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Özgün Araştırma Makalesi

Evaluation of Salivary Visfatin, Leptin and Oxidative Stress Markers in Obese And Non-Obese Periodontitis Patients

Obez Olan ve Olmayan Periodontitis Hastalarında Tükürük Visfatin, Leptin ve Oksidatif Stres Belirteçlerinin Değerlendirilmesi

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ABSTRACT

Aim: This study aimed to assess visfatin, leptin, total antioxidant capacity (TAOC), nitric oxide (NO) and total oxidant capacity (TOC) levels at obese and non-obese periodontitis patients in saliva.

Material and Methods: Seventy-eight individuals were included in study, 20 obese periodontal healthy patients (Group I), 18 obese periodontitis patients (Group II), 20 non-obese periodontal healthy patients (Group III) and 20 non-obese periodontitis patients (Group IV). Body mass index (BMI), periodontal clinical parameters and waist circumference measurements were registered. Griess and ELISA methods were used for biochemical analysis.

Results: It was found that leptin and TAOC levels were higher in Group I and III than in Group II and IV; visfatin, TOC and NO levels were higher in Group II and IV than Group I and III (P<0,01). NO level was higher in Group I than in Group III (P<0,01). In group II the levels of visfatin and NO were significantly higher than in group IV (P<0,01, P<0,05, respectively). In whole group (adjusted for age, probing depth, and BMI) leptin showed negative correlation with NO and TOC, and this explained %79 and %74 variance of NO and TOC respectively.

Conclusions: While changes in leptin levels can affect oxidative stress and the severity of periodontal destruction; in obese patients, changes in visfatin and NO may also affect the severity of periodontal disease.

Keywords: Leptin; Obesity; Oxidative Stress; Periodontitis; Visfatin

ÖZET

Amaç: Bu çalışmanın amacı obez olan ve olmayan periodontitis hastalarında tükürükte leptin, visfatin, total antioksidan kapasite (TAOK), nitrik oksit (NO), total oksidan kapasite (TOK) düzeylerini değerlendirmektir.

Gereç ve Yöntem: Çalışmaya 20 obez periodontal sağlıklı (Grup I), 18 obez periodontitisli (Grup II), 20 obez olmayan periodontal sağlıklı (Grup III) ve 20 obez olmayan periodontitisli (Grup IV) olmak üzere toplam 78 birey katılmıştır. Vücut kitle indeksi (VKİ), periodontal klinik parametreler ve bel çevresi ölçümleri kaydedildi. Biyokimyasal analiz için Griess ve ELISA yöntemleri kullanıldı.

Bulgular: Leptin ve TAOK düzeylerinin Grup I ve III'te, Grup II ve IV'e göre daha yüksek olduğu; visfatin, NO ve TOK düzeylerinin de Grup II ve IV'te, Grup I ve III'e göre daha yüksek olduğu belirlendi (P<0.01). NO düzeyi Grup I'de daha yüksekti (P<0.01). Grup II'deki visfatin ve NO düzeyleri Grup IV'e göre anlamlı derecede yüksekti (sırasıyla P<0.01, P<0.05). Tüm gruplarda (yaş, sondalama derinliği ve VKİ'ye göre) leptin; NO ve TOK ile negatif korelasyon gösterdi ve bu durum, NO ve TOK'un sırasıyla %79 ve %74 varyansını açıklamaktaydı.

Sonuç: Leptin seviyelerindeki değişiklikler oksidatif stresi ve periodontal yıkımın şiddetini etkileyebiliryorken; obez hastalarda visfatin ve NO değişiklikleri de periodontal hastalığın şiddetini etkileyebilmektedir.

