodontal destruction are modified by numerous host-related and environmental factors, with the destructive process being dependent upon the balance between bacteria and these modifying factors (1).

Obesity, defined as the deposition of abnormal or excessive fat in adipose tissue and adipose cells, was originally proposed as a factor affecting the periodontal disease process through an obesity-associated adiposity supposedly related to systemic immunologic and inflammatory alterations (2,3). In terms of health consequences, obesity is a state of 'increased chronic oxidative stress' and 'low-grade subclinical systemic inflammation' that causes major adverse metabolic effects, including dyslipidemia and insulin resistance, and leads ultimately to endothelial dysfunction and atherogenesis (4,5). In the first of a very few histomorphologic studies examining the relationship between obesity and periodontal disease, Perlstein and Bissada (6) induced periodontitis by applying ligature to obese and nonobese Zucker rats; the findings indicated that obese rats had a more pronounced inflammatory response to plaque accumulation and higher alveolar bone resorption than did nonobese rats. Another experimental study in rats concluded that systemic low-grade inflammation was a common link between periodontitis and obesity and that the combination of these two diseases caused an increase in the expression of proinflammatory mediators from hepatocytes and adipose tissue (7). More recent studies have verified a proinflammatory state during obesity by measuring increased production of several cytokines and inflammatory mediators in serum (4) and in gingival crevicular fluid (8,9). In conjunction with this proinflammatory state, a study with a murine model (10) showed the pathways for production of reactive oxygen species and oxidative stress (OS) to be upregulated in liver and adipose tissue of mice fed a high-fat diet. Aside from the studies mentioned above, most periodontal-disease-related studies concerning obesity and OS have used serum levels to identify a pro-oxidant state in obesity (11,12), whereas there are no clinical studies evaluating the effects of a pro-oxidant state, caused by obesity, on periodontal tissues or gingival crevicular fluid.

Although the precise mechanisms of operation remain unclear, OS is known to contribute to various diseases by affecting cellular functions through the oxidation of proteins, lipids and DNA (13–19). OS can be assessed by measuring the products of oxidative damage found in proteins, lipids and DNA, or alterations in total antioxidant capacity (TAOC). When attacked by reactive oxygen species, polyunsaturated lipids undergo a series of nonenzymatic reactions that produce a wide range of intermediate and end products. Of these, malondialdehyde (MDA) is the most specific molecule and the one most often used in the measurement of biological lipid oxidation (20). Protein carbonylation, or protein carbonyl tissue content indicated as protein carbonyl (PC) level, is another nonenzymatic oxidative post-translational modification that is often used as a biomarker of OS (21). TAOC, on the other hand, provides an overview of the biological interaction between an individuals' antioxidant status and how well these antioxidants are able to protect host cells during periods of OS (22). Because of the potential synergistic effects of different antioxidant molecules, the measurement of TAOC can provide a more accurate assessment of antioxidant status than the separate measurement of individual molecules (22).

In light of this scientific information, this study intended to evaluate obesity-related OS alterations in gingival crevicular fluid of individuals with different periodontal status. To the best of our knowledge, this study is the first to investigate the effect of obesity-induced OS on healthy and diseased periodontal tissues using clinical assessments of oxidative change (i.e. of MDA, PC and TAOC levels) in gingival crevicular fluid.

Material and methods

Study population

This case–control study was conducted on 93 individuals (45 normal weight; 48 Class I obese) recruited from the Periodontology Department of the Faculty of Dentistry and the Endocrinology and Metabolic Diseases Department of the Faculty of Medicine at Ondokuz Mayis University in Samsun, Turkey, between 1 September 2012 and 31 March 2014. Participants were randomly selected among individuals referred to the Periodontology Department for either dental treatment or dental check-up. The study protocol was approved by the Local Ethics Committee, and written informed consent was obtained from all study participants in accordance with the Helsinki Declaration (revised in 2000) (ClinicalTrials.gov Identifier: NCT02508987).

General inclusion criteria were as follows: (i) $\geq$ 18 years of age and having $\geq$ 16 teeth; (ii) no periodontal therapy in the 6 mo before data collection; (iii) no systemic problems or chemotherapy within the 6 wk before data collection; and (iv) no previous history of smoking. Exclusion criteria were as follows: (i) medical history of cancer, rheumatoid arthritis, diabetes mellitus or cardiovascular disease; (ii) compromised immune system; (iii) pregnancy, menopause or lactation; (iv) ongoing drug therapy that might affect the clinical characteristics of periodontitis; (v) use of systemic antimicrobials during the 6 wk before data collection; and (vi) dental treatment during the 6 mo before data collection.

