ORIGINAL ARTICLE

The effect of obesity on total antioxidant/oxidant status and oxidative stress index in patients with chronic periodontitis

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Key Words
Obesity, Oxidative stress, Periodontitis, Total antioxidant status

Abstract
Objective: The aim of this study was to evaluate the possible effects of obesity on the local and systemic total oxidant status (TOS), total antioxidant status (TAS), and oxidative stress index (OSI) in patients with chronic periodontitis.
Methods: The study included 88 subjects assigned to four groups of 22 subjects each, as follows: O+CP+ (patients with obesity and periodontitis); O-P+ (patients with normal weight and periodontitis); O+P- (periodontally healthy patients with obesity); and the control group consisting of periodontally healthy patients with normal weight (O-P-). Serum and salivary samples were obtained a week before the recording of clinical periodontal parameters. Local and systemic TOS and TAS levels were determined biochemically.
Results: TOS and OSI levels were higher and TAS levels were lower in the serum and saliva of the obese individuals with periodontitis. However, TAS, TOS and OSI levels were found to be comparable in patients with obesity or periodontitis.
Conclusion: Our results suggest that obesity might play a destruction provoking role in the pathogenesis of periodontitis by negatively affecting oxidative parameters.

INTRODUCTION

Periodontitis is a specific chronic inflammatory disease characterized by periodontal tissue destruction and loss, and it occurs as a result of the complex interactions between periodontopathogenic bacteria and host immune-inflammatory response [1]. However, although bacteria are the primary factor in the development of the disease, their presence alone is insufficient to explain the occurrence and severity of the disease. While bacteria cause direct tissue destruction with the various toxic compounds and enzymes they secrete, their actual effect is the indirect stimulation and modification of the host response. Various systemic, environmental, and genetic factors such as stress, smoking, and obesity may affect this interaction between the pathogen bacteria and host response [1, 2].

Obesity, a condition that results from excess adipose tissue, has become a major global health problem. Obesity, particularly central obesity, is a significant risk factor in the development of diabetes mellitus (DM), dyslipidemia, cardiovascular diseases, hypertension, non-alcoholic fatty liver disease, as well as cancer [3, 4]. Recent studies have shown that obesity is also related to oral diseases, especially periodontitis [5-7].

It is known that both periodontitis [8] and obesity [9] cause subclinical low levels of inflammation. Adipose tissue does not just function as simple triglyceride storage, but also works as an active endocrine organ. Adipocytes secrete excess amounts of adipokines such as leptin, adiponectin and C-reactive protein (CRP), and adipocytokines such as interleukin 6 (IL-6) and tumor necrosis factor (TNF)-α [5]. In addition, it has been shown that adipose tissue cells produce reactive oxygen species (ROS) as intensely as adipokines and increase oxidative stress [10-12]. C-reactive protein sensitivity and oxidative stress markers have been found to be higher in obese individuals, and this association is compatible with body mass index (BMI), body fat ratio, low-density lipoprotein oxidation and triglyceride levels [13]. However, antioxidant defense markers are inversely proportional to and lower than body fat ratio and central obesity [14, 15]. Amirkhizi *et al* [16] reported that in individuals with long-term obesity, antioxidant sources and enzyme activities such as superoxide dismutase (SOD) and catalase (CAT) decrease. Because of the association between high BMI and the increase in oxidative stress and decrease in antioxidant capacity, obesity is considered an independent risk factor in terms of oxidative stress increase [10, 11, 17].

Studies have reported that the increase in oxidative stress and/or the decrease in antioxidant capacity play an important role in the pathogenesis of periodontal disease [18-21]. The increased production of ROS by excessive adipose tissue might adversely affect periodontal health by increasing gingival oxidative stress even further [22, 23].

To our knowledge, no human or rat study has been performed in order to test the effects of obesity on chronic periodontitis with regard to local and systemic antioxidant and oxidant status. Thus, the aims of this study were to compare the serum and salivary total antioxidant status (TAS), total oxidant status (TOS), and oxidative stress index (OSI) levels of patients with obesity, chronic periodontitis and with patients suffering both obesity and chronic periodontitis.

MATERIAL AND METHODS

Study groups

Eighty-eight participants (47 males and 41 females; age range 27-51 years) were enrolled in this study. Using the BMI criteria of the World Health Organization (WHO) [5], 44 participants of normal weight and 44 obese, systemically healthy participants were selected from the Department of Periodontology, Faculty of Dentistry, and from the Department of Endocrinology and Metabolic Diseases, Faculty of Medicine, Ataturk University, Erzurum, Turkey. Patients with DM, hypertention and dyslipidemia were exluded. The participants were divided into four study groups (22 subjects each) as follows:

-O+P+ group; patients with obesity and periodontitis,

-O-P+ group; patients with normal weight and periodontitis,

-O+P- group; periodontally healthy patients with obesity,

-O-P- group; control group consisting of periodontally healthy patients with normal weight.

