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Original

The levels of visceral adipose tissue-derived serpin, omentin-1 and tumor necrosis factor-α in the gingival crevicular fluid of obese patients following periodontal therapy

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Abstract: The aim of this clinical study was to determine levels of visceral adipose tissue-derived serpin (vaspin), omentin-1, and tumor necrosis factor-alpha (TNF-α) in the gingival crevicular fluid (GCF) of obese and non-obese periodontitis patients following nonsurgical periodontal therapy. Seventy-six subjects were separated into four groups according to periodontal and anthropometric measurements: a periodontal-healthy group, a chronic periodontitis (CP) group, a periodontal-healthy with obesity group, and a CP with obesity group. Nonsurgical periodontal treatment was administered to periodontitis patients. Before treatment and at 6 weeks after treatment, GCF samples were analyzed and clinical periodontal parameters were examined. Enzyme-linked immunosorbent assays were used to measure the levels of vaspin, omentin-1, and TNF-α. Obese and non-obese CP patients displayed higher levels of vaspin and TNF-α ($P < 0.008$), which declined following treatment ($P < 0.025$), and lower omentin levels ($P < 0.008$), which increased after treatment ($P < 0.025$). There was a negative correlation between the total amount of vaspin and omentin-1 in all groups. Obese and non-obese patients had opposing levels of vaspin and omentin-1 in the GCF; therefore, these may represent diagnostic and prognostic indicators of periodontal disease and therapeutic outcome.
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Keywords: visceral adipose-specific serpin, human; omentin-1 protein, human; tumor necrosis factor-alpha; periodontal disease; obesity.

Introduction

Obesity is a medical condition characterized by excessive, health-threatening levels of body fat (1). Numerous studies have suggested a correlation between obesity and chronic periodontitis (2-9). In addition, clinical periodontal parameters, such as gingival inflammation and loss of attachment, are associated with a high body mass index (BMI) and waist-to-hip ratio (WHR) (10,11). At the same time, an intensified local inflammatory response (increased levels of acute phase protein and pro-inflammatory cytokines) has been observed in obese individuals (12,13). Furthermore, a greater risk of periodontal disease was related to BMI and WHR, irrespective of periodontal disease indicators (14). Higher body mass and secretion of white adipose tissue are the likely reason why obesity is connected to periodontal disease.

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Visceral adipose tissue-derived serpin (vaspin) belongs to the family of serine protease inhibitors and is produced by white adipose tissue of obese people and Otsuka Long-Evans Tokushima Fatty rats (15,16). The relationship between vaspin and inflammatory processes remains controversial. Hida et al. reported that ageing, diabetes progression, and weight loss reduced vaspin expression and that vaspin administration enhanced insulin sensitivity and glucose tolerance (15). Conversely, metabolic syndrome caused by a high-fat diet was associated with decreased levels of vaspin, which correlated negatively with the lipid profile, TNF-α, and body weight of rats (17).

Omentin, the adipocytokine secretory protein, is primarily secreted in visceral adipose tissue. Omentin is encoded by two genes, omentin-1 and omentin-2, and the predominant isoform in human plasma is omentin-1 (18). Omentin increases the sensitivity of adipocytes to insulin, and increasing obesity and insulin resistance have been correlated with lower levels of omentin (18,19). Furthermore, individuals with coronary artery disease and rheumatoid arthritis have reduced omentin levels (20,21). In addition, omentin suppresses the ERK/NF-κB pathway to promote vasodilation in blood vessels of rats and diminishes the expression of adhesion molecules triggered by TNF-α in endothelial cells (22,23).

The role of vaspin and omentin-1 in chronic periodontitis (CP) has not been examined. Based on the assumption that the pathogenesis of periodontitis and obesity involves vaspin and omentin-1, the present study investigated the relevance of vaspin and omentin-1 as diagnostic and prognostic indicators of periodontal disease. We assessed changes in the levels of vaspin and omentin-1 in the GCF of CP patients following nonsurgical periodontal treatment. We also examined the correlation between vaspin/omentin-1 and the pro-inflammatory adipocytokine, TNF-α. The relationships between biochemical markers and clinical parameters were evaluated.

