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The Effects of Obesity on Local and Circulating Levels of Tumor Necrosis Factor-α and Interleukin-6 in Patients with Chronic Periodontitis

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Abstract

Background and aims. The aim of this study was to evaluate the possible effects of obesity on the local (salivary) and systemic TNF-α and IL-6 levels in patients with chronic periodontitis (CP).

Materials and methods. This study included 88 subjects assigned to four groups of 22 subjects each, as follows: group O+P+ (patients with obesity and CP), group O-P+ (patients with normal weight and CP), group O+P- (periodontally healthy patients with obesity), and the control group, group O-P- (periodontally healthy patients with normal weight). Serum and salivary samples were obtained a week before the recording of clinical periodontal parameters. Local and systemic TNF-α and IL-6 levels were determined biochemically.

Results. In serum and saliva, both TNF-α and IL-6 levels were the lowest in O-P- group ($P < 0.05$). The highest TNF-α and IL-6 levels were observed in O+P+ group, while only IL-6 levels were statistically significant ($P < 0.05$).

Conclusion. Obesity upregulated the salivary and serum levels of TNF-α and IL-6. In patients with periodontitis, who were also obese, the serum and saliva levels of IL-6 were significantly high. Obesity might play a destructive and provocative role in the pathogenesis of periodontitis by negatively affecting IL-6 levels.

Key words: **Interleukin, obesity, periodontitis, saliva, tumor necrosis factor.**

Introduction

Periodontal diseases are chronic inflammatory diseases whose primary etiological agents are pathogenic bacteria in microbial dental plaque localized on the tooth surface adjacent to the gingival margin. Although bacteria are a prerequisite for the occurrence of the disease, the main factor determining the course and severity of tissue destruction caused by the disease is the host response that develops against several antigenic and chemotactic virulence factors.[1,2]

Many studies have revealed the role of increased levels of tumor necrosis factor-α (TNF-α) and interleukin-6, which are proinflammatory cytokines, in the host response that develops against pathogenic bacteria.[3-6] Host response is a dynamic process and is affected by several environmental and acquired risk factors, such as smoking and stress, as well as local factors.[7] Many studies have drawn attention to the role of the increase in local proinflammatory cytokine levels caused by these risk factors and their effects on the pathogenesis of periodontitis.[8,9] It has been reported that obesity, which is characterized by abnormal and excessive fat accumulation in the adipose tissue and has been shown to be one of the most important societal health problems today, affects the pathogenesis of periodontitis in a destructive-provocative way.[10-13] Nishada et al[14] reported that obesity is the second most important risk determinant, following smoking, in the pathogenesis of inflammatory periodontal diseases.

Obesity has been confirmed as a chronic disease whose development is affected by many factors, such as molecular interactions and social, behavioral, physiological, metabolic, and cellular factors.[15,16] Its prevalence has reached alarming rates in both developed and developing countries in several parts of the world.[17] Obesity is characterized by a systemically low-grade chronic inflammatory condition and is reported to be a fundamental risk factor in cancer development and many chronic diseases such as diabetes mellitus (DM) and hypertension.[18]

Although it has been suggested in many studies[12,13] that obesity affects the pathogenesis of periodontal disease, the mechanisms that have a role in this process have not been entirely elucidated. The traditional point of view on this subject is that several cytokines (TNF-α, IL-1 and IL-6) released in excessive amounts from the excess adipose tissue may have a role in periodontal tissue destruction, causing the hyperinflammatory response.[11] It has been pointed out that proinflammatory cytokines such as TNF-α, IL-1 and IL-6 stimulate the production of reactive oxygen species (ROS).[19,20] However, it has also been reported that several oxidation-reduction reactions that form ROS and increase oxidative stress trigger the release of many factors, including TNF-α and IL-6 cytokines, by activating several transcription factors (NF-κβ and AP-1).[21,22] In addition, it has been reported that obesity causes vascular changes. TNF-α has a very important role in the regulation of plasminogen activator inhibitor-1 (PAI-1) of liver and endothelial cells. Elevated PAI-1 levels cause an increase in clot formation and a decrease in blood flow, which can lead to ischemic changes in several tissues,[23] including periodontal tissues.

To the best of our knowledge, only one study in the literature has reported on the effects of obesity on levels of local proinflammatory cytokines in individuals with periodontitis.[24] No study was found that assessed the possible effects under discussion in saliva samples. Thus, the objective of this study was to assess the possible effects of obesity on serum and salivary TNF- α and IL-6 levels in individuals with chronic periodontitis (CP).

