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Glutathione levels in plasma, saliva and gingival crevicular fluid after periodontal therapy in obese and normal weight individuals

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Background and objective: The purpose of this study was to investigate the effects of obesity on reduced and oxidized glutathione (GSH and GSSG) levels in the gingival crevicular fluid, plasma and saliva of patients with chronic periodontitis and to evaluate the changes after nonsurgical periodontal therapy.

Material and methods: The study included 60 patients: 30 patients with chronic periodontitis (15 obese patients and 15 normal weight patients) and 30 healthy control subjects (15 obese patients and 15 normal weight patients). Gingival crevicular fluid, plasma and saliva samples were collected, and clinical periodontal measurements were recorded at baseline and at the first month after periodontal therapy from patients with chronic periodontitis. GSH and GSSG levels were analyzed with spectrophotometry.

Results: The GSH levels in the plasma, saliva and gingival crevicular fluid in obese individuals with chronic periodontitis were lower than in normal weight individuals at baseline ($p < 0.01$). There was a significant difference in the GSH/GSSG ratio in plasma and gingival crevicular fluid between the obese and normal weight groups at baseline ($p < 0.01$). The GSH levels in plasma, gingival crevicular fluid and saliva were significantly increased in both chronic periodontitis groups after nonsurgical periodontal therapy ($p < 0.01$). A significant positive correlation was found between GSH levels in saliva, plasma and gingival crevicular fluid in all groups ($p < 0.001$).

Conclusions: The study revealed that obesity in patients with chronic periodontitis is associated with decreased GSH levels and the GSH/GSSG ratio. Moreover, nonsurgical periodontal therapy may be helpful for improvement in glutathione values in obese and normal weight individuals with chronic periodontitis.

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Obesity is a medical condition characterized by the abnormal or excessive deposition of fat in the adipose tissue, which may have an adverse effect on health (1). Obesity is a risk factor for several chronic diseases such as coronary heart diseases, type 2 diabetes, hypertension, dyslipidemia and recent oral diseases (particularly

periodontitis) (1–3). Obesity can cause and affect systemic oxidative stress, which is the imbalance between the production of reactive oxygen species (ROS) and antioxidant defenses (4). Thus, oxidative stress might be a major mechanism underlying obesity-related complications (5). In addition, obesity may be defined as a state of chronic oxidative stress and recently as a disruption of redox signaling and control (6).

Periodontitis is a chronic inflammatory disease that results in tissue destruction caused by periodontopathogens and long-term release of ROS (7). A recent review showed that increased oxidative stress and decreased antioxidative status play a critical role in the progression of periodontal disease (7).

Reduced glutathione (GSH) is the most important intracellular antioxidant for ROS detoxification and a good marker for investigating the antioxidant defense mechanism in chronic inflammatory diseases caused by oxidative stress (8,9). Generally, oxidative stress based on redox status is examined by determining the glutathione redox ratio (reduced/oxidized glutathione, GSH/GSSG) (10). Previous studies have reported that GSH levels decreased in patients with periodontitis in the gingival crevicular fluid, plasma and saliva when compared to healthy subjects, and the GSH levels increased after therapy (11–15). The GSH level is expected to be inversely related to the severity of the periodontal disease (15). In addition, Barnes *et al*. (16) reported that increased levels of GSSG in saliva were related with increased oxidative stress in the patients with periodontal disease.

Albuali (5) found that GSH concentrations in the blood in obese individuals were lower than those of normal weight control individuals. Tomofuji *et al*. (17) showed that the GSH/GSSG ratio in gingival tissues decreased in obese rats compared to lean rats. Similar results were reported in obese rats fed with a high-fat diet without exercise training (18). It is suggested that local alterations in the redox balance and a systemic increase in ROS following obesity may induce gingival oxidative damage, and this may lead to progression of periodontal inflammation (17,18). However, to the best of our knowledge, no literature data are available that show the effect of obesity on glutathione values in patients with periodontitis. We hypothesized that high ROS levels in circulation may decrease GSH levels in the gingival crevicular fluid, plasma and saliva in obese patients with periodontal disease and, thus, that periodontal therapy could have positive effects on these levels. The aim of this study was to evaluate the clinical periodontal parameters and to analyze GSH and GSSG levels, the GSH/GSSG ratio in saliva, gingival crevicular fluid and plasma in obese and normal weight patients with chronic periodontitis at baseline and after nonsurgical periodontal therapy.