Materials and Methods

Study population

Seventy-six subjects were recruited (40 males and 36 females), who were admitted to the Periodontology Department of Bulent Ecevit University between July 2014-May 2015. Approval for this study was obtained from the Ethics Committee of Bulent Ecevit University (2014-115-17/06) and informed consent was obtained from all participants. This study was also registered at ClinicalTrials.gov (NCT02563171) on September 28, 2015.

Criteria for inclusion and exclusion

Inclusion criteria included 30-49 years of age, at least twenty natural teeth not including third molars, and glycosylated hemoglobin (HbA1c) and fasting plasma glucose (FPG) levels lower than 6.5% and 100 mg/dL, respectively. Exclusion criteria included aggressive periodontitis, periapical pathologies, excessive forces from orthodontics and occlusion, systemic diseases (such as diabetes mellitus, cancer, and HIV), disorders affecting adipokine levels or periodontal conditions, chronic high-dose steroid treatment, radiation or immunosuppressive treatment, pregnancy, lactation, smoking in the last five years, drug allergy or sensitivity, a history of periodontal treatment, and treatment with drugs (e.g., anti-inflammatories or antibiotics) in the last six months.

Primary and secondary outcome variables

Levels of vaspin, omentin and TNF-α following periodontal therapy were the primary outcome variables. Probing pocket depth (PPD), clinical attachment level (CAL), gingival index (GI), and bleeding on probing (BOP) were secondary outcome variables.

Obesity evaluation

Anthropometric measurements were made by a specialist. Obesity was evaluated based on the BMI, which is the body weight divided by height in square meters (kg/m^2), and the WHR, which is the ratio of waist-to-hip circumference (cm/cm) (24). The World Health Organization has specified six weight categories on the basis of BMI: underweight (BMI < 18.5 kg/m^2), normal weight ($18.5 \leq$ BMI < 25 kg/m^2), overweight ($25 \leq$ BMI < 30 kg/m^2), class I obesity ($30 \leq$ BMI < 35 kg/m^2), class II obesity ($35 \leq$ BMI < 40 kg/m^2), and class III obesity (BMI > 40 kg/m^2) (25). Furthermore, a WHR ≥ 0.85 (females) and ≥ 0.90 (males) is indicative of abdominal obesity (25). Individuals with a BMI ≥ 20 but <25 kg/m^2 and a WHR below obesity level were placed in the normal weight group, while individuals with a BMI ≥ 30 but <40 kg/m^2 and a WHR greater than the obesity level were placed in the obese group (24).

Periodontal examinations

Periodontal disease was diagnosed based on the standards of the international world workshop for classification of periodontal disease and conditions (26). Participants were subjected to radiographic and full-mouth periodontal assessments, covering PPD, CAL, plaque index (PI) (27), GI (28), and BOP (29). Following treatment with scaling and root planing (SRP), CP patients were subjected to another full-mouth periodontal assessment.

Participants who had a GI of 0, PPD of <3 mm, and no clinical loss of attachment and bone loss were placed in the healthy group. Participants with clinical signs of inflammation (red color and swelling of the gingival margin), a GI greater than 2, a PPD and CAL greater than 5 mm, and bone loss affecting 30% of the existing teeth were placed in the CP group.

Participants were divided into four groups according to periodontal and anthropometric measurements: group CTRL comprised nineteen individuals of normal weight with clinically healthy periodontium; group CP consisted of nineteen individuals of normal weight with generalized chronic periodontitis; group O-CTRL comprised nineteen obese individuals with clinically healthy periodontium; and group O-CP comprised nineteen obese individuals with generalized chronic periodontitis.