Materials and Methods

Study Groups

Eighty-eight participants (47 males and 41 females; an age range of 27–59 years) were enrolled in the study between July 2011 and April 2012. According to body mass index (BMI) criteria of the World Health Organization (WHO),[11] 44 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, University of Atatürk, Erzurum, Turkey. The patients were divided into four study groups (22 each): group O+P+ (obese patients with CP), group O-P+ (normal weight patients with CP), group O+P- (obese but periodontally healthy patients), and as control group, group O-P- (normal weight and periodontally healthy patients). The inclusion criterion consisted of having a minimum of 20 natural teeth. The exclusion criteria consisted of a history of periodontal therapy within a year of the examination; the use of antibiotics, anti-inflammatory drugs, antioxidants, or corticosteroids within six months of the examination; smoking; pregnancy; use of hormone therapy; or lactation. The participants also had to meet specific inclusion criteria regarding diabetic condition; patients with DM as indicated by oral glucose tolerance test (OGGT) and glycated hemo-

globin (HbA1c) criteria for the diagnosis of DM according to the American Diabetes Association (ADA) were excluded.[25] This study was approved by the Atatürk University, Faculty of Medicine Ethics Committee in accordance with the Helsinki Declaration of 1975, revised in 2000. Written informed consent was obtained from each individual before participation.

Clinical Assessments

Assessment of Obesity: Obesity was diagnosed using the BMI and waist circumference (WC) criteria of the WHO. BMI, which is a cheap, easy, and confidential method frequently used for the diagnosis of overall obesity, was calculated as weight (kg)/height^2 (m^2). WC, which provides better information about abdominal obesity, was accepted as normal or high (obese). Participants with BMI ≥30 kg/m^2 and WC >88 cm for women and >102 cm for men were diagnosed as obese.[11] 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.

Assessment of Periodontal Parameters: Periodontal assessment of the participants was performed by two trained and calibrated examiners (measurements were carried out twice, first by 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). The assessments included plaque index (PI),[26] gingival index (GI),[27] 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.[28] Intraexaminer 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 samples with blood 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 hours before the sampling. Unstimulated saliva samples were collected after the subject kept his/her mouth open for five minutes. The samples were transferred to microcentrifuge tubes and centrifuged immediately to remove cell debris (×1,000 g for 10 minutes at 4°C), and then stored at −80°C until analysis.

Serum sampling: Venous blood was collected from the antecubital fossa and kept at room temperature for 30 min, then centrifuged at ×3,000 g for 10 minutes to obtain the serum. Serum aliquots were stored at −80°C until analysis.

Laboratory Assessments

Measurement of Cytokine Levels: IL-6 and TNF-α levels in serum and saliva samples were assayed using commercial standard sandwich enzyme-linked immunosorbent (ELISA) assay kits [Human IL-6 ELISA Kit (EK0410) and Human TNF-α ELISA Kit (EK0525); Boster Biological Technology, Ltd., Fremont, CA] according to the manufacturer's instructions. Specimens were thawed and assayed immediately to ensure minimal deterioration, and each patient's samples were assayed at the same time as the matched control samples. The standards ranged from 0 to 300 pg/mL human IL-6 and from 0 to 1000 pg/mL human TNF-α. The lower limits of detection were <0.3 pg/mL and <1 pg/mL for IL-6 and TNF-α, respectively. Intra- and inter-assay coefficients of variation for IL-6 and TNF-α were <10%.

Statistical Analyses

The Kolmogorov–Smirnov test was used to determine the compatibility of the data to normal distribution. It was determined that the demographic findings, clinical findings and laboratory findings were not normally distributed. Comparisons between the 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 the analyses were performed using SPSS 17.0 (IBM, Chicago, IL). $P<0.05$ was accepted as statistically significant.

Results

Demographic Findings

There were no differences in gender or age between

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

Variable	O-P- (n=22)	O-P+ (n=22)	O+P- (n=22)	O+P+ (n=22)
Male-to-female ratio	12 : 10	11 : 11	12 : 10	12 : 10
Age	35.42 ± 4.87	36.63 ± 4.33	34.63 ± 4.40	36.90 ± 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	77.50 ± 15.73	113.68 ± 9.51 †	112.00 ± 9.14 †
OGTT (mg/dL)	171.42 ± 9.35	172.50 ± 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-to-female ratio.
† Significant difference compared to the O-P- and O-P+ groups (P < 0.01).

the groups. The mean and standard deviation values of BMI, WC, OGGT and HbA1c levels of the control and study groups are provided in Table 1. All the parameters were statistically and significantly higher in the obese groups (O+P-, O+P+; P < 0.01).

Periodontal Findings

All the clinical parameters (PI, GI, BOP, PD and CAL) were statistically higher in the CP groups (O-P+, O+P+) compared to the periodontally healthy groups (O-P-, O+P-; P < 0.001). There were no differences between the CP groups (O-P+, O+P+) or between the obese groups (O+P-, O+P+; P > 0.05); Table 2).