Anahtar Kelimeler: Leptin; Obezite; Oksidatif Stres; Periodontitis; Visfatin

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INTRODUCTION

Periodontitis is a complex disease affected by local and systemic factors, and the responses of tissue to biofilms also play a role in its etiology.[1,2] Systemic diseases such as leukocyte dysfunctions, cardiovascular diseases, diabetes and obesity can affect the susceptibility to and severity of periodontitis by causing changes in the immune response to microorganisms.[2] Pro and anti-inflammatory cytokines and, oxidant and antioxidant compounds that have altered levels in inflammation are possible factors that are involved in the relationship between systemic diseases and periodontitis.[1,3] Recent studies indicate that some adipokines such as leptin, visfatin, and chemerin play a crucial role in the pathophysiological relationship between systemic diseases and periodontitis, are also involved in this complicated mechanism.[4,5]

Leptin regulates immune and inflammatory responses, coagulation, hematopoiesis, modulates lipid and bone metabolism, insulin sensitivity and pancreatic beta cell function, and stimulates energy expenditure. Leptin acts through its receptors, which are widely distributed in many tissues.[6] Leptin, which is increased in the blood circulation in obesity, increases the inflammatory state by inducing oxidative stress in some systemic diseases. In some cases, the protective role of leptin has been mentioned because it increases antioxidant activity.[7,8] This may be because leptin has different effects on different cell types. In some studies it has been shown that the levels of leptin in serum are increased in parallel with the severity of periodontal destruction in periodontitis while salivary and gingival clevicular fluid (GCF) levels are decreased.[9] These results led to the idea that leptin may play a preventive role against periodontal diseases. However, the mechanism by which leptin accomplishes this is not clear.[9,10]

Visfatin which is also known as nicotinamide phosphoribosyltransferase is a new adipokine that is released more intensely by macrophages than by adipocytes.[11] Visfatin is a pleomorphic adipocytokine that plays a significant role in the inflammatory progression through the inhibition of neutrophil apoptosis and the activation of immune system cells such as dendritic cells, T cells, macrophages, B cells and monocytes.[12] It has been suggested that visfatin inhibits the apoptosis of polymorphonuclear leukocytes (PMNLs) during the inflammatory process, allowing them to stay longer in the area thus leading to prolongation of the inflammatory process.[11, 12] It has been shown that visfatin acts an essential role in the pathogenesis of systemic diseases such as diabetes, atherosclerosis and rheumatoid arthritis, cardiovascular diseases, cancer, and it is also an effective adipokine related to obesity and systemic diseases.[13, 14] It has also been studied the role of visfatin in the pathogenesis of periodontitis in recent years. It is known that visfatin is increased in proportion to the severity of destruction in saliva and GCF in periodontitis patients and that it is more exaggerated when inflammation there is increased in gingival tissues; furthermore it is related to some microbiological factors involved in periodontitis.[15, 16]

It is a known fact that the levels of serum leptin and visfatin increase in obesity, which is a chronic disease characterized by abnormal or extreme amounts of fat accumulation in the adipose tissue.[17, 18] Disturbed patterns of adipokine secretion in obesity are involved in chronic inflammatory states and increase oxidative stress.[17] In obesity, adipocytes secrete pro-inflammatory cytokines, such as inducible nitric oxide synthase (iNOS), interleukin 6 (IL-6), and tumor necrosis factor-α (TNF-α), which stimulate acute phase reactants, such as C reactive protein (CRP), and this causes low grade inflammation.[17] It has also been demonstrated that adipose tissue in obesity produces excessive reactive oxygen species (ROS), which results in an increase in circulating ROS.[18] The resulting ROS shows us it plays a role in many physiological and pathological processes. Increased serum oxidative damage products increase the inflammatory response and contribute to further oxidative damage by increasing neutrophil adhesion and chemotaxis.[19] Although the causal mechanism between obesity and periodontitis is not fully understood, it has been suggested that increased ROS in obesity may increase the susceptibility to periodontitis by affecting the gingival oxidative state by altering the local redox balance.[10, 20] In addition, in clinical trials have shown that a hyper oxidative status in obese individuals may affect periodontal destruction.[20] However, it is unclear whether this hyper oxidative state is affected only by systemic

ROS or whether locally detected adipokines are associated with this oxidative state.[8, 19, 21] A far as we know, there are no studies in the literature evaluating oxidative damage and adipokines in together in periodontitis and / or obesity. Therefore, we aimed to evaluate the levels of leptin, visfatin, total antioxidant capacity (TAOC) nitric oxide (NO) and total oxidant capacity (TOC) in the saliva from obese and non-obese patients with either healthy periodontal tissues or periodontitis and the relationship between these clinical parameters and biochemical parameters to each other.