Obesity was diagnosed according to World Health Organization criteria (23) using body mass index (BMI), waist circumference (WC) and waist/hip ratio (WHR). BMI is defined as a person's weight in kilograms (kg) divided by the square of height in

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meters (m) and is classified as follows: underweight ($\leq$ 18.49 kg/m^2); normal weight (18.50–24.99 kg/m^2); overweight (25.00–29.99 kg/m^2); Obesity class I (30.00–34.9 kg/m^2); Obesity class II (35.00–39.99 kg/m^2); and Obesity class III (morbid obesity) ($\geq$ 40.00 kg/m^2).

WC was measured at the midpoint between the lower border of the rib cage and the iliac crest. WC and WHR (the cut-off values were > 0.85 for female subjects and > 1.0 for male subjects) were determined to be convenient and simple measurements that were unrelated to height but correlated closely with BMI, and were also found to be approximate indices of intra-abdominal fat accumulation/distribution (23).

Periodontal status was assessed by clinical examination and classified according to criteria proposed by the 1999 International World Workshop for a Classification of Periodontal Disease and Conditions (24), as follows:

- Periodontally healthy. Mean gingival index (GI) < 1; mean percentage bleeding on probing $\leq$ 25%; no sites of attachment loss;
- Gingivitis. Mean GI > 1; mean percentage bleeding on probing > 25%; no sites of attachment loss related to chronic inflammatory periodontal disease;
- Generalized chronic periodontitis: $\geq$ 30% of sites with probing depth $\geq$ 5 mm and with clinical attachment level $\geq$ 5 mm at the same time; $\geq$ 30% alveolar bone loss present on radiography.

Study participants were then grouped according to obesity and periodontal status, as follows:

- Group NH: normal weight + periodontally healthy;
- Group NG: normal weight + gingivitis;
- Group NCP: normal weight + generalized chronic periodontitis;
- Group OH: obese + periodontally healthy;
- Group OG: obese + gingivitis;
- Group OCP: obese + generalized chronic periodontitis.

All clinical examinations and gingival crevicular fluid collection were performed by a single examiner. All laboratory procedures were performed by another researcher blinded to the study.

Clinical assessment

Periodontal status was determined by evaluating the following clinical parameters: the Silness & Löe plaque index (25) (PI); Löe & Silness GI (26); probing depth; clinical attachment level; and BOP. Measurements were performed on six sites per tooth (mesiobuccal, midbuccal, distobuccal, mesiolingual, midlingual and distolingual) using a Williams periodontal probe (Nordent Manufacturing Inc., Elk Grove Village, IL, USA) calibrated in mm. Statistical analysis was subsequently performed using those sites that fit the criteria for gingival crevicular fluid sampling described below.

Gingival crevicular fluid sampling and processing

All samples were collected between 8 AM and 10 AM on the day following periodontal status assessment. Samples were collected from the deepest sites of two molar teeth, two premolar teeth and two incisors in the chronic periodontitis groups. In the gingivitis groups sample were collected from the teeth with BOP, whereas teeth without BOP were chosen in the healthy groups. Gingival crevicular fluid samples were collected from the same six sites in the gingivitis and periodontally healthy groups in order to maintain consistency of sampling. Accordingly, a total of 108 gingival crevicular fluid samples were taken from the OG group (18 individuals $\times$ six sites) and 90 from each of the remaining five groups (15 individuals per group $\times$ six sites).

Gingival crevicular fluid samples were collected using Periopaper strips (Oraflow Inc., Plainview, NY, USA). Before sample collection, each site was gently air-dried, all supragingival plaque was removed and the area was carefully isolated to prevent samples from being contaminated with saliva. The paper strip was inserted into the gingival crevice up to 1 mm or until mild resistance was felt, and was left in place for 30 s. Care was taken to avoid mechanical injury of gingival tissue, and any strip contaminated with blood or exudate was discarded. The gingival crevicular fluid sample volume (μL) was measured using a calibrated Periotron 8000 (Periotron® 8000; Pro Flow Inc., Amityville, NY, USA). Strips were individually placed in 500-μL plastic Eppendorf microcentrifuge tubes that were then labeled, sealed with paraffin and stored at −80°C until required for processing.