Intra-examiner reproducibility

A Williams periodontal probe (Hu-Friedy, Chicago, IL, USA) was used to measure PPD and CAL and to record PI, GI, and BOP scores. The same examiner (F.O.D.) performed the periodontal assessments, group classification, sampling site selection, and GCF collection blind to the research design. Before the study, measurements were calibrated on ten randomly selected individuals. Measurements were made on two distinct occasions separated by a 2-day interval. Reproducibility was determined by whether the baseline measurements were within 10% of the measurements taken after two days at the mm level (30).

Periodontal therapy

Upon completion of baseline GCF sampling, nonsurgical periodontal treatment involving SRP with manual scalers and curets (Hu-Friedy) was performed on CP patients under local anesthesia. Depending on the needs of each individual, this treatment was carried out by the same examiner (F.O.D) in 2-3 visits with a duration of 45-60 minutes over a period of two weeks. Advice on oral hygiene was given to all individuals, particularly on the use of the modified Bass method, regular tooth brushing, and suitable devices for interdental cleaning such as dental floss and an interdental brush.

Sample collection

GCF samples were taken from two sites of the mesio-buccal or disto-buccal tooth surfaces with one root. These sites were non-inflamed at baseline in control individuals. In CP patients, these sites were heavily inflamed with maximum GI, BOP, and PPD scores as well as radiographic evidence of alveolar bone loss at baseline and six weeks after SRP. Two days prior to GCF sampling, we ensured that GCF was not contaminated by probing-induced bleeding of inflamed areas. Before GCF collection, cotton rolls were used to isolate the sites in question, and saliva and supragingival plaque were removed to avoid contamination of GCF. GCF was sampled using the intracrevicular technique, which uses filter paper (31). Strips of filter paper were introduced in the crevice until mild resistance was met and were kept there for 30 s. Strips that were contaminated with blood or saliva were discarded. After GFC collection the strips were placed into separate Eppendorf tubes for each individual and aggregated into a single sample. Samples were stored at −80°C until analysis.

Measurement of vaspin, omentin-1, and TNF-α

Tubes containing sample strips were vortexed in 300 μL of phosphate-buffered saline (pH 7.4), then homogenized for 60 s followed by centrifugation at 3,000 × *g* for 15 min at 4°C. Supernatants were collected. Total quantities of vaspin, omentin, and TNF-α were measured using commercially available sandwich enzyme-linked immunosorbent assay kits (vaspin: Hangzhou Eastbiopharm Co., Ltd., Hangzhou, China; omentin: Hangzhou Eastbiopharm Co., Ltd.; TNF-α: Boster Biological Technology Co., Ltd., Pleasanton, CA, USA). Assays were performed twice according to the manufacturer's recommendation.

Total vaspin values were calculated in ng, while omentin-1 and TNF-α were calculated in pg. The minimum and maximum standard detection limits for vaspin, omentin-1, and TNF-α assays were 0.05 ng/mL and 10 ng/mL, 2 pg/mL and 600 pg/mL, and 7.8 pg/mL and 500 pg/mL, respectively. Vaspin, omentin-1 and TNF-α had a sensitivity of <0.01 ng/mL, <1.03 pg/mL and <1 pg/mL, respectively. Furthermore, vaspin and omentin-1 were both associated with intra-assay and inter-assay variation coefficients of 10% and 12% while TNF-α was associated with coefficients of 5.5% and 7.5%. Color intensity was measured at 450 nm and standard curves were used to calculate the results.

Statistical analysis

Sample size and study power were determined based on the primary outcome variable (GCF vaspin, omentin and TNF-α levels). However, sample size could not be calculated because accurate data on GCF vaspin and omentin levels was lacking. Instead, we used the TNF-α levels reported by Zimmerman et al. (24) (minimum difference of 0.65 with 0.51 pg standard deviation between obese and non-obese periodontitis patients) to determine the required sample size for appropriate study power. For