Material and methods

Study population

The study protocol was approved by the Ethics Committee of the Faculty of Medicine (2013-25-12/02), Bulent Ecevit University, Turkey, in accordance with the 1975 Declaration of Helsinki, as revised in 2002. Individuals were informed about the protocol of the study and gave their written consent for the procedures. This study is registered at ClinicalTrials.gov as NCT02498561. Individuals were selected from a population who 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) (19) and waist/hip ratio (WHR) (20). The BMI was categorized using the World Health Organization classification: normal weight was equated to BMI = 18.50–24.99 kg/m^2, obese $\geq$ 30 kg/m^2 (19). Obesity was defined as WHR $\geq$ 0.85 for women and $\geq$ 0.90 for men while normal weight was defined as WHR lower than those for obesity were (20). Sixty individuals fulfilled the inclusion criteria (30 obese and 30 normal weight). The obese and normal weight groups were categorized into two subgroups, i.e., (i) clinically periodontally healthy, and (ii) generalized chronic periodontitis. The diagnosis was based on their periodontal conditions according to the criteria proposed by the 1999 International World Workshop for a Classification of Periodontal Diseases and Conditions (21). The study included 15 obese individuals with chronic periodontitis (OCP; eight men and seven women, aged 34–60 years; mean age: 47.13 $\pm$ 7.17 years), 15 normal weight individuals with chronic periodontitis (eight men and seven women, aged 26–51 years; mean age: 38.47 $\pm$ 7.50 years), 15 obese individuals without periodontal disease (OPH; seven men and eight women, aged 25–50 years; mean age: 41.33 $\pm$ 6.47 years), and 15 normal weight individuals without periodontal disease (PH; eight men and seven women, aged 30–50 years; mean age: 37 $\pm$ 7.40 years).

Clinical measurements

The periodontal status of the patients was determined by measuring the probing depth, clinical attachment level, gingival index (GI) (22), bleeding on probing (BOP) (23) and plaque index (PI) (24). The level of periodontal bone loss was determined with full-mouth periapical radiographs. All 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 millimeters by the same examiner (FOD).

Inclusion criteria

Inclusion criteria for the patients were as follows: (i) never-smokers; (ii) no history of systemic disease; (iii) no patients had been under periodontal treatment and medicine for at least

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6 mo before the study; (iv) no pregnancy or lactation; (v) no diabetes mellitus (subjects who had glycated hemoglobin A1c [HbA1c] levels < 5.7%) or any other chronic inflammatory disease or infection; (vi) no alcohol or antioxidant vitamin consumption; (vii) possess ≥ 20 teeth excluding third molars and teeth with advanced decay; (viii) GI = 0, probing depth ≤ 3 mm, and no signs of bone loss by clinical and radiographic examination for the periodontally healthy groups; and (ix) clinical signs of inflammation (red color and swelling of the gingival margin) GI ≥ 2, probing depth and clinical attachment level ≥ 5 mm and bone loss affecting > 30% of the existing teeth on clinical and radiographic examination for the chronic periodontitis groups.

Collection of samples

All samples were obtained in the morning following overnight fasting, during which patients were requested not to drink (except water) or eat. Before the samples were collected, the individuals were checked for protocol adherence.

Whole saliva samples were collected before the gingival crevicular fluid samples and the clinical periodontal measurements.

Patients' mouths were rinsed with distilled water. Then they were seated comfortably with eyes open, head tilted. Collection of unstimulated saliva samples was started after five min. Subjects were instructed to spit into plastic tubes five times per min for 5 min. About 2 mL whole saliva was centrifuged immediately to remove cell debris (10,000 $g \times 10$ min at 4°C). The supernatants (50 μL each) were stored at −40°C until analyzed.

To prevent contamination of the gingival crevicular fluid with blood associated with the probing of inflamed sites, the gingival crevicular fluid samples were collected 2 d after the clinical measurements and sampling site selections, in the morning between 08:00 and 10:00 h. Gingival crevicular fluid samples were collected from a mesiobuccal and distopalatal site on each tooth (molars, premolars, canines/incisors). In the chronic periodontitis group, the samples were obtained from patients in areas with ≥ 5 mm clinical attachment level, ≥ 6 mm probing depth and ≥ 30% bone loss. In the healthy group, gingival crevicular fluid samples were collected from teeth that exhibited probing depth < 3 mm without clinical attachment level and BOP. Six gingival crevicular fluid samples were collected from each patient. The sample area was isolated with cotton rolls, saliva contamination elimination was ensured and it was slightly air-dried. Gingival crevicular fluid was sampled with paper strips (Periopaper; Ora Flow Inc., Amityville, NY, USA). Paper strips were placed in the crevice until mild resistance was felt (intracrevicular method) and left in position for 30 s (26). Strips contaminated with blood or saliva were discarded. Each sampled strip was placed in a disposable tube and stored at −40°C until analyzed. For laboratory analysis, 200 μL phosphate-buffered saline (pH 7.4) was added to each tube containing a sampled strip. Centrifugation was carried out at 10,000 × g for 5 min, and then supernatant was used to determine the GSH and GSSG levels.

Five milliliters of venous blood was taken from the antecubital vein using a standard venipuncture method. The blood sample was collected in vacutainer tubes and anticoagulated with EDTA. The blood samples were centrifuged at 1000 g for 10 min at 4°C to obtain plasma, and the upper plasma phase was drawn with a pipette, transferred into disposable tubes and stored at −40°C until analyzed.