Analysis of gingival crevicular fluid by ELISA for MDA, PC and TAOC

Elution of gingival crevicular fluid was performed according to Curtis *et al*. (27), with a slight modification. A total of 200 μL of 2% bovine serum albumin (0.01 M, pH 7.2) in phosphate-buffered saline was added to each tube, and the samples were incubated at 4°C for 60 min. Following incubation, a sterile drill was used to bore a hole in the bottom of each tube, which was then placed inside a 1.5-mL tube, and the nested tubes were centrifuged (Shimadzu UV160A, SNo: 28,006,648; Shimadzu, Kyoto, Japan) at 10,000 ***g*** for 10 min at 4°C. This process was repeated twice to obtain 400 μL of gingival crevicular fluid eluate. The supernatants were stored at −80°C until required for analysis. Commercial kits were used to analyze the levels of MDA (Bioxytech, MDA-586, Cat. No. 21,044; OxisResearch, Burlingame, CA, USA), PC (Oxiselect Protein Carbonyl ELISA Kit; Cat. No. STA-310; Cell Biolabs Inc., San Diego, CA, USA) and TAOC (ImAnOx-TAS/TAC Kit; Cat. No. KC 5200; Immundiagnostik AG, Bensheim, Germany) in gingival crevicular fluid. All assays were performed according to the manufacturers' instructions. Spectrophotometry was conducted at wavelengths of 450 and 550 nm. The concentrations and total amounts of MDA, PC and TAOC obtained in

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gingival crevicular fluid collected over a 30-s period were recorded and analyzed statistically.

Sample size calculation

The sample size required to ensure adequate power for this study was calculated based on the results of the studies by Baltacioglu *et al*. (14) and Pradeep *et al*. (16) for the total amount of PC. According to Baltacioglu *et al.,* there was a difference of at least 495 (standard deviation = 196) pg of PC between gingivitis and periodontitis patients (Cohen's d = 1.67; effect-size $r = 0.641$), and Pradeep *et al*. also reported a difference, of at least 113.54 (standard deviation = 23.38) pg of PC, between individuals with periodontitis and healthy subjects (Cohen's d = 3.40; effect-size $r = 0.862$). Based on these values, we calculated that a minimum sample size of 13 patients in each group for analysis of PC levels would allow for a type II error level of $\beta = 0.05$ (95% power) and a two-tailed type I error level of $\alpha = 0.05$ (5% probability). To account for possible dropouts, we included at least 15 patients in each group.

Statistical analysis

Statistical analysis was performed using the statistical software program SPSS (SPSS v.21.0 Inc., Chicago, IL, USA), with the results presented as means and standard deviations. A Shapiro–Wilk test performed before parametric analysis showed normal distribution of data, and the Levene test showed nonhomogeneity of variance; therefore, the Welch ANOVA was used to identify statistical differences among the six groups, and TamhaneT2 tests were used for post-hoc group comparisons. The nonparametric chi-square test was conducted for comparisons of gender. When investigating the changes in OS markers in study groups, the effects of probing depth, clinical attachment level, PI, GI and BOP were adjusted using analysis of covariance. The relationship between clinical indices and biochemical parameters was evaluated using the Pearson correlation test. A p value of < 0.05 was considered statistically significant.

Results

Clinical findings

The descriptive measurements of anthropometric parameters and clinical assessments of the study groups are summarized in Table 1. There were no significant differences between the study groups in terms of either gender or age ($p > 0.05$). BMI, WC and WHR measurements were significantly higher in obese individuals when compared with normal-weight individuals with the same periodontal status ($p < 0.05$ for each of the determined parameters). Clinical parameters varied significantly according to periodontal status for both obese and normal-weight individuals ($p < 0.05$ for each of the parameters measured).

The clinical assessments of the sampling sites of the study groups are summarized in Table 2. The intragroup comparisons of PI, GI, BOP, probing depth, clinical attachment level and gingival crevicular fluid volume of the sites sampled were significantly higher in the NCP group compared with the NG ($p < 0.001$) and NH ($p < 0.001$) groups; in the NG group compared with the NH group ($p < 0.001$); in the OCP group compared with the OG ($p < 0.001$) and OH ($p < 0.001$) groups; and in the OG group compared with the OH group ($p < 0.001$).