Table 1 Demographic values in the study population

	Sex[a] (male:female)	Age[a] (years)	BMI (kg/m^2)	WHR	HbA1c[a] (%)	FPG[a] (mg/dL)
CTRL	10:9	39 (34-43)	22.98 (21.35-24.39)	0.80 (0.78-0.82)	5.10 (4.70-5.30)	88 (84-94)
O-CTRL	10:9	40 (34-44)	33.92[b] (32.68-35.54)	0.97[b] (0.94-1.01)	5.10 (4.90-5.50)	94 (86-99)
CP	8:11	40 (36-43)	24.21 (21.58-24.65)	0.81 (0.79-0.83)	5.20 (5.00-5.50)	89 (86-100)
O-CP	9:10	42 (36-46)	33.97[b] (32.53-34.98)	0.97[b] (0.94-1.03)	5.30 (5.00-5.50)	91 (89-105)

Data are expressed as the median (25th-75th percentiles). CTRL: normal weight individuals with a healthy periodontium, CP: normal weight individuals with generalized chronic periodontitis, O-CTRL: obese individuals with a healthy periodontium, O-CP: obese individuals with generalized chronic periodontitis, BMI: body mass index, WHR: waist-to-hip ratio, HbA1c: glycosylated hemoglobin, FPG: fasting plasma glucose. [a]No significant difference among groups ($P > 0.008$); [b]Significant difference between CTRL and CP groups ($P < 0.008$).
Kruskal-Wallis/Bonferroni-adjusted Mann-Whitney. Bonferroni correction $\alpha = 0.05/6 = 0.008$.

Table 2 Clinical parameters before and after treatment (full-mouth periodontal examination)

	Before treatment					After treatment				
	PPD (mm)	CAL (mm)	GI	PI	BOP (%)	PPD[cd] (mm)	CAL[cd] (mm)	GI[cd]	PI[cd]	BOP[cd] (%)
CTRL	2.00 (1.78-2.18)	2.12 (1.98-2.28)	0.10 (0.05-0.13)	0.09 (0.04-0.16)	7.33 (5.95-8.67)					
O-CTRL	2.09[a] (1.86-2.31)	2.14[a] (1.92-2.38)	0.12[a] (0.06-0.15)	0.11[a] (0.08-0.16)	7.14[a] (6.52-8.67)					
CP	4,37 (3.87-4.48)	4.62 (4.18-4.74)	2.29 (1.87-2.67)	2.42 (1.92-2.67)	73.48 (67.36-76.98)	2.48 (2.42-2.79)	2.97 (2.77-3.24)	0.81 (0.45-1.02)	0.53 (0.32-0.98)	8.50 (7.11-11.67)
O-CP	4.27[b] (4.02-4.48)	4.52[b] (4.27-4.86)	2.21[b] (1.79-2.44)	2.16[b] (1.98-2.58)	69.44[b] (64.00-80.16)	2.52 (2.28-3.02)	3.02 (2.84-3.45)	0.76 (0.43-1.11)	0.46 (0.26-0.72)	8.70 (7.58-9.62)

Data are expressed as median (25th-75th percentiles). CTRL: normal weight individuals with a healthy periodontium, CP: normal weight individuals with generalized chronic periodontitis, O-CTRL: obese individuals with a healthy periodontium, O-CP: obese individuals with generalized chronic periodontitis, PPD: probing pocket depth, CAL: clinical attachment levels, GI: gingival index, PI: plaque index, BOP: Bleeding on probing, GCF: Gingival crevicular fluid. [a]No statistically significant difference from CTRL group ($P > 0.008$); [b]No statistically significant difference from CP group ($P > 0.008$); [c]No statistically significant difference between groups ($P > 0.008$); [d]Statistically significant differences from before treatment ($P < 0.025$). Kruskal-Wallis/Bonferroni-adjusted Mann-Whitney (Bonferroni correction $\alpha = 0.05/6 = 0.008$). Wilcoxon signed-rank test ($P < 0.05$).