Biochemical analysis

The GSH and GSSG levels of the gingival crevicular fluid, saliva and plasma samples were determined with the commercially available optimized enzyme recycling method Glutathione Assay Kit (item no. 703002; Cayman Chemical Company, Ann Arbor, MI, USA). The data were expressed as μM/L. The mean interassay coefficient of variation percentage and the intra-assay coefficient of variation percentage were 3.6% and 1.6%, respectively. The dynamic range of the kit is 0–16 μM GSH and 0–8 μM GSSG. The samples, which have shown higher concentrations, were diluted and measured in duplicate. Finally, the concentrations were multiplied by the dilution factor according to the manufacturer's instructions.

Periodontal treatment

All patients were motivated and instructed in daily plaque control. Nonsurgical periodontal therapy was performed on patients with chronic periodontitis. Periodontal therapy for patients were performed at different periods appropriate to their periodontal condition with the mean frequency of four recall visits for 4 wk. Treatment included an intensive hygiene phase, full-mouth scaling and root planing (performed in all four quadrants), and maintenance and monitoring of oral hygiene. Clinical data and samples were obtained at baseline and at 1 mo after the final scaling and root planing appointment.

Statistical analyses

The primary outcome variable (change in gingival crevicular fluid GSH and GSSG levels) was used to decide the sample size calculation and determine the power of the study. We based our estimates on the pilot study, which included 10 patients in each group. We estimated that a sample size of 13 patients in each group would allow for a type II error level of $\beta = 0.20$ (80% power) and a type I error level of $\alpha = 0.05$ (5% probability). To account for possible dropouts, we included 15 patients in each group.

Statistical analysis was performed using a commercially available software program (SPSS 19.0; IBM Corp., Armonk, NY, USA). The Shapiro–Wilk test was used to investigate whether the data were normally distributed. Continuous variables with unequal variances were compared with Welch and Tamhane's T2 *post hoc* test for the BMI, GSH and GSSG

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levels, and GSH/GSSG ratio. The comparison of the age, probing depth, clinical attachment level, GI, PI, BOP and HbA1c was analyzed using the Kruskal–Wallis nonparametric test followed by *post hoc* group comparisons with the Bonferroni-adjusted Mann–Whitney *U*-test. The percentage differences between the chronic periodontitis groups for changes of gingival crevicular fluid GSH levels, GSSG saliva levels, gingival crevicular fluid GSH/GSSG ratios and BMI values were analyzed by the independent samples *t*-test; plasma GSH levels, saliva GSH levels, GSSG plasma levels, GSSG gingival crevicular fluid levels, plasma and saliva GSH/GSSG ratios were analyzed by Mann–Whitney *U*-test. A paired Student's *t*-test or a Wilcoxon rank-sum test was used to compare the measurements at two points (baseline and after therapy). The Spearman's rank correlation test was also used to detect the relationship between the biochemical and clinical findings.

Results

Clinical findings

Clinical findings are summarized in Table 1. BMI and WHR values were significantly higher in individuals with obesity than in normal weight individuals ($p < 0.05$), and there were no significant differences after therapy ($p > 0.05$). The full-mouth probing depth, clinical attachment level, BOP, PI and GI were statistically higher in the chronic periodontitis groups than PH groups ($p < 0.05$). The mean probing depth and clinical attachment level statistically significant decreased in the chronic periodontitis groups after nonsurgical periodontal therapy ($p < 0.05$) (Table 1). There was no significant difference in levels of HbA1c among all groups.

Biochemical findings

In all groups, the mean GSH and GSSG values were detected in the micromolar (μM) range. The GSH levels in gingival crevicular fluid, plasma and saliva were found to be significantly lower in both chronic periodontitis groups (gingival crevicular fluid; OCP: 6.62 ± 0.85 μM; chronic periodontitis: 9.31 ± 0.82 μM; plasma; OCP: 7.85 ± 1.47 μM; chronic periodontitis: 14.35 ± 1.07 μM; saliva; OCP: 9.41 ± 1.06 μM; chronic periodontitis: 12.12 ± 2.50 μM) compared with both PH groups (plasma; OPH: 24.60 ± 2.23 μM; PH: 33.73 ± 2.01 μM; gingival crevicular fluid; OPH: 25.58 ± 2.68 μM; PH: 35.70 ± 2.83 μM; saliva; OPH: 15.82 ± 1.50 μM; PH: 16.23 ± 1.23 μM) and these values were lower in the OCP group than the chronic periodontitis group at baseline ($p < 0.01$; Fig. 1). The plasma and gingival crevicular fluid GSH levels in the PH groups were significantly different from those of the OPH and chronic periodontitis groups at baseline ($p < 0.01$; Fig. 1). In both chronic periodontitis groups, GSH levels after periodontal therapy increased in plasma, saliva and gingival crevicular fluid compared to baseline ($p < 0.01$; Fig. 1).