MATERIAL AND METHODS

A total of 78 people who applied to Health Sciences University, Gülhane Faculty of Dentistry, Department of Periodontology were enrolled in the study: 20 obese patients with periodontal healthy (Group I), 18 obese periodontitis patients (Group II), 20 non-obese patients with periodontal healthy (Group III) and 20 non-obese periodontitis patients (Group IV). The patients who did not smoke, were systemically healthy, did not use any antibiotics or anti-inflammatory drugs and did not receive any periodontal treatments within the last 6 month were included in the study. Obese patients with healthy periodontal tissues and obese patients with periodontitis (Group I and Group II) had a waist circumference for men >94 cm or for women >80 cm and a Body Mass Index (BMI) of ≥30. Non-obese patients with periodontal healthy and non-obese periodontitis patients (Group III and Group IV) had a waist circumference for men <94 cm or for women <80 cm and a BMI of ≤30. The Ethics Committee of Gulhane Medical Faculty approved the study (2016-Session 3/Number:173). Before starting the study, all patients gave oral and written informed consent.

In the main hypotheses of this research, it was planned to compare three or more independent groups. The sample size was calculated at the 95% confidence level by using the "G. Power-3.1.9.2" program. Similar studies were examined to determine the effect size and the sample size was calculated from the appropriate study.[21] However, due to the small size obtained, it was not used and was recalculated considering the high effect size. As a result of the analysis, α=0.05, the standardized effect size was calculated as 76 with a theoretical power of 0.80 and when Cohen's (1988) effect size was taken as 0.40 (high grade) due to the lack of benefit from previous studies in this field and expert opinions.[22]

The clinical periodontal parameters including probing depth (PD), bleeding on probing (BOP), plaque index (PI)[23] , gingival index (GI)[24], and clinical attachment level (CAL) measured using a periodontal probe[3] (Hu-Friedy®, Chicago, IL) were recorded in all subjects. BMI was calculated according to WHO guidelines. Periodontal diagnosis was made according to the classification of the American Academy of Periodontology (AAP) in 2017, but patients with periodontitis were not divided into stages and grades. While individuals with BOP<20%, a PD≤3 and no clinical attachment loss were considered to have periodontal healthy, patients with periodontitis, which was diagnosed clinically and radiographically, had a PD>3 mm and a CAL ≥3 mm in in two or more teeth. Saliva samples were obtained with unstimulated saliva collection procedures.[25] Individuals were told to hold their heads in a tilted forward position and to have their mouths open to allow passive drainage of saliva in a test tube for ten minutes. An average of 5 ml of unstimulated saliva was collected from everyone into an empty glass test tube and transferred into Eppendorf tubes with pipettes. Collected saliva samples were kept at -80 ºC immediately until they were processed in the biochemistry laboratory.

Analysis of Adipokines

All biochemical analyzes were performed at Gulhane Faculty of Medicine in the Department of Medical Biochemistry. After the saliva samples dissolved, they were centrifuged for 10 minutes at 6000 rpm (1700 G). An enzyme-linked immunosorbent assay (ELISA) method was preferred to define the levels of leptin and visfatin in the saliva,. For leptin levels, the Human Leptin ELISA kit [¥] (DRG Products® Germany) was used; for visfatin levels, the Human visfatin ELISA kit[∞] (Elabscience® China) was used. Consistent with the manufacturer's instructions, the measurements were performed. For leptin, the intra-assay coefficient of variation (CV) was <10%, the inter-assay CV was 8.66-11.55 %, and the analytic sensitivity of these assays yielded 1.00 ng/ml. For visfatin, the intra-assay CV was <10%, the

inter-assay CV was 3.63-5.21 %, and the analytic sensitivity of these assays yielded 0.19 ng/ml.

Analysis of Nitric Oxide with the Griess Method

The Griess method based on the reaction of nitrite/nitrate and sulfonamide with N-ethylenediamine to form a pink complex followed by measurement of the absorbance with a spectrophotometer at a 540 nm wavelength, was used to determine the NO content in saliva, BioPhotometer D30 Single Beam[Ъ] (Eppendorf®, South America) was used for the study.