The only statistically significant differences in clinical parameters between obese and normal-weight individuals with the same periodontal status were observed for the probing depth ($p < 0.001$) and clinical attachment level ($p < 0.001$) measurements of the OCP and NCP groups, both

Table 1. Anthropometric measurements and full-mouth clinical assessments of the study groups

	Study groups						
Clinical parameter	NH ($n = 15$)	NG ($n = 15$)	NCP ($n = 15$)	OH ($n = 15$)	OG ($n = 18$)	OCP ($n = 15$)	p value
Age (years)	39.60 ± 5.84	42.40 ± 4.33	42.47 ± 2.99	45.47 ± 6.66	42.00 ± 10.91	43.75 ± 1.81	0.225*
Gender (m/f)	6/9	7/8	9/6	9/6	8/10	5/10	0.127§
BMI (kg/m^2)	22.90 ± 0.94	22.84 ± 1.47	22.97 ± 1.21	32.62 ± 1.62	32.43 ± 1.76	33.05 ± 1.21	0.001*
WC (cm)	81.06 ± 14.96	80.93 ± 9.73	83.07 ± 13.58	113.80 ± 26.09	111.20 ± 17.74	114.44 ± 17.50	0.001*
WHR	0.77 ± 0.07	0.78 ± 0.42	0.79 ± 0.12	1.05 ± 0.17	1.11 ± 0.16	1.06 ± 0.13	0.001*
PI	0.41 ± 0.43	2.28 ± 0.61	2.27 ± 0.61	0.52 ± 0.51	1.94 ± 0.77	2.42 ± 0.57	0.001*
GI	0.18 ± 0.28	1.81 ± 0.62	1.97 ± 0.55	0 ± 0	1.46 ± 0.45	1.94 ± 0.53	0.001*
BOP (%)	0.36 ± 1.42	11.10 ± 11.79	72.44 ± 17.42	0.37 ± 1.14	12.01 ± 15.55	80.20 ± 15.97	0.001*
PD (mm)	1.33 ± 0.32	2.19 ± 0.60	5.52 ± 1.03	1.53 ± 0.44	2.12 ± 0.65	7.49 ± 1.52	0.001*
CAL (mm)	1.33 ± 0.32	2.19 ± 0.60	6.67 ± 1.05	1.55 ± 0.44	2.12 ± 0.65	7.97 ± 1.37	0.001*

All values, except for gender, are given as mean ± SD.
*Welch ANOVA. §Chi-square test.
BMI, body mass index; BOP, bleeding on probing; CAL, clinical attachment level; f, female; GI, gingival index; m, male; NCP, normal weight+generalized chronic periodontitis; NG, normal weight+gingivitis; NH, normal weight+periodontally healthy; OCP, obese+generalized chronic periodontitis; OG, obese+gingivitis; OH, obese+periodontally healthy; PD, probing depth; PI, plaque index; WC, waist circumference; WHR, waist–hip ratio.

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Table 2. Clinical assessments of sampling sites of the study groups

Clinical parameters	Study groups						
	NH (n = 15)	NG (n = 15)	NCP (n = 15)	OH (n = 15)	OG (n = 18)	OCP (n = 15)	p value
PI	0 ± 0	1.45 ± 0.50	2.37 ± 0.18	0 ± 0	1.60 ± 0.50	2.63 ± 0.23	0.001*
GI	0 ± 0	1.21 ± 0.15	2.01 ± 0.40	0 ± 0	1.23 ± 042	2.12 ± 0.53	0.001*
BOP (%)	0 ± 0	66.00 ± 6.91	92.13 ± 3.98	0 ± 0	71.00 ± 6.91	95.92 ± 2.02	0.001*
PD (mm)	1.17 ± 0.19	1.65 ± 0.18	5.68 ± 0.28	1.39 ± 0.23	1.91 ± 0.35	6.33 ± 025	0.001*
CAL (mm)	1.17 ± 0.19	1.65 ± 0.18	7.28 ± 0.88	1.39 ± 0.23	1.91 ± 0.35	8.42 ± 0.67	0.001*
GCF (μL)	0.26 ± 0.027	0.40 ± 0.018	0.97 ± 0.082	0.26 ± 0.021	0.41 ± 0.01	1.03 ± 0.04	0.001*

Values are given as mean ± SD.
*Welch ANOVA.
BOP, bleeding on probing; CAL, clinical attachment level; GCF, gingival crevicular fluid; GI, gingival index; NCP, normal weight+generalized chronic periodontitis; NG, normal weight+gingivitis; NH, normal weight+periodontally healthy; OCP, obese+generalized chronic periodontitis; OG, obese+gingivitis; OH, obese+periodontally healthy; PD, probing depth; PI, plaque index.

of which were higher in the OCP group.