No significant difference was found between the chronic periodontitis and OCP groups in the GSSG levels in saliva, plasma and gingival crevicular fluid at baseline ($p > 0.05$; Fig. 2). The plasma levels of GSSG were significantly lower in the PH group compared to the OPH, chronic periodontitis and OCP groups at baseline ($p < 0.05$; Fig. 2). The gingival crevicular fluid levels of GSSG were significantly higher in the OCP group compared to the PH and OPH groups and in the chronic periodontitis group compared to the PH group at baseline ($p < 0.05$; Fig. 2). In both chronic periodontitis groups, all GSSG levels reduced after periodontal therapy compared to baseline ($p < 0.05$; Fig. 2).

The GSH/GSSG ratio in plasma (OCP: 0.32 ± 0.17; chronic periodontitis: 0.58 ± 0.12) and gingival crevicu-

Table 1. Clinical characteristics of the study groups

Parameters		OCP ($n = 15$)	OPH ($n = 15$)	CP ($n = 15$)	PH ($n = 15$)
BMI (kg/m^2)		35.80 ± 3.89	36.95 ± 5.18	22.87 ± 1.03[a]	22.26 ± 1.67[a]
WHR		1.0 ± 0.20	0.98 ± 0.05	0.81 ± 0.10[a]	0.80 ± 0.07[a]
HbA1c (%)		5.18 ± 0.26	5.17 ± 0.31	5.19 ± 0.28	5.02 ± 0.22
PI	Baseline	2.40 ± 0.40[b]	0.68 ± 0.20	2.44 ± 0.30[b]	0.20 ± 0.19
	After SRP	0.89 ± 0.31[c]	NA	0.62 ± 0.29[c]	NA
GI	Baseline	2.39 ± 0.41[b]	0.00 ± 0.00	2.12 ± 0.21[b]	0.00 ± 0.00
	After SRP	0.84 ± 0.38[c]	NA	0.92 ± 0.28[c]	NA
BOP (%)	Baseline	90.26 ± 7.23[b]	0.00 ± 0.00	90.84 ± 6.62[b]	0.00 ± 0.00
	After SRP	38.41 ± 18.20[c]	NA	27.03 ± 13.20[c]	NA
PD (mm)	Baseline	4.34 ± 1.08[b]	1.46 ± 0.16	3.82 ± 1.23[b]	1.48 ± 0.14
	After SRP	3.82 ± 1.05[c]	NA	3.22 ± 1.07[c]	NA
CAL (mm)	Baseline	5.02 ± 1.16[b]	1.46 ± 0.16	4.65 ± 1.33[b]	1.48 ± 0.14
	After SRP	4.23 ± 0.92[c]	NA	4.01 ± 1.05[c]	NA

BMI, body mass index; BOP, bleeding on probing; CAL, clinical attachment level; CP, normal weight individuals with chronic periodontitis; GI, gingival index; HbA1c, glycated hemoglobin A1c; NA, not applicable; OCP, obese individuals with chronic periodontitis; OPH, obese individuals without periodontal diseases; PD, probing depth; PH, normal weight individuals without periodontal diseases; PI, plaque index; SRP, scaling and root planing; WHR, waist/hip ratio.

Data are expressed as the mean ± standard deviation.

[a]Statistically significant difference from obese groups ($p < 0.05$).

[b]Statistically significant difference from periodontal healthy control groups ($p < 0.05$).

[c]Statistically significant difference from baseline ($p < 0.05$).

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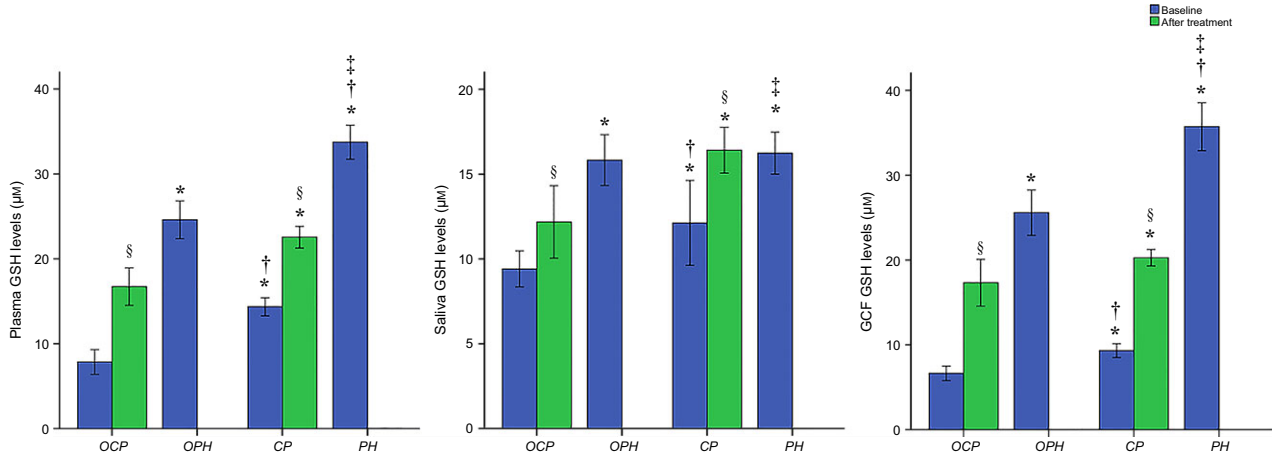