Exclusion criteria included a history of periodontal therapy during the year before the study; the use of antibiotics, anti-inflammatory agents, antioxidants or corticosteroids during the six months before the study; smoking; and pregnancy or lactation. None of the participants had DM according to an oral glucose tolerance test (OGGT) and glycosylated hemoglobin (HbA1c) criteria of the American Diabetes Association (ADA) [24]. Written informed consent was obtained from each individual prior to participation in the study. The study protocol was approved by the Ataturk University Institute of Health Sciences Ethics Committee.

Clinical assessments

Assessment of obesity: BMI was used to assess overall adiposity, and waist circumference (WC) was used to assess abdominal adiposity. BMI was obtained by dividing the subject's weight in kilograms (kg) by the square of the height in meters (m^2).

The BMI criteria are as follows: normal weight, 18.5-24.99 kg/m^2; overweight, 25-29.99 kg/m^2; obesity, ≥ 30 kg/m^2. WC values of the participants were divided into two categories, normal or high, using the cut off points of > 88 cm for females and > 110 cm for males. Thus, participants with a BMI ≥ 30 kg/m^2 and WC > 88 (females) or > 110 cm (males) were diagnosed as obese [5].

The BMI values of all of the participants were measured with a digital scale designed to calculate height, weight, and BMI. A tape measure was used to determine WC values.

Assessment of periodontal parameters: periodontal assessment of the participants was performed by two trained examiners (measurements were carried out twice, firstly by author OK and then by CFC, and the second examiner did not see the recordings of the first examiner) at six sites per tooth (mesiobuccal, buccal, distobuccal, mesiolingual, lingual and distolingual) with a Williams probe with Michigan markings (Hu-Friedy; Chicago, IL, USA) and included plaque index (PI) [25], gingival index (GI) [26], bleeding on probing (BOP), probing depth (PD) and clinical attachment level (CAL). PD was determined by measuring the distance from the free gingival margin to the base of the pocket. CAL was measured as the distance between the cementoenamel junction and the base of the pocket. BOP was considered positive when it occurred within 15 seconds after probing, and it was expressed as the percentage of sites showing bleeding. Periodontal disease was defined as two or more tooth sites with PD ≥ 4 mm or CAL of 4 mm that bled on probing [27]. Intra-examiner variability in using the dental examination criteria was tested by performing duplicate examinations on 20 randomly selected participants on consecutive days. Agreement was 90% for PD, 88% for BOP and 92% for CAL.

Collection of samples

Saliva sampling: to avoid irritation and contamination of the saliva sampling with bleeding during periodontal probing, serum and saliva samples were obtained a week before the clinical periodontal examination. The sampling procedure was carried out early in the morning. The participants were instructed not to eat or drink anything except water and not to brush their teeth within at least 12 h before the sampling. Unstimulated saliva samples were collected after the subject kept his/her mouth open for 5 min. The samples were transferred to microcentrifuge tubes and centrifuged immediately to remove cell debris (1,000g for 10 min at 4°C), and then stored at -80°C until analysis.

Serum sampling: venous blood was collected from the antecubital fossa and allowed to stand at room temperature for 30 min, then centrifuged at 3,000g for

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10 min to obtain the serum. Serum aliquots were stored at -80°C until analysis.

Laboratory assessments

Assay of total antioxidant status: TAS of the serum and saliva samples was measured using an automated colorimetric measurement method for TAS developed by Erel [28]. A commercially available TAS assay kit (REL Assay Diagnostics; Gaziantep, Turkey) was used for quantitative TAS detection in clinical samples. This method is based on the bleaching of the characteristic color of a more stable 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical cation by antioxidants. The results were expressed as millimole Trolox equivalent per liter (mmol Trolox Eq/l). All samples were run in duplicate, and the mean values were used for statistical analysis. The intra- and inter-assay coefficients of variation were lower than 3% [28].

Assay of total oxidant status: TOS of the clinical samples was measured using the colorimetric method described by Erel [29]. A TOS assay kit (REL assay diagnostics; Gaziantep, Turkey) was used for the quantitative detection of TOS in the serum and saliva samples. In this method, oxidants present in the sample oxidize the ferrous ion-o-dianisidine complex to ferric ion, which produces a colored complex with xylenol orange in an acidic medium. The color intensity, measured spectrophotometrically, is related to the total oxidant molecules present in the sample. The assay was calibrated with hydrogen peroxide (calibrator concentration 10 μM, analytical range 0-300 μM), and the results are expressed in terms of micromolar hydrogen peroxide equivalent per liter (μmol H_2O_2 Eq/l). The assay measurements were performed in duplicate and the average values were used for statistical evaluation. The intra- and inter-assay coefficients of variation were lower than 3% [29].