Analysis of Total Oxidant Capacity and Total Antioxidant Capacity

A colorimetric measurement kit was used to determine the TOC and TAOC levels in the saliva samples. The Total Oxidant Status Assay Kit [Ф] (Rel Assay Diagnostics ®, Turkey) was used for the TOC measurement, and the measurements were performed in line with the manufacturer's instructions. Measurements were expressed as μmol H_2O_2 Eq/L by calibrating with 10 μM hydrogen peroxide (measurement range of 0-300 μM). The Total Antioxidant Status Assay kit [Д] (Rel Assay Diagnostics ®, Turkey) was used for the quantitative TAOC measurement, and measurements were performed in accordance with the manufacturer's recommendations. The TAOC measurement results were expressed as millimole Trolox equivalents/Liter (mmol Trolox Eq/L).

Statistical Analysis

SPSS statistical software version 18[Ж] (IBM, Armonk, NY) were used for the statistical analyses. To evaluate the normality of the data distribution, Shapiro Wilk's test was used. Kruskall Wallis-H test and Mann-Whitney U test were used for the comparison of the clinical and biochemical parameters in all groups. To evaluate the relations of the biochemical and clinical parameters, the Spearman rank test was performed. To study the relationship between oxidative stress markers and adipokines levels, Linear regression analyses were used. The results were presented as the value of B unstandardized regression coefficient with 95% confidence interval and change R-squared coefficient after each variable was entered. Regression models were adjusted for BMI, PD, and age. P<0.05 was accepted as statistically significant.

RESULTS

The outcomes of the clinical parameters and demographic characteristics of the patients included in the study were presented in Table 1. Accordingly, it was determined that the measured periodontal parameters in all periodontitis patients (Group II and Group IV) were statistically higher than those in individuals with healthy periodontal tissues (Group I and Group III) (P<0.01). In addition, among the randomized patients included in the study, the mean PI, PD and CAL values in Group II were significantly higher than those in Group IV (P<0.01). In addition, the mean PI, BOP, PD and CAL values in Group II were significantly higher than those in Group IV (P<0.01). As expected, the average BMI and waist circumference index in all the obese subjects (Group I and Group II) were higher than those in the non-obese patients (Group II and Group IV) (P<0.01). In terms of the average age among the groups, the mean age of the periodontitis groups was significantly higher than the periodontal healthy groups (P<0.01), but no significant difference detected between Group I and Group III and between Group II and Group IV. In addition, the mean age in Group IV was higher than in Group II (P <0.01).

Significantly, the mean salivary leptin levels were statistically higher in Group I (6.01 ± 2.41 ng/ml) than in Group II (1.77 ± 0.83 ng/mL); and also higher in Group III (5.97 ± 2.62 ng/mL) than in Group IV (1.66 ± 0.85 ng/mL) (p <0.01). No statistically significant difference was found between the mean salivary leptin levels in Group I and those in Group III or between those in Group II and those in Group IV (Fig. 1).

Statistically, the mean salivary levels of visfatin were significantly higher in Group II (37.03 ± 8.46 ng/ml) than in Group I (22.16 ± 3.57 ng/ml); and they were also higher in Group IV (25.8 ± 4.85 ng/ml) than in Group III (20.11 ± 2.15 ng/ml) (P<0.01). In addition, the mean values in Group II were statistically significantly higher than those in Group IV (P<0.01). No statistically significant difference was found between the mean salivary visfatin levels in Group I and those in Group III (Fig. 1).

Table 1. Means and standard deviation of clinical parameters in four groups and their comparison