Fig. 1. Comparison of the plasma, GCF and saliva levels of GSH (μM) in normal weight patients with CP (baseline and after therapy), obese patients with chronic periodontitis (baseline and after therapy) and healthy controls. *Statistically significant difference from OCP (Welch and Tamhane's T2 *post-hoc* test). †Statistically significant difference from OPH (Welch and Tamhane's T2 *post-hoc* test). ‡Statistically significant difference from CP (Welch and Tamhane's T2 *post-hoc* test). §Statistically significant difference from baseline (paired samples *t* test). Data are presented as bar graphs with mean and standard deviation. CP, normal weight individuals with chronic periodontitis; GCF, gingival crevicular fluid; GSH, reduced glutathione; OCP, obese individuals with chronic periodontitis; OPH, obese individuals without periodontal diseases; PH, normal weight individuals without periodontal diseases.

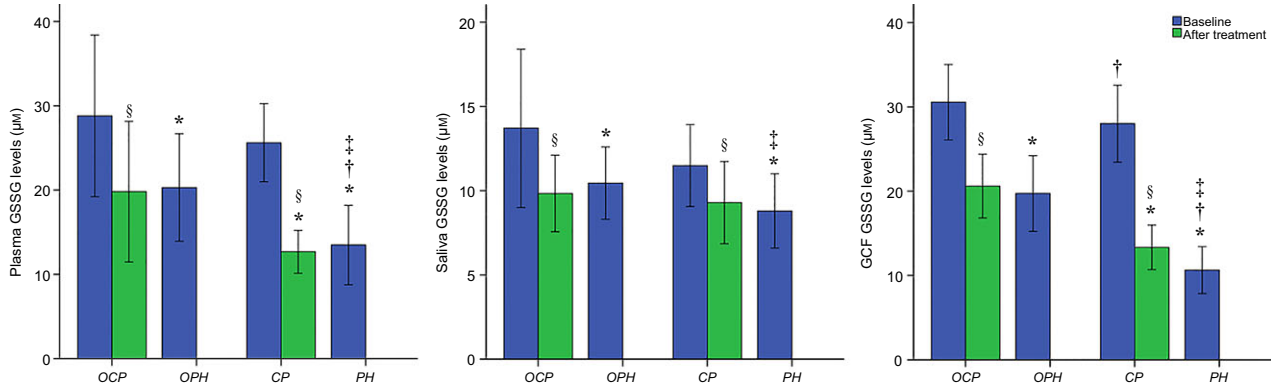

Fig. 2. Comparison of the plasma, GCF and saliva levels of GSSG (μM) in normal weight patients with CP (baseline and after therapy), obese patients with CP (baseline and after therapy) and healthy controls. *Statistically significant difference from OCP (Welch and Tamhane's T2 *post-hoc* test). †Statistically significant difference from OPH (Welch and Tamhane's T2 *post-hoc* test). ‡Statistically significant difference from CP (Welch and Tamhane's T2 *post-hoc* test). §Statistically significant difference from baseline (paired samples *t* test). Data are presented as bar graphs with mean and standard deviation. CP, normal weight individuals with chronic periodontitis; GCF, gingival crevicular fluid; GSSG, oxidized glutathione; OCP, obese individuals with chronic periodontitis; OPH, obese individuals without periodontal diseases; PH, normal weight individuals without periodontal diseases.

lar fluid (OCP: 0.22 ± 0.05; chronic periodontitis: 0.34 ± 0.08) in both chronic periodontitis groups were significantly decreased compared with both PH groups (plasma; OPH: 1.31 ± 0.37; PH: 2.85 ± 1.13; gingival crevicular fluid; OPH: 1.37 ± 0.38; PH: 3.68 ± 1.43) and a significant difference was found between both of the chronic periodontitis groups at baseline ($p < 0.01$; Fig. 3). The saliva GSH/GSSG ratio in both chronic periodontitis groups (OCP: 0.76 ± 0.28; chronic periodontitis: 1.10 ± 0.34; $p < 0.05$) were lower than in both PH groups (OPH: 1.58 ± 0.35; PH: 1.95 ± 0.46), but the difference between the chronic periodontitis and OCP groups did not reach statistical significance at baseline ($p > 0.05$; Fig. 3). All GSH/GSSG ratios after nonsurgical periodontal therapy increased in both chronic periodontitis groups compared to baseline ($p < 0.01$; Fig. 3).

The percentage of changes in plasma GSH levels (120.59%; 61.95%), gingival crevicular fluid GSH levels (160.79%; 125.15%), GSSG levels (29.07%; 51.73%) and GSH/GSSG ratios (286.44%; 390.06%) in the OCP group were significantly different from the chronic periodontitis group ($p < 0.05$), whereas no significant differences were observed in BMI (1.11%; 1.74%), respectively ($p > 0.05$; Fig. 4).