Determination of oxidative stress index: OSI is a current parameter developed to demonstrate degree of oxidative stress more clearly. This index, which is a proportional value between TAS and TOS, is affected directly by oxidant and antioxidant status. The percentage ratio of TOS to TAS was accepted as OSI and calculated according to following formula [30], in which TAS values were converted to μmol/l:

$$\text{OSI (arbitrary unit)} = \frac{\text{TOS (μmol } H_2O_2 \text{ Eq/l)}}{\text{TAS (μmol Trolox Eq/l)}} \times 100$$

Statistical analyses

The Kolmogorov-Smirnov test was used to determine the data compatibility to the normal distribution. It was determined that the demographic findings, clinical findings, and laboratory findings were not normally distributed. Thus, comparisons between groups were made with the Kruskal-Wallis and Mann-Whitney U tests. Correlations between demographic, clinical, and laboratory parameters were analyzed with Pearson's correlation test. All analyses were performed using a The SPPS software version 17.0 (Chicago, IL). $P < 0.05$ was accepted as statistically significant.

RESULTS

Demographic and periodontal findings

There were no differences in gender or age among the groups. The mean and standard deviation values of BMI, WC, OGGT and HbA1c levels of the control and study groups are given in Table 1. All of the parameters were significantly higher in the obese groups (O+P- and O+P+; $P < 0.01$).

All of the clinical parameters (PI, GI, BOP, PD, CAL) were statistically higher in the periodontitis groups (O-P+ and O+P+) than in the periodontally healthy groups (O-P- and O+P-, $P < 0.001$). There were no differences between the chronic periodontitis groups and between the obese groups ($P > 0.05$ both, Table 2).

Laboratory findings

***Serum findings*:** serum TOS levels of all study groups were significantly higher than that of the control group ($P < 0.01$). The highest TOS level was observed in the O+P+ group, and this value was not significantly different compared to the other study groups ($P > 0.05$).

Serum TAS levels of all the study groups were significantly lower than that of the control group ($P < 0.01$). The highest TAS levels were observed in the control group, and the lowest in the O+P+ group. The O+P+ group had lower values than the O-P+ group, but the difference was not statistically significant ($P > 0.05$).

Serum OSI of all of the study groups were significantly higher than that of the control group. The highest OSI was observed in the O+P+ group, and this value was significantly higher than those of the other study groups ($P < 0.05$).

Saliva findings: the highest salivary TOS levels were observed in the O+P+ group, and the lowest in the control group. The O+P+ group's TOS levels were significantly higher compared to all of the other groups, and the O-P+ and O+P- groups' levels were significantly higher than those of the O-P- group ($P < 0.01$). The O-P+ group had higher values than the O+P- group, but the difference was not statistically significant ($P > 0.05$).

The highest salivary TAS values were observed in the control group and the lowest in the O+P+ group. The salivary TAS levels of the O+P+ group were significantly lower than those of all other groups, and the salivary TAS levels of the O-P+ and O+P- groups were significantly lower than control group ($P < 0.05$).

Table 1. Demographic and obesity-related data of the study participants

Variable	O-P- (n = 22)	O-P+ (n = 22)	O+P- (n = 22)	O+P+ (n = 22)
Male:female ratio	12:10	11:11	12:10	12:10
Age	35.42 ± 4.87	36.63 ± 4.33	34.63 ± 4.4	36.9 ± 4.54
Body mass index (kg/cm^2)	23.53 ± 2.4	23.71 ± 1.65	34.98 ± 3†	35.07 ± 2.92†
Waist circumference (cm)	75.13 ± 11.88	77.5 ± 15.73	113.68 ± 9.51†	112 ± 9.14†
Oral glucose tolerance test (mg/dl)	171.42 ± 9.35	172.5 ± 8.71	181.95 ± 11.47†	180.63 ± 16.67†
HbA1c (%)	4.9 ± 0.27	5.1 ± 0.64	5.9 ± 0.37†	5.9 ± 0.38†

Data are mean ± SD (except male:female ratio). $^{\dagger}P < 0.01$ compared to the O-P- and O-P+ groups