There were no statistically significant effects of the confounding variables probing depth, clinical attachment level, GI, PI and BOP as a covariate on the levels of MDA, PC and TAOC in gingival crevicular fluid in our study groups ($p > 0.05$, analysis of covariance).

Analysis of gingival crevicular fluid

The levels of MDA, PC and TAOC in gingival crevicular fluid and the statistical comparisons are listed in Tables 3 and 4. Among normal-weight individuals, the concentration and the total amount of MDA in gingival crevicular fluid and the levels of PC parameters (PC concentrations, total amounts and PC content, expressed as pM per mg protein) in gingival crevicular fluid were highest in the NCP group, followed by the NG and NH groups, with statistically significant differences found between the NCP and NG groups, the NCP and NH groups and the NG and NH groups. Similar results were found for obese individuals (OCP > OG > OH), with statistically significant differences in the concentration and the total amount of MDA in gingival crevicular fluid and in the levels of PC parameters in gingival crevicular fluid found between the OCP group and the OG and OH groups and between the OG and OH groups. Both concentrations and total amounts of MDA in gingival crevicular fluid and of PC parameters in gingival crevicular fluid were higher in each of the obese groups when compared with the normal-weight groups of similar periodontal status; however, the differences were only statistically significant between the OCP and NCP groups.

Among normal-weight individuals, the concentrations and total amounts of TAOC in gingival crevicular fluid were lowest in the NCP group, with the differences being statistically significant between the NCP group and the NG and NH groups and between the NG and NH groups. Similar results were found for obese individuals (OCP < OG < OH), with statistically significant differences between the OCP group and the OG and OH groups and between the OG and OH groups. Both the concentrations and total amounts of TAOC in gingival crevicular fluid were lower in each of the obese groups when compared with the normal-weight groups of similar periodontal status; however, the differences were only statistically significant between the OCP and NCP groups (Tables 3 and 4).

Correlations between gingival crevicular fluid parameters and clinical periodontal assessments

For both obese and normal-weight individuals, the values obtained of PI, GI, BOP, probing depth and clinical attachment level correlated positively with the levels of MDA and PC in gingival crevicular fluid and negatively with the levels of TAOC in gingival crevicular fluid (Tables 5 and 6).

Discussion

Periodontitis constitutes one of the main causes of adult tooth loss. This fact is closely related to the multifactorial nature of the disease, the development and severity of which are affected by a variety of risk and modifying factors. Obesity is considered to be one of the numerous risk factors affecting the progression of periodontal disease, and the association between obesity and severity of periodontal disease has also been previously demonstrated (28–35).

Several studies conducted with periodontally healthy individuals and individuals with periodontitis have stated that oxidative injury/OS related to the processes of inflammatory response and periodontal tissue destruction lead to an increase lipid peroxidation and MDA levels in gingival crevicular fluis and salivary lipid peroxidation and MDA levels (13,15,18). In line with these earlier studies, our study found gingival crevicular fluid MDA levels to be significantly higher in individuals with gingivitis and chronic periodontitis when compared with periodontally healthy individuals with the same BMI status. On the other hand, a comparison of the concentrations and total amounts of MDA in gingival crevicular fluid of obese vs. normal-weight individuals with the same periodontal status found gingival

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Table 3. Levels of the biochemical parameters malondialdehyde (MDA), protein carbonyl (PC) and total antioxidant capacity (TAOC) in gingival crevicular fluid from sites sampled in the study groups