Correlations

When all groups were examined together, there was a strong positive

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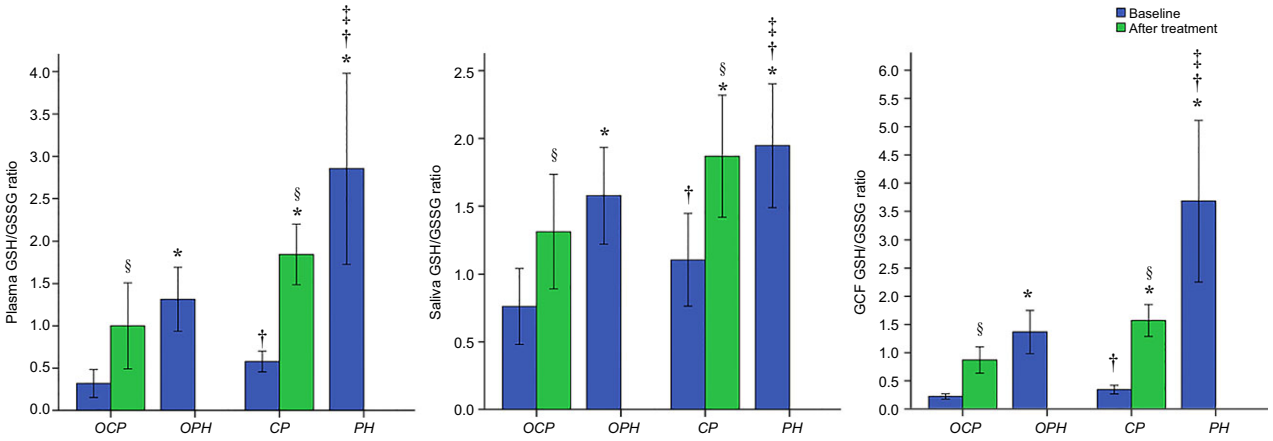

Fig. 3. Comparison of the plasma, GCF and saliva glutathione redox ratio (GSH/GSSG) in normal weight patients with CP (baseline and after therapy), obese patients with CP (baseline and after therapy) and healthy controls. *Statistically significant difference from OCP (Welch and Tamhane's T2 *post-hoc* test). †Statistically significant difference from OPH (Welch and Tamhane's T2 *post-hoc* test). ‡Statistically significant difference from CP (Welch and Tamhane's T2 *post-hoc* test). §Statistically significant difference from baseline (paired samples *t* test). Data are presented as bar graphs with mean and standard deviation. CP, normal weight individuals with chronic periodontitis; GCF, gingival crevicular fluid; GSH/GSSG, reduced/oxidized glutathione ratio; OCP, obese individuals with chronic periodontitis; OPH, obese individuals without periodontal diseases; PH, normal weight individuals without periodontal diseases.

correlation among the GSH of gingival crevicular fluid, saliva and plasma ($p < 0.001$; Table 2). Strong negative correlations reached between the gingival crevicular fluid GSH and GSSG, between the saliva GSH and GSSG, between the plasma GSH and GSSG ($p < 0.001$; Table 2). In addition, GI and clinical attachment level values were correlated with the GSH and GSSG levels ($p < 0.05$; Table 2).

Discussion

There are many reports on the relationship between obesity and periodontal disease, and studies have suggested that obesity could be a major risk factor for periodontal disease (27–31). However, the biological mechanisms of obesity on the periodontium remain unclear. One of the mechanisms of obesity is probably related to increasing gingival inflammation through disruption of the redox balance and to increasing the ROS levels in blood (28). For the first time, we evaluated the effect of nonsurgical periodontal therapy on the levels of GSH, GSSG and the GSH/GSSG ratio in plasma, saliva and gingival crevicular fluid in obese individuals with periodontal disease. The results demonstrated that the GSH levels and the GSH/GSSG ratio were significantly lower in obese individuals with periodontal disease than in normal weight individuals, and the levels significantly increased after therapy for both periodontitis groups.

In this study, gingival crevicular fluid and whole unstimulated saliva, which are useful in the determination of tissue destruction, were collected. Stimulation of saliva can result in an increase in the volume and concentration of saliva and in an increase in the amount of gingival crevicular fluid from periodontal pockets (32). Accordingly, it was suggested to impair the antioxidant concentration