Table 3 Clinical parameters before-after treatment (sampled sites periodontal examination) in the study groups

	Before treatment						After treatment					
	PPD (mm)	CAL (mm)	GI	PI	BOP (%)	GCF (µL)	PPD[cd] (mm)	CAL[cd] (mm)	GI[cd]	PI[cd]	BOP[cd] (%)	GCF[cd] (µL)
CTRL	2.00 (2.00-2.50)	2.50 (2.00-2.50)	0.00 (0.00-0.00)	0.00 (0.00-1.00)	0.00 (0)	0.26 (0.25-0.28)						
O-CTRL	2.50[a] (2.00-2.50)	2.50[a] (2.00-3.00)	0.00[a] (0.00)	0.50[a] (0.00-1.00)	0.00[a] (0)	0.27[a] (0.23-0.29)						
CP	7.00 (6.50-7.50)	7.00 (6.50-7.50)	2.00 (2.00-3.00)	2.50 (2.00-3.00)	100.00 (100)	0.47 (0.42-0.50)	2.50 (2.50-3.00)	3.00 (2.50-3.50)	0.00 (0.00-1.00)	0.00 (0.00-1.00)	0.00 (0-100)	0.34 (0.29-0.37)
O-CP	5.50[b] (5.00-6.50)	6.00[b] (5.50-6.50)	2.50[b] (2.00-3.00)	2.50[b] (2.00-3.00)	100.00[b] (100)	0.49[b] (0.43-0.52)	2.50 (2.50-3.00)	3.00 (2.50-3.50)	0.50 (0.00-1.00)	0.50 (0.00-1.00)	0.00 (0-100)	0.34 (0.32-0.38)

Data are expressed as the median (25th-75th percentiles). CTRL: Normal-weight individuals with a healthy periodontium, CP: Normal-weight individuals with generalized chronic periodontitis, O-CTRL: obese individuals with a healthy periodontium, O-CP: Obese individuals with generalized chronic periodontitis, PPD: Probing pocket depth, CAL: Clinical attachment levels, GI: Gingival index, PI: Plaque index, BOP: Bleeding on probing, GCF: Gingival crevicular fluid. [a]No statistically significant difference from CTRL group ($P > 0.008$); [b]No statistically significant difference from CP group ($P > 0.008$); [c]No statistically significant difference between groups ($P > 0.008$); [d]Statistically significant differences from before treatment ($P < 0.025$). Kruskal-Wallis/Bonferroni-adjusted Mann-Whitney (Bonferroni correction $\alpha = 0.05/6 = 0.008$). Wilcoxon signed-rank test ($P < 0.05$).

TNF-α levels, a sample of 14 participants was used in every group to accommodate a type II error level of β of 0.20 (80% power) and a two-tailed type I error level of α of 0.05 (5% probability). Five additional participants were recruited for each group to allow for potential withdrawals. A power of 89% in identifying outcome discrepancies prior to and following therapy was obtained through *a posteriori* power calculation.

The normal distribution of data was assessed with the Shapiro-Wilk test. The Kruskal-Wallis nonparametric test was used to analyze comparisons between biochemical and clinical parameters. The lack of normality

prompted the application of the Bonferroni-adjusted Mann-Whitney *U* test for post-hoc group comparisons. Regarding the Bonferroni correction, $\alpha = 0.05/6 = 0.008$ was considered statistically significant. The baseline and post-therapy values were compared using the Wilcoxon signed-rank test with Bonferroni correction (paired observations), where $\alpha = 0.025$ (0.05/2) was considered statistically significant. The BOP percentages and gender ratios were compared between groups using χ^2 analysis. Furthermore, the correlations between the total quantity of vaspin, omentin-1, TNF-α, BMI, and WHR with clinical periodontal parameters were evaluated using Spearman's rank correlation test. Statistical analyses were performed using SPSS software (SPPS Inc., version 19.0, Chicago, IL, USA). A *P* value < 0.05 was considered significant.