Table 2. Clinical periodontal parameters of the study groups

Variable	O-P- (n = 22)	O-P+ (n = 22)	O+P- (n = 22)	O+P+ (n = 22)
Plaque index	0.03 ± 0.018 (0.04)	2.29 ± 0.193 (2.27)*****	0.05 ± 0.021 (0.04)	2.36 ± 0.18 (2.3)*****
Gingival index	0.06 ± 0.019 (0.05)	1.82 ± 0.181 (1.76)*****	0.08 ± 0.021 (0.09)	1.77 ± 0.17 (1.75)*****
Bleeding on probing	0.06 ± 0.016 (0.04)	85.07 ± 4.956 (84.67)*****	0.07 ± 0.014 (0.06)	83.2 ± 5.06 (84.12)*****
Probing depth	1.28 ± 0.195 (1.19)	4.02 ± 0.286 (4.14)*****	1.29 ± 0.281 (1.22)	4.07 ± 0.322 (4.18)*****
Clinical attachment level	1.54 ± 0.212 (1.48)	4.42 ± 0.333 (4.4)*****	1.6 ± 0.273 (1.52)	4.48 ± 0.353 (4.6)*****

Data are mean ± SD (Median). *****$P < 0.01$ compared to the O-P- and O+P- groups.

Table 3. Serum and Salivary TOS, TAS and OSI Values of the Study Groups

Variable	O-P- (n = 22)	O-P+ (n = 22)	O+P- (n = 22)	O+P+ (n = 22)
Serum				
TOS (µmol H_2O_2 Eq/l)	12.19 ± 2.77 (13.05)	15.96 ± 4.01 (16.33)*****	15.65 ± 3.08 (15.24)*****	16.74 ± 6.34 (18.22)*****
TAS (mmol Trolox Eq/l)	1.34 ± 0.32 (1.40)	1.16 ± 0.29 (1.05)*****	1.11 ± 0.21 (1.16)*****	1.01 ± 0.25 (0.97)*****
OSI (arbitrary unit)	0.95 ± 0.39 (0.9)	1.39 ± 0.56 (1.43)*****	1.45 ± 0.37 (1.5)*****	1.9 ± 0.72 (1.79)***†‡**
Saliva				
TOS (µmol H_2O_2 Eq/L)	4.41 ± 1.54 (4.86)	6.8± 3.49 (7.2)*****	6.02± 2.55 (6.5)*****	8,33 ± 5.27 (10.04)***†‡**
TAS (mmol Trolox Eq/l)	0.92 ± 0.22 (1.02)	0.77 ± 0.14 (0.81)*****	0.79 ± 0.16 (0.8)*****	0.57 ± 0.12 (0.64)***†‡**
OSI (arbitrary unit)	0.55 ± 0.29 (0.6)	0.9 ± 0.39 (0.85)*****	0.81 ± 0.46 (0.75)*****	1.53 ± 0.56 (1.41)***†‡**

Data are mean ± SD (Median). $P < 0.05$ compared to the: *O-P- group; †O-P+ group; ‡O+P- group.

The O-P+ group had lower salivary TAS values than the O+P- group, but the difference was not statistically significant ($P > 0.05$).

The salivary OSI of all study groups were significantly lower than those of the control group. The highest salivary OSI was observed in the O+P+ group; this value was significantly higher compared to the other study groups ($P < 0.05$).

All of the serum and salivary TOS, TAS, and OSI values are shown in Table 3.

DISCUSSION

To the best of our knowledge, this is the first study investigating the effects of obesity on local and systemic TOS, TAS and OSI values in chronic periodontitis patients. According to our findings, local and systemic TOS and OSI levels were considerably higher, and TAS levels were significantly lower, in obese individuals with periodontitis compared to the other groups. It was also found that obesity and periodontitis resulted in increased local and systemic OSI levels.

Studies have shown that obese individuals are very likely to be diagnosed with DM [9, 12, 17], which in turn, is associated with increased oxidative stress [9, 12]. For this reason, in order to keep patients with DM excluded from the present study, all of the participants' OGGT and HbA1c levels were evaluated according to the ADA criteria (OGGT < 200 mg/dl and HbA1c < %7) during forming the study groups [24]. In addition, we preferred to include only individuals who had never smoked, as it has been shown that smoking significantly affects the oxidative and antioxidant parameters of individuals with periodontitis [31, 32].