Groups/ Clinical Parameters	Group I (Obese and Periodontal Healthy) n=20	Group II (Obese and Periodontitis) n=18	Group III (Non-Obese and Periodontal Healthy) n=20	Group IV (Non-Obese and Periodontitis) n=20	Intergroups P values	
PI (0 to 3)	1.20±0.34	2.40±0.30	0.96±0.32	2.02±0.23	I-II	0.00**
					I-III	0.06
					II-IV	0.00**
					III-IV	0.00**
GI (0 to 3)	1.12±0.47	1.70±0.22	0.86±0.61	1.67±0.30	I-II	0.00**
					I-III	0.23
					II-IV	0.99
					III-IV	0.00**
BOP (%)	11.31±2.4	69.68±5.58	8.86±2.49	57.54±7.5	I-II	0.00**
					I-III	0.40
					II-IV	0.00**
					III-IV	0.00**
PD (mm)	2.17±0.32	4.57±0.93	1.69±0.48	3.70±0.82	I-II	0.00**
					I-III	0.12
					II-IV	0.00**
					III-IV	0.00**
CAL (mm)	-	6.75±1.14	-	5.56±0.83	I-II	0.00**
					I-III	1.00
					II-IV	0.00**
					III-IV	0.00**
Age (Years)	36.75±4.57	42.72±5.90	37.75±3.37	46.20±4.39	I-II	0.00**
					I-III	0.90
					II-IV	0.10
					III-IV	0.00**
Waist Circ. (cm)	115.55±11.25	115.61±6.98	87.90±5.37	94.05±7.41	I-II	1.00
					I-III	0.00**
					II-IV	0.00**
					III-IV	0.00**
BMI (kg/m²)	37.16±3.81	36.88±2.85	24.87±1.48	26.58±2.37	I-II	0.99
					I-III	0.00**
					II-IV	0.00**
					III-IV	0.00**

**P<0.01, statistically significant

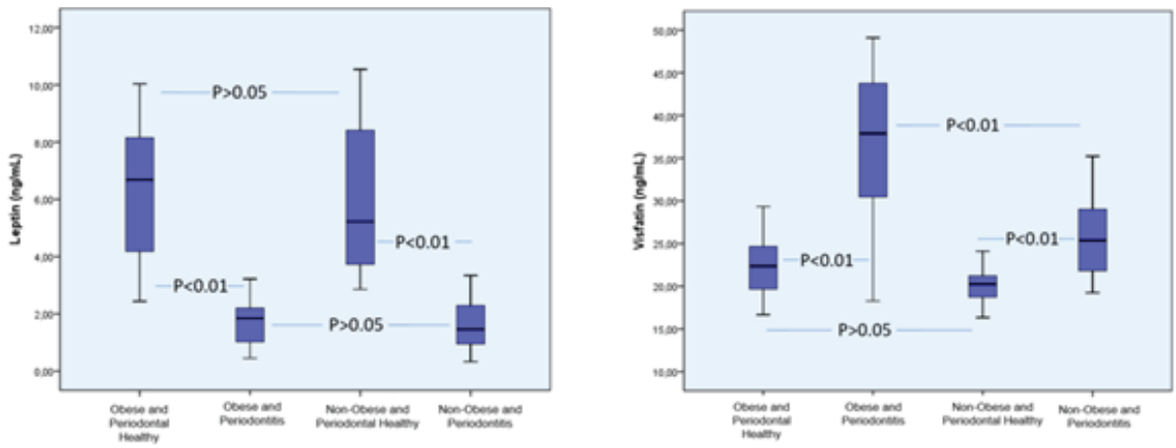

Figure 1. The comparision of leptin levels (A) and visfatin levels (B) in different groups (obese and periodontal healthy, obese and periodontitis, non-obese and periodontal healthy, non-obese and periodontitis)