Results

Clinical findings

Descriptive statistics associated with the study population are presented in Table 1, and an overview of clinical findings is provided in Tables 2 and 3. Periodontitis patients exhibited significantly higher PPD, CAL, BOP, PI, and GI based on full-mouth and sample site measurements ($P < 0.05$). As anticipated, clinical parameters were reduced in periodontitis patients in the whole mouth and at sample sites following SRP ($P < 0.05$). There were no significant differences in periodontal clinical parameters of CP patients in the whole mouth and at sample sites following therapy ($P > 0.05$). Non-obese individuals had a lower BMI and WHR than obese individuals ($P < 0.05$). However, age, gender ratio, HbA1c and FPG were similar between groups.

Biochemical findings

Total quantities of vaspin, omentin-1, and TNF-α are presented in Fig. 1. Obese CP patients had significantly higher levels of vaspin compared with non-obese individuals without periodontitis ($P < 0.008$). Furthermore, vaspin levels were significantly reduced in individuals with periodontitis after periodontal therapy ($P < 0.025$). Additionally, following SRP, there was significant difference in vaspin levels between CP groups ($P < 0.05$).

In contrast to individuals without periodontitis and non-obese individuals, CP patients and obese individuals had lower levels of omentin-1 ($P < 0.008$). Furthermore, omentin-1 levels significantly increased in individuals with periodontitis following SRP ($P < 0.05$). In comparison to non-obese individuals, obese individuals with CP had significantly reduced omentin-1 levels following SRP ($P < 0.05$).