Albeit many studies have investigated the relationship between obesity and periodontitis, the biological mechanisms that explain these associations have not yet been fully explained [5-7, 10]. It is accepted that various cytokines that are secreted intensively from

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adipose tissue (identified generally as adipokines) and hormones increase periodontal destruction by causing a hyperinflammatory response [5, 9]. Increased adipose tissue causes increased ROS production as well as adipokines [10-12, 19]. Pro-inflammatory cytokines secreted intensively from the cells, such as the adipocytes and pre-adipocytes that make up adipose tissue, such as TNF-α, IL-1 and IL-6 are potent stimulators of ROS production [33]. Increased adipose tissue also increases the secretion of tissue angiotensin (Ang) II. Ang II increases the activity of NADPH oxidase enzyme, which plays an important role in the production of ROS from adipocytes [34]. In addition, it has been found that factors such as increased mitochondrial and peroxisomal oxidation of fatty acids, excessive oxygen consumption, and a lipid-rich diet are connected with increased ROS in obese individuals [19]. In addition to excessive ROS production and increased oxidative stress, obesity is also associated with decreased antioxidant capacity [10, 11, 14-16].

Current experimental studies have demonstrated that increased systemic ROS levels and decreased antioxidant capacity in obese rats may affect the pathogenesis of periodontal disease by increasing gingival oxidative stress [22, 23]; however, it is still unclear how obesity increases gingival oxidative stress.

TAS is a reliable parameter of measuring total antioxidant capacity, which includes the effects of all undiscovered and discovered antioxidants [28, 30]. Chapple *et al* [35] showed that, as the severity of periodontal destruction increases in individuals with chronic periodontitis the decrease in TAS becomes more evident. Our TAS findings showed that obesity and periodontitis decrease systemic and local TAS levels and that this decrease is more evident when individuals with periodontitis are also obese. Our findings are consistent with studies showing significantly decreased serum [36-38] and saliva [39] TAS levels in subjects with periodontitis compared to periodontally healthy subjects. In contrast, Brock *et al* [40] reported a statistically significant decrease in gingival crevicular fluid (GCF) and plasma TAS levels in individuals with periodontitis and a nonsignificant decrease in saliva and serum TAS levels. Esen *et al* [41] found that serum TAS levels were lower in individuals with periodontitis, while, interestingly, GCF TAS levels were similar to those of the control group. Although there is an adaptive increase in antioxidants during the first phase of oxidative outburst, antioxidant defense might decrease over time as ROS production becomes chronic [38]. This finding might account for the different results reported in the various studies evaluating TAS levels in individuals with periodontitis.

TOS measurement is a parameter that has recently come into use to determine lipid peroxidation and oxidative stress levels. TOS is a more practical method than measuring different oxidant molecules one by one. Moreover, it reveals the final oxidative status, which also includes the interactions of oxidants with each other [29, 30]. Only a few studies in the literature have evaluated TOS levels in individuals with periodontitis [41-43]. Akalin *et al* [42] and Wei *et al* [43] reported that local (saliva and GCF) and systemic (serum) TOS levels increase in individuals with chronic periodontitis. In a recent study, Esen *et al* [41] evaluated the effects of chronic periodontitis (CP) and rheumatoid arthritis (RA) on serum TOS levels and found that the GCF TOS levels of the CP alone and RA+CP groups were significantly higher than those of the control and RA alone groups. They reported no significant difference in serum TOS levels. Our findings are consistent with the findings of studies that reported an increase in local and systemic TOS levels in individuals with periodontitis. If individuals with periodontitis are also obese, the local and systemic TOS levels increase even more.

OSI, a proportional value between TAS and TOS, is a recently investigated parameter that is affected directly by oxidant and antioxidant status and that can present the degree of oxidative stress more clearly [30]. Therefore, OSI may be a useful and practical parameter for evaluating oxidative injury in periodontal tissues. In their recent study, Baltacioglu *et al* [44] suggested that increased TOS and OSI and decreased TAS play important roles in the pathogenesis of periodontitis and are closely associated with clinical periodontal status. In their above mentioned study, Esen *et al* [41] evaluated GCF and serum OSI and reported that the local OSI levels of CP groups (CP and RA+CP) were significantly higher; however, there was no significant difference in systemic OSI levels. They also found that when individuals with CP also had RA, the local and systemic OSI levels were not affected. The findings of that study suggested that compared to RA, obesity affects both systemic and local oxidative and antioxidant parameters more clearly.

In the present study, OSI was used for the first time to evaluate the association between obesity and periodontitis in terms of oxidative injury. Obesity seems to affect both serum and saliva OSI values in the presence of chronic periodontitis, and because OSI serves as an element of balance, its use is likely to be more important than TAS and TOS values in interpreting the study results. Further research of the association between obesity and periodontitis in terms of oxidative destruction is needed, using larger study groups. If our findings can be supported by new studies, efforts that aim to treat obesity might also benefit periodontal treatment strategies.

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