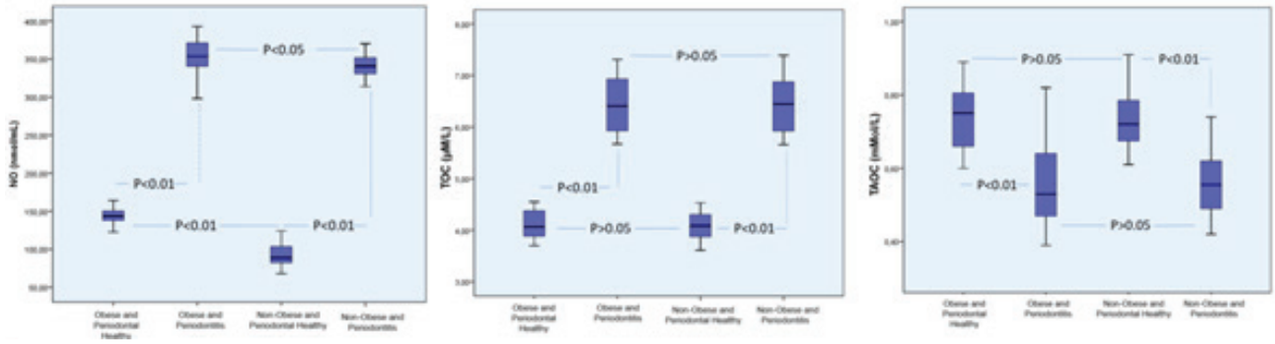

Figure 2. The comparison of nitric oxide levels (A), total oxidative capacity levels (B) and total antioxidant capacity (C) in different groups (obese and periodontal healthy, obese and periodontitis, non-obese and periodontal healthy, non-obese and periodontitis)

Statistically, the mean salivary levels of NO were significantly higher in Group II (355.18 ± 23.45 nmol/ml) than in Group I (143.56 ± 9.67 nmol/ml); and they were also higher in Group IV (341.48 ± 14.46 nmol/ml) than in Group III (93.39 ± 15.7 nmol/ml) (P<0.01). In addition, the mean values in Group II were found statistically significantly higher than those in Group IV (P<0.05); and they were also higher in Group I than in Group III (P<0.01) (Fig 2).

Statistically, the mean salivary levels of TOC were significantly higher in Group II (6.45 ± 0.53 µM/L) than in Group I (4.12 ± 0.27 µM/L); and they were also higher in Group IV (6.43 ± 0.52 µM/L) than in Group III (4.09 ± 0.27 µM/L) (P<0.01). No statistically significant difference was found between the mean salivary TOC levels in Group I and those in Group III or between I those in Group II and those in Group IV (Fig 2).

Statistically, the mean salivary levels of TAOC were significantly higher in Group I (0.74 ± 0.08 mmol/L) than in Group II (0.55 ± 0.11 mmol/L); and they were also higher in Group III (0.73 ± 0.08 mmol/L) than in Group IV (0.56 ± 0.09 mmol/L) (P<0.01). No statistically significant difference was found between the mean salivary TAOC levels in Group I and those in Group III or between those in Group II and those in Group IV (Fig 2).

After adjustment for factors, such as age, PD and BMI, which may affect correlations, used a linear regression analysis; the data are provided in Table 2. According to this analysis (adjusted for age, PD and BMI) leptin showed a negative correlation with NO and TOC in the whole group, and this explained the 79% and 74% variance in NO and TOC, respectively.

Table 2. Multivariation logistic regression analysis of TOC and NO with adipokines (visfatin and leptin) as an independent variables.

Dependent and independent variables n=78	B	95%CI	p value	ΔR^2
Dependent variable: TOC				
Leptin	-0.152	-0.218/-0.087	**<0.01**	0.746
Visfatin	-0.01	-0.028/0.026	0.948	0.671
Dependent variable: NO				
Leptin	-14.102	-19.771/-8.433	**<0.01**	0.791
Visfatin	2.047	-0.298/4.391	0.086	0.731
Dependent variable: TAOC				
Leptin	0.001	-0.009/0.011	0.772	0.411
Visfatin	-0.003	-0.006/0.001	0.137	0.428

(Adjusted for age, PPD and BMI)
P <0.01, statistically significant correlations between TOC, NO and adipokines.
Bold represents statistically significant P value differences.

Table 3. Correlations of intra-group biochemical parameters with clinical parameters in periodontitis (obese and non-obese) groups.

Biochemical/Clinical	Parameters n=38	PI	GI	BOP	PD	CAL	Age (Years)	Waist Circ.	BMI (kg/m²)
Leptin (ng/mL)	**r**	0.007	-0.136	-0.21	0.226	0.114	0.098	0.057	0.136
	P	0.966	0.415	0.901	0.173	0.494	0.557	0.732	0.415
Visfatin (ng/mL)	**r**	0.310	0.012	0.620	0.458	0.450	-0.248	0.525	0.516
	P	0.058	0.945	**0.00****	**0.00****	**0.00****	0.134	**0.00****	**0.00****
NO (nmol/Ml)	**r**	0.283	-0.085	0.410	0.060	0.135	-0.295	0.141	0.293
	P	0.086	0.611	**0.012***	0.721	0.420	0.072	0.399	0.074
TOC (µM/L)	**r**	-0.193	-0.274	-0.282	0.124	0.101	0.038	-0.094	-0.015
	P	0.246	0.096	0.091	0.459	0.459	0.823	0.575	0.929
TAOC (mMol/L)	**r**	-0.025	0.080	0.086	-0.183	-0.92	-0.029	-0.047	-0.047
	P	0.881	0.631	0.612	0.271	0.583	0.864	0.780	0.777

** P <0.01, statistically significant correlations between biochemical and clinical parameters.

* P <0.05, statistically significant correlation between NO and BOP.