Laboratory Findings

Serum and Saliva Findings: The serum and salivary TNF-α levels were consistent with each other. The highest levels were observed in the O+P+ group, and the lowest levels were observed in the control group. The serum and salivary TNF-α levels of the study groups were found to be significantly higher than those of the control group (serum and saliva: P<0.05). When both serum and saliva findings were assessed, the differences were statistically insignificant (P>0.05), although the TNF-α level of the O+P- group was higher than that of the O-P+ group.

Serum and salivary IL-6 levels were also correlated with each other. The highest levels were observed in the O+P+ group, and the lowest levels were observed in the control group. The IL-6 level of the O+P+ group was found to be higher at the statistically significant level than those of the other groups. In addition, the O-P+ and O+P- groups were found to have statistically significantly higher IL-6 levels than the O-P- group (serum and saliva: P < 0.01). Although the O+P+ group had a higher IL-6 level than the O-P+ group, the difference was not statistically significant (P > 0.05; Table 3).

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)
PI	0.03 ± 0.018 (0)	2.29 ± 0.193 (2.27) *	0.05 ± 0.021 (0)	2.36 ± 0.18 (2.3) *
GI	0.06 ± 0.019 (0)	1.82 ± 0.181 (2) *	0.08 ± 0.021 (0.5)	1.77 ± 0.17 (2) *
BOP	0.06 ± 0.016 (0)	85.07 ± 4.956 (84.5) *	0.07 ± 0.014 (0)	83.20 ± 5.06 (84.5) *
PD	1.28 ± 0.195 (1)	4.02 ± 0.286 (4) *	1.29 ± 0.281 (1)	4.07 ± 0.322 (4) *
CAL	1.54 ± 0.212 (1.5)	4.42 ± 0.333 (4) *	1.60 ± 0.273 (1)	4.48 ± 0.353 (4) *

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

Table 3. Serum and salivary TNF-α and IL-6 levels of the study groups

Variable	O-P- (n=22)	O-P+ (n=22)	O+P- (n=22)	O+P+ (n=22)
Serum				
TNF-α (pg/mL)	62.12 ± 9.19 (58.45)	71.40 ± 11.99 (74.12) *	73.01 ± 7.49 (74.33) *	75.15 ± 13.13 (71.77) *
IL-6 (pg/mL)	17.22 ± 6.10 (15.32)	25.05 ± 4.13 (26.65) *	26.11 ± 9.49 (29.77) *	32.14 ± 11.59 (36.12) *†‡
Saliva				
TNF-α(pg/mL)	40.55 ± 7.96 (38.23)	47.21 ± 5.30 (48.77) *	48.02 ± 6.07 (46.01) *	50.11 ± 10.84 (54.44) *
IL-6 (pg/mL)	11.77 ± 4.47 (9.75)	17.41 ± 3.07 (18.11)*	17.91 ± 7.47 (16.67) *	22.62 ± 9.31 (19.65) *†‡

Data are mean ± SD (Median).
* Significant difference compared to the O-P- group (P < 0.01).
† Significant difference compared to the O-P+ group (P < 0.05).
‡ Significant difference compared to the O+P- group (P < 0.05).

Discussion

To the best of our knowledge, this study is the first to assess local TNF- α and IL-6 levels in the saliva samples of obese and non-obese individuals with or without CP. Saliva is an important body fluid that can be examined; it provides important information regarding the severity of several oral diseases, including periodontal diseases. This fluid can also be used to determine response to therapy. The content of saliva is complex and dynamic and may be affected by diseases. The sampling procedure is simple and cheap, and there is a rich amount of saliva; therefore, it is a reasonable body fluid for tests. Saliva is collected by non-invasive techniques, and thus, the risk of infection is minimal.[29,30] Several studies have reported that stimulating salivary flow by mastication increases the gingival crevicular fluid (GCF) flow from the periodontal pocket, affecting the composition of the saliva.[31] Therefore, we examined non-stimulated saliva samples to assess the effects of obesity on local proinflammatory cytokine levels.

Our findings revealed that when obesity was taken into account in individuals with periodontitis, local and systemic levels of IL-6 in particular increased significantly compared with individuals with only obesity or only periodontitis. Our findings also suggested that obesity and periodontitis cause a similar effect on increasing serum and saliva cytokine levels.