Biochemical parameters	Study groups					
	NH (n = 15)	NG (n = 15)	NCP (n = 15)	OH (n = 15)	OG (n = 18)	OCP (n = 15)
MDA concentration (μM/L)	566.28 ± 14.10	736.22 ± 31.08	826.44 ± 79.02	572.79 ± 22.36	745.70 ± 26.75	966.27 ± 48.35
MDA total amount (μM)	142.59 ± 16.08	298.52 ± 18.90	802.09 ± 88.17	147.50 ± 13.18	306.28 ± 15.62	995.33 ± 57.45
PC concentration (pM/mL)	197.36 ± 25.33	670.40 ± 54.43	1974.67 ± 266.97	220.39 ± 38.03	780.52 ± 176.84	2314.87 ± 268.35
PC total amount (pM)	0.05 ± 0.008	0.27 ± 0.01	1.92 ± 0.31	0.06 ± 0.01	0.32 ± 0.07	2.39 ± 0.36
PC (pM/mg prot)	621.38 ± 55.22	1915.07 ± 103.96	5405.02 ± 516.58	644.58 ± 85.13	2211.53 ± 425.84	7055.47 ± 526.09
TAOC concentration (μM/mL)	351.60 ± 21.66	187.48 ± 14.02	72.43 ± 6.79	338.65 ± 35.79	180.26 ± 10.32	52.10 ± 4.59
TAOC total amount (μM)	88.01 ± 4.61	75.82 ± 3.34	69.70 ± 3.37	86.52 ± 2.74	53.58 ± 3.70	73.92 ± 2.20

Values are given as mean ± SD.
NCP, normal weight+generalized chronic periodontitis; NG, normal weight+gingivitis; NH, normal weight+periodontally healthy; OCP, obese+generalized chronic periodontitis; OG, obese+gingivitis; OH, obese+periodontally healthy; prot, protein.

Table 4. Comparisons of biomarkers between groups

Study group comparisons	Biomarkers						
	MDA concentration (μM/L)	MDA total amount (μM)	PC concentration (pM/mL)	PC total amount (pM)	PC (pM/mg prot)	TAOC concentration (μM/mL)	TAOC total amount (μM)
NH-NG	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*
NH-NCP	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*
NG-NCP	0.009*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*
OH-OG	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*
OH-OCP	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*
OG-OCP	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*
NH-OH	0.998	0.999	0.620	0.749	0.999	0.985	0.994
NG-OG	0.999	0.981	0.408	0.305	0.248	0.855	0.704
NCP-OCP	0.001*	0.001*	0.041*	0.025*	0.001*	0.001*	0.001*

The numbers are p values.
*Welch ANOVA ($p < 0.05$) followed by *post-hoc* Tamhane Test.
MDA, malondialdehyde; NCP, normal weight+generalized chronic periodontitis; NG, normal weight+gingivitis; NH, normal weight+periodontally healthy; OCP, obese+generalized chronic periodontitis; OG, obese+gingivitis; OH, obese+periodontally healthy; PC, protein carbonyl; prot, protein; TAOC, total antioxidant capacity.

crevicular fluid MDA levels to be higher in obese individuals than in normal-weight individuals; however, the difference was statistically significant only between individuals with generalized chronic periodontitis.

To the best of our knowledge, to date, there are no published data on MDA levels in the gingival crevicular fluid or periodontal tissue of obese individuals with different statuses. However, a cross-sectional clinical study conducted by Baydaa and Yas (36), which compared GI, PI, probing depth, clinical attachment level, calculus index and salivary MDA levels of normal-weight, overweight and obese individuals without regard to periodontal status found salivary MDA levels to be significantly higher in obese individuals than in normal-weight and overweight individuals. Recent studies by Selvakumar *et al.* (37) and Bhale *et al.* (38) concluded that increases in serum MDA levels observed in obese individuals, regardless of periodontal status, were reflective of increases in OS as a result of obesity. These results, although obtained from serum, are compatible with the changes observed in our study for gingival crevicular fluid, a biological fluid found in the gingival sulcus that originates from blood plasma and contains metabolic elements of bacteria and host cells defined as transudate or exudates (39).

In our study, the concentrations and total amounts of PC in gingival crevicular fluid were significantly higher in individuals with chronic periodontitis than in individuals with gingivitis and in periodontally healthy individuals, with increases in levels

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Table 5. Correlations between periodontal clinical assessments and biochemical parameters in normal-weight individuals (n = 45)

	Periodontal parameters				
Biochemical parameters	PI	GI	BOP (%)	PD (mm)	CAL (mm)
MDA conc. (μM/L)	0.824*	0.814*	0.894*	0.778*	0.764*
MDA total (μM)	0.777*	0.881*	0.834*	0.969*	0.968*
PC conc. (pM/mL)	0.816*	0.886*	0.855*	0.963*	0.962*
PC total (pM)	0.733*	0.845*	0.772*	0.963*	0.970*
PC (pM/mg prot)	0.825*	0.895*	0.861*	0.975*	0.969*
TAOC conc. (μM/mL)	−0.911*	−0.936*	−0.975*	−0.883*	−0.856*
TAOC total (μM)	−0.809*	−0.844*	−0.891*	−0.756*	−0.721*

Values are given as r.
*Pearson Correlation Test, significant at the 0.01 level.
BOP, bleeding on probing; CAL, clinical attachment level; conc., concentration; GI, gingival index; PC, protein carbonyl; PD, probing depth; PI, plaque index; prot, protein; MDA, malondialdehyde; TAOC, total antioxidant capacity.