Bold represents statistically significant P value differences.

In Table 3 is shown that intra-group correlations of clinical parameters with biochemical parameters in obese and non-obese periodontitis groups. While there were no clinical parameters associated with leptin, visfatin was found to be positively correlated with the values of BOP, PD, CAL, BMI and waist circumference (r=0.620, P<0.05;, r=0.458, P<0.01; r=0.450, P<0.01; r=0.516, P<0.01; and r=0.525, P<0.01, respectively) in the intra-group comparison.

DISCUSSION

Some systemic diseases, such as diabetes and obesity, that affect oxidative damage may increase the severity of the destruction associated with periodontal inflammation when the oxidant/antioxidant balance is in favor of oxidative damage.[3, 6] Many clinical trials have reported that the severity of periodontitis increases in obesity.[10, 15] In this study, we observed that the CAL, BOP, PD and PI values were higher in obese periodontitis patients than in non-obese periodontitis patients by selecting randomized patients. It has been reported that adipokines released from increasing adipose tissues in obesity may affect the systemic oxidative balance.[11, 19] For the first time in this study, the total oxidant and antioxidant capacity values in saliva were evaluated together with adipokines, and the relationship of these factors with periodontal parameters were investigated.

In our study, it was observed that the levels of leptin in saliva were lower in periodontitis patients, while they were higher in periodontal healthy individuals. This is compatible with many previous clinical trials.[26] Studies have shown that leptin levels increase in the serum during periodontal inflammation in parallel with clinical periodontal parameters but decrease in saliva and GCF.[5, 8, 10] In addition, some studies have shown that leptin levels in healthy periodontal tissues are higher than in periodontitis tissues, indicating the role of leptin in the local metabolism, defense, and regeneration of periodontal tissues.[26, 27] Although the mechanism is unknown, the authors suggested that leptin might protect the gingiva from periodontitis. One interesting result of the present study is the negative relationship between leptin and TOC levels, which might explain this protective role of leptin. In the linear regression analysis, when adjustments for age, PD and BMI were made, leptin showed a negative correlation with NO and TOC, and this explained the 79% and 74% variance in NO and TOC, respectively. In some studies, it has been shown that leptin reduces ROS production by reducing energy consumption and mitochondrial membrane potential[2]; it protects against oxidative stress at low concentrations and reduces ROS production in rats fed daily leptin[7] In addition, leptin may have a protective role against drug-induced lipid peroxidation[7] and plays a cleaner role for OH radicals.[2] Although the association of leptin with oxidative damage in periodontitis is unknown, our results propose that leptin levels may influence the susceptibility to periodontal destruction by affecting the oxidative state.

In the present study, it was determined that all periodontitis patients had higher visfatin levels than

periodontal healthy patients. In some studies it has been reported that visfatin increases in saliva during periodontal inflammation[6] and decreases after periodontal treatment[15]; furthermore it is highly expressed in periodontal tissues during periodontitis[14], and its expression may be associated with some microbiological factors.[14, 15] Visfatin is a molecule that is expressed in inflammatory conditions not only in adipose tissue but also in many tissues and cells including periodontal tissues and it regulates the inflammatory response.[5, 14] Therefore, it is a known fact that visfatin plays an essential role in the pathogenesis of many inflammatory diseases.[3, 4] Visfatin levels in obesity are found to be associated with an increased susceptibility to systemic diseases such as diabetes, cardiovascular diseases, metabolic syndrome and rheumatoid arthritis.[11] In our study, the higher salivary visfatin levels that were found in the obese periodontitis group than in the non-obese periodontitis group indicate that visfatin may be a helpful molecule for investigating whether there is increased periodontal destruction in obesity. Although visfatin appeared to be positively correlated with NO and TOC when all the groups were evaluated together, the regression analysis did not confirm this relationship. According to the available literature data and the findings of our study, we think that visfatin may not directly affect oxidative damage but may affect destruction by other mechanisms.