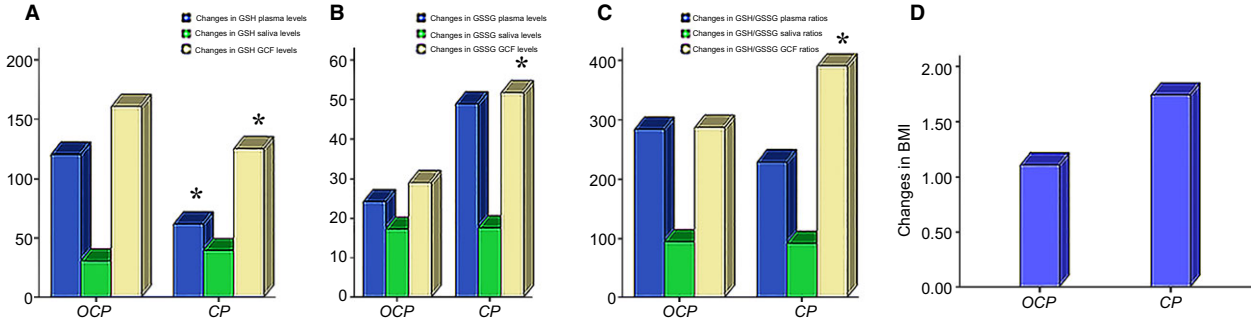

Fig. 4. Percentage of changes after periodontal therapy in (A) GSH levels, (B) GSSG levels, (C) GSH/GSSG ratio and (D) BMI. *Statistically significant difference from OCP. BMI, body mass index; CP, normal weight individuals with chronic periodontitis; GSH, reduced glutathione; GSH/GSSG, reduced/oxidized glutathione ratio; GSSG, oxidized glutathione; OCP, obese individuals with chronic periodontitis.

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Table 2. The Spearman's rank correlation (*r*) among groups with respect to BMI, CAL, GI, GSH and GSSG levels in all subjects

		GI	CAL	GCF-GSH	P-GSH	S-GSH	GCF-GSSG	P-GSSG	S-GSSG
BMI	*r*	0.059	0.032	−0.240	−0.344	−0.214	0.278	0.246	0.206
	p	0.653	0.808	0.065	0.007	0.100	0.031	0.059	0.115
CAL	*r*	0.860		−0.838	−0.813	−0.723	0.661	0.444	0.386
	p	< 0.001		< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.002
GCF-GSH	*r*	−0.920	−0.838		0.960	0.795	−0.869		
	p	< 0.001	< 0.001		< 0.001	< 0.001	< 0.001		
P-GSH	*r*	−0.903	−0.813	0.960		0.816		−0.658	
	p	< 0.001	< 0.001	< 0.001		< 0.001		< 0.001	
S-GSH	*r*	−0.823	−0.723	0.795	0.816				−0.488
	p	< 0.001	< 0.001	< 0.001	< 0.001				< 0.001
GCF-GSSG	*r*	0.778	0.661	−0.869				0.705	0.409
	p	< 0.001	< 0.001	< 0.001				< 0.001	0.001
P-GSSG	*r*	0.581	0.444		−0.658		0.705		0.266
	p	< 0.001	< 0.001		< 0.001		< 0.001		0.040
S-GSSG	*r*	0.429	0.386			−0.488	0.409	0.266	
	p	0.001	0.002			< 0.001	0.001	0.040	

BMI, body mass index; CAL, clinical attachment level; GCF, gingival crevicular fluid; GI, gingival index; GSH, reduced glutathione; GSSG, oxidized glutathione; P, plasma; S, salivary.
Statistically significant correlations ($p < 0.05$).

of stimulating saliva (33). In addition, this study is based mainly on the total amount of biomarkers in gingival crevicular fluid because it may be a better and more reliable indicator for diagnostic purposes. The total amount of a constituent in the gingival crevicular fluid instead of its concentration is more appropriate when examining the relationship between the gingival crevicular fluid constituents and periodontal diseases (34). However, we used BMI as the primary measure of adiposity for evaluating the association between obesity and periodontitis (35). BMI and obesity are directly related to the severity, prevalence and development of periodontal disease (36,37).

Glutathione provides powerful antioxidant protection against oxidative damage and plays a crucial role in cellular redox homeostasis (8). Glutathione exists in reduced and oxidized forms. GSSG accumulates inside the cells while GSH is decreased by oxidative stress (9,38). In addition, the authors suggested that chronic inflammatory diseases are associated with a decrease in GSH levels (9). The maintenance of a high and stable GSH/GSSG ratio is essential for cellular survival, and a decrease in this ratio is indicative of oxidative stress (13,15). The GSH and GSSG concentrations in the gingival crevicular fluid were significantly lower in patients with chronic periodontitis than in the control subjects (11,13,39). The authors suggested that suppression of the effect against ROS of gingival crevicular fluid in patients with periodontitis was lower than in healthy controls (13). However, authors have claimed that the gingival crevicular fluid probably contained a locally derived antioxidant from local three sources such as plaque bacteria, neutrophils and crevicular epithelium (11). The GSH levels in saliva and gingival crevicular fluid may be decreased by the GSH metabolism of some periodontopathogen bacteria (40). Our study showed that the GSH levels and the GSH/GSSG ratio in gingival crevicular fluid were significantly decreased in both chronic periodontitis groups compared to both periodontally healthy groups; however, the GSSG levels were elevated at baseline. These findings supported that the GSH levels in gingival crevicular fluid can play an important role in the pathogenesis of periodontitis.