Table 6. Correlations between periodontal clinical assessments and biochemical parameters in obese individuals (n= 48)

	Periodontal parameters				
Biochemical parameters	PI	GI	BOP (%)	PD (mm)	CAL (mm)
MDA conc. (μM/L)	0.907*	0.870*	0.911*	0.886*	0.878*
MDA total (μM)	0.832*	0.810*	0.792*	0.976*	0.975*
PC conc. (pM/mL)	0.865*	0.844*	0.836*	0.955*	0.949*
PC total (pM)	0.790*	0.781*	0.736*	0.973*	0.972*
PC (pM/mg prot)	0.863*	0.845*	0.828*	0.963*	0.962*
TAOC conc. (μM/mL)	−0.921*	−0.899*	−0.955*	−0.839*	−0.825*
TAOC total (μM)	−0.870*	−0.874*	−0.889*	−0.914*	−0.901*

Values are given as r.
*Pearson Correlation Test, significant at the 0.01 level.
BOP, bleeding on probing; CAL, clinical attachment level; conc., concentration; GI, gingival index; PC, protein carbonyl; PD, probing depth; PI, plaque index; prot, protein; MDA, malondialdehyde; TAOC, total antioxidant capacity.

occurring in line with increases in periodontal pathology in both obese and normal-weight groups. A cross-sectional clinical study by Baltacıoğlu *et al*. (14) reported that the levels of PC in gingival crevicular fluid and serum of individuals with chronic periodontitis were significantly higher than those of periodontally healthy individuals and suggested increases in PC levels to be a sign of oxidative damage caused by periodontal disease. Similarly, other researchers also reported the levels of PC in both saliva and gingival crevicular fluid to be significantly higher in individuals with chronic periodontitis compared with periodontally healthy individuals (16,40).

In line with our findings for MDA, our study also found the levels of PC in gingival crevicular fluid of obese individuals to be higher than those of normal-weight individuals with the same periodontal status, although the difference was only significant between the groups with chronic periodontitis. As this is the first study to differentiate gingival crevicular fluid PC levels in obese individuals according to periodontal status, it is not possible to compare our results with those of other studies. However, clinical studies by Atabek *et al*. (41), Krzystek-Korpacka *et al*. (42) and Kolyva *et al*. (43), which measured the levels of PC in serum of obese and normal-weight individuals in order to evaluate oxidative damage caused by obesity, reported significant increases in advanced protein oxidation products in obese individuals and concluded that obesity causes protein oxidation. Furthermore, a number of experimental animal studies have found PC levels of obese rats to increase following increases in tissue and serum OS, but to decrease significantly following weight loss (44,45). These findings demonstrate obesity to have a significant effect on protein oxidation, selectively causing oxidative damage to proteins. Our findings are in line with these earlier studies.

Numerous studies have reported that periodontal disease impairs the balance between oxidants and antioxidants and have attributed this imbalance to decreases in antioxidant capacity in gingival crevicular fluid serum and saliva (14,15,17,46,47). In contrast, a cross-sectional clinical study by Su *et al*. (40) reported salivary TAOC to be higher in individuals with periodontitis than in periodontally healthy individuals; the authors attributed this finding to an increase in antioxidant response as a local response to an increase in OS occurring with periodontal pathology. In the present study, statistically significant decreases in the concentrations and total amounts of TAOC in gingival crevicular fluid were found in conjunction with increases in periodontal destruction among both obese and normal-weight individuals. These periodontal-status-related alterations are in line with previous studies suggesting that the progression of periodontal disease impairs the balance between oxidants and antioxidants, thereby reducing the capacity of antioxidants to regulate OS.