In our study, TOC was found to increase and TAOC was found to decrease in periodontitis consistent with previous studies.[21, 28] Nevertheless, NO, which is an indicator of oxidative damage and plays a significant role in obesity, was also evaluated in our study. NO is a molecule that regulates blood pressure, blood flow and vascular tone and plays a protective role against microorganisms, while also having oxidative damage and destructive properties.[29, 30] Because the half-life of NO is short, it is usually determined by evaluating nitrite levels as we did.[30, 31] Numerous studies on periodontitis have reported that NO is increased in the GCF and saliva.[13, 18] In microbiological studies it has also been reported that the iNOS level may be negatively related to periodontopathogens and NO may thus play a protective role in periodontal health.[31] It has been shown that NO and TOC increase in obesity by leading to endothelial dysfunction and oxidative damage, and in this way obesity plays a role in the etiology of systemic diseases.[2, 8, 10] Atabay et al.[19] observed oxidative stress markers in the GCF in obese and non-obese periodontitis patients and also observed that in obese patients, TAOC levels were lower and MDA and protein carbonyl levels were higher. The authors reported that increasing the level of oxidative stress in obesity might affect periodontal destruction and severity of the disease. Dursun et al.[21] reported that TAOC levels decreased, and TOC levels increased in the GCF and serum of obese females compared with non-obese females. Researchers have found that gingival inflammation is more prevalent in young obese women and the increased local oxidative stress that occurs with obesity may be related to periodontal disease. In our study, NO levels were found higher in obese periodontal healthy patients than in non-obese periodontal healthy patients; similarly, they were higher in the obese periodontitis group than in the non-obese periodontitis group. Our findings show us that NO may be an important molecule that affects periodontal inflammation in obesity.

In recent studies, visfatin has been reported to be increased in saliva in proportion to destruction parameters such as the pocket depth and attachment loss in periodontal inflammation.[6, 14] Then the parameters assessed in our study were related to periodontal destruction in the periodontitis groups; notably, visfatin was associated with the PD and CAL in particular. The importance of visfatin in periodontal disease was confirmed once again, as the evaluations of all patients and intra-group comparisons revealed that visfatin was highly correlated with the destruction parameters. And also, the positive correlation between visfatin, BMI and waist circumference in the periodontitis group supports our belief that increased visfatin levels in obesity may affect tissue destruction.

Although periodontitis is a disease that is known to increase in severity and prevalence with age, in our research, NO and visfatin levels were found higher in the obese periodontitis group with a younger mean age than in the non-obese periodontitis group. And again in our study, in the comparison of patients with healthy periodontal tissues, there was no mean age difference between obese and non-obese patients. In addition to this data, in the periodontitis

group, none of the parameters were correlated with age suggesting that the results we obtained were independent of age.

CONCLUSION

The reduction in salivary leptin levels and the increase in salivary visfatin levels in periodontitis indicate that these two adipokines act in different ways during periodontal pathogenesis. The change in leptin levels may affect the susceptibility to periodontal destruction by affecting the oxidative state. The detection of different levels of salivary visfatin and NO in obese and non-obese periodontitis patients shows that the severity of periodontal destruction in obese patients may be affected by these molecules. However, studies involving larger patient populations are needed to support these results.

Footnotes

[3] Williams periodontal probe, Hu-Friedy®, Chicago, IL.
[¥] Human Leptin ELISA kit, DRG Products® Germany.
[ᵒᵒ] Human Visfatin ELISA kit, Elabscience® China.
[Φ] Total Oxidant Status Assay Kit, Rel Assay Diagnostics ®, Turkey.
[Д] Total Antioxidant Status Assay kit, Rel Assay Diagnostics ®, Turkey.
[ᵇ] BioPhotometer D30 Single Beam, Eppendorf®, South America.
[ж] SPSS PASW 18 software package, IBM, Armonk, NY.

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