Grant *et al.* (13) found that GSH levels and GSH/GSSG ratio in gingival crevicular fluid increased in patients with periodontitis after periodontal treatment but these changes in the GSH levels did not have a statistical significance. Another study showed a significant improvement in glutathione redox status in patients with chronic periodontitis after non-surgical periodontal therapy (39). In this study, the GSH levels and the GSH/GSSG ratio in gingival crevicular fluid were significantly increased after periodontal therapy while the GSSG levels were significantly decreased in both chronic periodontitis groups. Our data suggest that this change in redox status can be due to a reduction in the amount of GSSG and an increase in the GSH levels through to oxidation of GSH to GSSG within gingival crevicular fluid with the elimination of inflammation after therapy.

Tsai *et al.* (14) reported that saliva GSH concentrations were lower in patients with periodontitis than in control groups and these levels significantly increased after treatment in patients with periodontitis. Otherwise, Panjamurthy *et al.* (12) showed that the plasma GSH concentration decreased in patients with periodontitis. Another study found that serum GSH levels were lower in patients with periodontitis than control groups. They suggested that GSH levels could be related to the severity of periodontal disease (15). Similarly, the present study showed that levels of the GSH in the saliva and plasma were significantly lower in both of the

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chronic periodontitis groups than in the control groups, and GSH levels significantly increased after therapy in the chronic periodontitis groups. These results are consistent with the results of previous studies.

Recent studies have reported that plasma GSH levels and the GSH/GSSG ratio were lower in obese individuals than in normal weight individuals (5,9,10). Furthermore, a significant negative correlation between the GSH concentration and BMI was indicated (9). The authors observed that obesity might cause a chronic increase in ROS and a reduction in cellular antioxidant defense (9). Boesing *et al.* (28) stated that obesity could contribute to the multifactorial effects of periodontitis through disruption of the redox balance and an increase in ROS in the circulation. A lower GSH/GSSG ratio in gingival tissues was reported in obese animal models in spite of higher blood ROS levels (17,18). It was suggested that local alterations in the redox balance were involved in the progression of obesity-induced gingival inflammation (17). Our study showed that the GSH levels were significantly reduced in plasma, saliva and gingival crevicular fluid in the OCP groups compared to the chronic periodontitis groups. When the OPH and PH groups were compared, the GSH levels were reduced, whereas that of GSSG was increased in the plasma and gingival crevicular fluid in obese individuals. In addition, a lower GSH/GSSG ratio in plasma and gingival crevicular fluid was observed in obese individuals compared with normal weight individuals. Moreover, a significant positive correlation was found between GSH levels in saliva, plasma and gingival crevicular fluid and a significant negative correlation between plasma GSH levels and BMI. It is possible that the decreased plasma GSH levels and the GSH/GSSG ratio could occur through a systemic increase in the ROS during obesity and then these values may lead to decreased saliva and gingival crevicular fluid values. Million *et al.* (41) stated a significant association between the increase in some bacterial groups and obesity. Another study reported that dental infection of *Porphyromonas gingivalis* could contribute to endothelial injury in obese mice (42). Saito and Shimazaki (43) suggested that the bacterial flora in dental plaque might differ in obese subjects. Our data also demonstrate that obesity may have an effect on decreased GSH levels by altering the quantity and composition of the bacteria.

We demonstrated that there is a significant difference in all clinical periodontal parameters and markers in both chronic periodontitis groups after therapy. Our data indicated that periodontal therapy could improve local changes in the gingival redox balance as well as resolution of periodontal inflammation in obesity. A recent study showed a significant improvement in all the clinical periodontal parameters in obese and normal weight groups after nonsurgical periodontal therapy (31). They emphasized that obesity did not have a negative impact on the periodontal clinical response after nonsurgical periodontal treatment. Furthermore, Ao *et al.* (42) suggested that periodontal therapy may prevent or minimize the side effects of bacterial infection in obese patients. Our study demonstrated the percentage increase in gingival crevicular fluid and plasma GSH levels of the OCP group compared to the chronic periodontitis group from baseline to post-periodontal treatment, but BMI values did not change. The improvement of lower GSH levels due to increased adiposity can be attributed to resolution of bacterial infection originating from obesity and periodontal therapy. These observations are in accordance with the present study results.

This study has some limitations. First, the limited number of samples may lead to difficulty in determining significant differences between the groups. Second, our study included moderate (obese class I) and severe (obese class II) obese individuals; therefore, our results could not be generalized for more severe cases of obesity (obese class III). Therefore, further studies are needed to clarify the relationship between periodontal inflammation and antioxidants in obese patients by eliminating limitations in long-term studies with larger groups of patients.

Conclusions

The results of this study demonstrate that obesity in patients with chronic periodontitis is associated with decreased GSH levels and GSH/GSSG ratio. Additionally, the GSH levels in gingival crevicular fluid and saliva may be used as a biomarker for detecting periodontal oxidative damage caused by obesity. Nonsurgical periodontal therapy on obesity-induced gingival inflammation leads to significant improvement in glutathione values in patients with chronic periodontitis. Thus, antioxidant-based approaches may be added to periodontal therapy to reduce the effect of obesity on glutathione redox status in patients with chronic periodontitis. Further long-term studies are needed to clarify the efficacy of antioxidant therapies in obese patients with chronic periodontitis.

Acknowledgements and conflicts of interest

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