When the normal weight groups in the present study were assessed together, the findings indicated that the increases in serum TNF-α levels in individuals with periodontitis were compatible with those reported by Duarte et al[6] and Bretz et al.[4] In addition, our findings showed that the increases in serum IL-6 levels were compatible with those of Marcaccini et al[5] and Duarte et al.[6] Conversely, other studies in the literature reported no significant increase in TNF-α or IL-6 levels in individuals with periodontitis.[32,33]

The limited number of studies dealing with salivary TNF-α and IL-6 levels in individuals with periodontitis has resulted in contradictory findings.[34-38] Frodge et al[34] reported that the salivary TNF-α levels of individuals with periodontitis increased more than twice as much as periodontally healthy individuals, and that saliva TNF-α level is a good indicator of periodontal disease. Ng et al[35] also touched on the increased cytokine levels. However, Ulker et al[36] reported that salivary TNF-α levels were not significantly higher in individuals with gingivitis, and Teles et al[37] and Mirrielees et al[38] determined that salivary TNF-α levels were not significantly higher in individuals with periodontitis.

The relationship between obesity and periodontitis has been revealed by many epidemiological and clinical studies; however, the pathological mechanisms underlying this relationship have not been precisely elucidated. Some studies have suggested that the local host response to periodontitis is provoked by several pathological events that develop due to the coordination of increased proinflammatory cytokine levels caused by adipose tissue.[10-13]

When the periodontally healthy groups in the present study were assessed together, the findings indicated that the serum TNF-α and IL-6 levels of the obese individuals were remarkably higher compared with the normal weight individuals, which is compatible with the finding of many studies that reported that obesity is a low-grade chronic inflammatory condition associated with increased proinflammatory cytokine levels and increased reactive C protein levels.[11,39] In addition, it has been shown that weight loss is related to decreases in serum levels of this cytokine.[40]

Lundin et al[41] reported that the GCF TNF-α level in periodontally healthy individuals with BMI ≥40 kg/m^2 was higher compared to that of non-obese individuals, and that the reason for this increase might be the rise in systemic TNF-α level caused by adipose tissue. Many studies have drawn attention to the relationship between systemic TNF-α level and insulin resistance.[42-44] Genco et al[42] emphasized that increased systemic TNF-α level and insulin resistance may have an important role in the relationship between obesity and periodontal disease. However, some studies in the literature have reported that IL-6 may have a more important role than TNF-α in the relationship between obesity and periodontitis.[43,44] Saxlin et al[45] suggested that IL-6 may be a potential mediator in the relationship between obesity and periodontitis. Modeer et al[46] reported no significant differences in GCF TNF-α levels between healthy obese and non-obese young adults. Our findings that both systemic and local TNF-α levels increased significantly in periodontally healthy obese individuals suggest, when interpreted with studies reporting similar results, that chronically high cytokine levels might increase the risk of future periodontal disease.

When our study groups composed of individuals with periodontitis were assessed together, it was observed that local and systemic IL-6 levels increased remarkably in obese patients compared with non-obese patients; however, there was no significant increase in TNF-α levels. Zuza et al[47] reported a

significant increase in serum levels of both cytokines, while Gürgan et al[48] only found a significant increase in IL-6 serum levels. On the other hand, Zimmerman et al[24] found no significant increases in the serum levels of the so-called cytokines, but they found that DOS TNF-α levels increased significantly. It was also found that local and systemic levels of TNF-α and IL-6 significantly decreased following periodontal treatment.[47,48]

Our findings indicated that serum and saliva levels of IL-6 in individuals with periodontitis increase significantly when obesity is taken into account compared with individuals who have only obesity or only periodontitis. Statistically significant increases in TNF-α serum and saliva levels were detected. Our findings indicating that obesity and periodontitis have a similar effect on increasing TNF-α and IL-6 levels contradict the findings of Zimmerman et al,[24] who reported that obesity, compared to periodontitis, caused a marked increase in GCF TNF-α levels in particular. That study suggested that TNF-α may have an important role in the relationship between obesity and periodontitis, while our study draws attention to the role of IL-6.

It can be seen that the findings of studies investigating the possible effect of obesity on the local and systemic levels of several cytokines having roles in the pathogenesis of periodontal diseases contradict one another. Among the reasons for this discrepancy are differences in statistical assessments, differences in working principles, sensitivity of ELISA kits used in the biochemical studies, obesity diagnosis measurements, obesity severity, periodontal disease severity, number of individuals in the study groups and demographic data.

The results of the present study can be summarized as follows: 1) Obesity and CP cause similar effects on increasing local (saliva) and systemic TNF-α and IL-6 levels; 2) when obesity is taken into account in patients with periodontitis, the serum and salivary levels of IL-6 in particular increase significantly. The observed increase in the levels of local cytokines of periodontally healthy obese patients suggest that obesity might increase the present risk in terms of possible periodontal disease development in the future; the increase in local cytokine levels in obese individuals with periodontitis, particularly IL-6 levels, suggest that obesity can increase the severity of the destruction by provoking the local host response to pathogenic bacteria in the pathogenesis of periodontitis.

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