The concentrations and total amounts of TAOC in gingival crevicular fluid were also found to be lower in obese individuals when compared with normal-weight individuals with the same periodontal status, with the differences statistically significant for those with chronic periodontitis. To the best of our knowledge, there is no published study evaluating the relationship between gingival crevicular fluid TAOC levels and periodontal clinical assessments in the combined presence of obesity and periodontal disease. The generally lower TAOC levels found in the gingival crevicular fluid of obese individuals when compared with normal-weight individuals

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in this study suggests that decreases in TAOC reflect increases in OS caused by an impairment of the balance between oxidants and antioxidants caused by obesity.

The present study also found significant, positive correlations between the levels of MDA and PC in gingival crevicular fluid and PI, GI, BOP, probing depth and clinical attachment level measurements in both obese and normal-weight individuals. These correlations are consistent with earlier studies reporting an association between periodontal-disease-related tissue destruction and increases in saliva and gingival crevicular fluid MDA levels (13,15,18) and gingival crevicular fluid PC levels (14) in systemically healthy normal-weight individuals. To the best of our knowledge, this study is one of a limited number of studies examining the relationship between obesity, OS and periodontal status using a combination of clinical assessment and biochemical analysis, and it is the first study to evaluate OS markers in gingival crevicular fluid of obese individuals with varying periodontal status. The higher levels of MDA and PC in gingival crevicular fluid found in obese individuals when compared with normal-weight individuals, together with the positive correlations between these levels and clinical periodontal assessments, suggest obesity to be a modifying factor in the increased tissue destruction occurring within the periodontal disease process by disrupting the oxidant/antioxidant balance in diseased periodontal tissue. This idea is supported by numerous previous studies that have assessed obesity as a state of 'increased chronic oxidative stress' (4). For example, one experimental animal study reported obesity to produce an exaggerated inflammatory response that was a contributing factor in periodontal tissue destruction of rats with experimental periodontitis (48), and two other studies with rats suggested that obesity can cause tissue to exhibit an exaggerated inflammatory response (49,50). Moreover, several studies have reported obesity to be associated with an increase in OS markers and a decrease in antioxidants, which could potentially increase the severity of tissue destruction caused by periodontal disease (30,51,52).

In contrast to the positive correlations found for oxidative markers, the present study found significant, negative correlations between the concentrations and total amounts of TAOC in gingival crevicular fluid and PI, GI, BOP, probing depth and clinical attachment level measurements in both obese and normal-weight individuals. Some studies comparing obese and normal-weight individuals (regardless of periodontal status) have also reported serum TAOC to correlate negatively with BMI (53,54). However, another study by Kwak & Yoon (55) reported a positive correlation between TAOC and BMI, suggesting that despite a well-documented connection between obesity and increases in OS (6), not all stress-inducing conditions will lead to reductions in TAOC.

Baltacıoğlu *et al*. (18) reported a negative correlation between TAOC levels and PI, GI, probing depth, clinical attachment level and gingival bleeding index measurements in the serum and saliva of individuals with periodontitis, as well as in periodontally healthy individuals (without considering BMI). Several studies have demonstrated an increase in products of oxidative damage in the peripheral blood of individuals with periodontitis compared with periodontally healthy controls (14,56), and there is extensive evidence of decreased antioxidant capacity in individuals with periodontitis (13,46,57). Thus, it might be argued that a more pro-oxidative state and diminished antioxidant capacity in obese individuals could facilitate the onset of an aggravated periodontal-disease process. This argument is in line with the increase in production of reactive oxygen species in periodontal tissue of obese animals found in the previously mentioned experimental studies (48–50).

In conclusion, the combination of obesity and periodontal disease could facilitate a pro-oxidative state that would diminish the antioxidant capacity of periodontal tissue. Oxidative stress can be the common link between the mutually effective conditions of obesity and periodontal disease. This would explain the increases in the levels of MDA and PC and the decreases in the levels of TAOC in gingival crevicular fluid found in our study for obese individuals in comparison with normal-weight individuals. Moreover, the fact that the differences in gingival crevicular fluid analyses between obese and normal-weight individuals were significant only for those with chronic periodontitis indicates that although obesity may modulate remarkable pathological changes in periodontal tissues, this should not be considered as a solitary factor sufficient for the initiation of periodontal deterioration.

Within the limits of the present study, obesity may be described as a state in which systemic, low-grade inflammatory stimulus can produce OS. In the presence of sufficient primary etiological factors capable of inducing periodontal disease, obesity may exacerbate periodontal tissue destruction and disease severity owing to an obesity-induced pro-oxidant status in periodontal tissue.

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