Similarly, TNF-α levels were significantly higher in

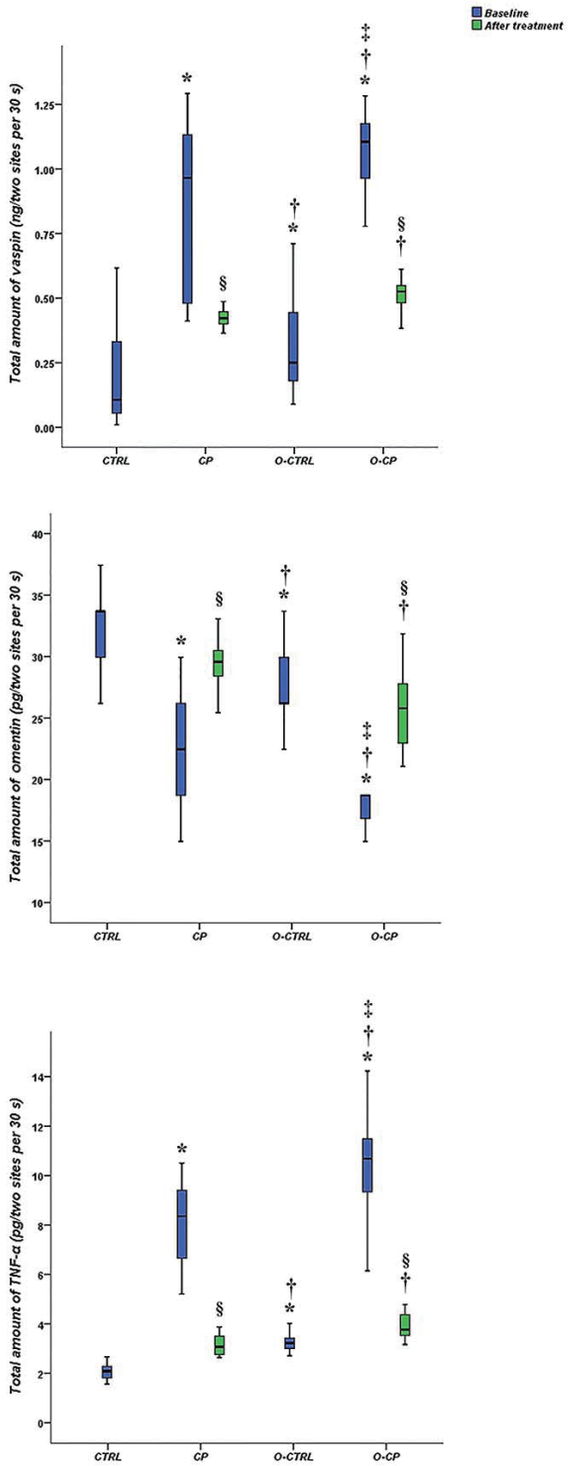

Fig. 1 Total amount of vaspin, omentin, and TNF-α in GCF. *Statistically significant difference from CTRL (Bonferroni-adjusted Mann-Whitney *U* test); †Statistically significant difference from CP (Bonferroni-adjusted Mann-Whitney *U* test); ‡Statistically significant difference from O-CTRL (Bonferroni-adjusted Mann-Whitney *U* test); §Statistically significant difference from baseline (Bonferroni-adjusted Wilcoxon signed-rank test). Data are presented as box and whisker plots. The median value is indicated by the line within the box plot. The box extends from the 25th to the 75th percentiles. Whiskers extend to show the highest and lowest values.

Table 4 Spearman's rank correlation (r) among groups with respect to vaspin, omentin, TNF-α, BMI, WHR and sampled site CAL and GI

	Vaspin to Omentin	Vaspin to TNF-α	Omentin to TNF-α	Vaspin to BMI	Omentin to BMI	TNF-α to BMI	Vaspin to WHR	Omentin to WHR	TNF-α to WHR	Vaspin to CAL	Omentin to CAL	TNF-α to CAL	Vaspin to GI	Omentin to GI	TNF-α to GI
CTRL	−0.197	0.251	−0.344	0.254	−0.359	0.311	0.133	−0.358	0.331	0.137	−0.381	0.114	NA	NA	NA
O-CTRL	−0.549*	0.686*	−0.513*	0.235	−0.481*	0.465*	0.344	−0.526*	0.459*	0.525*	−0.409	0.381	NA	NA	NA
CP	−0.495*	0.521*	−0.521*	0.354	−0.370	0.390	0.360	−0.343	0.275	0.542*	−0.512*	0.587*	0.546*	−0.533*	0.723*
O-CP	−0.562*	0.530*	−0.485*	0.632*	−0.586*	0.521*	0.628*	−0.565*	0.462*	0.527*	−0.522*	0.538*	0.505*	−0.474*	0.603*
All groups	−0.823*	0.849*	−0.870*	0.298*	−0.413*	0.398*	0.331*	−0.446*	0.424*	0.771*	−0.740*	0.783*	NA	NA	NA

*Statistically significant ($P < 0.05$). CTRL: normal weight individuals with a healthy periodontium, CP: normal weight individuals with generalized chronic periodontitis, O-CTRL: obese individuals with a healthy periodontium, O-CP: obese individuals with generalized chronic periodontitis, TNF-α: tumor necrosis factor-alpha, BMI: body mass index, WHR: waist-to-hip ratio, CAL: clinical attachment levels, GI: gingival index, NA: not applicable.

Table 5 Spearman's rank correlation (r) among groups with respect to vaspin, omentin, TNF-α and number of deep sites in CP group

	Vaspin to 4≤ PPD ≤5	Vaspin to PPD ≥6	Omentin to 4≤ PPD ≤5	Omentin to PPD ≥6	TNF-α to 4≤ PPD ≤5	TNF-α to PPD ≥6	BMI to 4≤ PPD ≤5	BMI to PPD ≥6	WHR to 4≤ PPD ≤5	WHR to PPD ≥6
CP	0.476*	0.483*	−0.465*	−0.477*	0.462*	0.496*	0.592*	0.579*	0.394	0.357
O-CP	0.581*	0.579*	−0.470*	−0.470*	0.550*	0.518*	0.836*	0.870*	0.860*	0.871*

CP: normal weight individuals with generalized chronic periodontitis, O-CP: obese individuals with generalized chronic periodontitis, PPD: probing pocket depth, TNF-α: tumor necrosis factor-alpha, BMI: body mass index, WHR: waist-to-hip ratio, *Statistically significant ($P < 0.05$).

CP and obese individuals compared with individuals without periodontitis and non-obese individuals ($P < 0.008$). Furthermore, individuals with periodontitis had significantly reduced TNF-α levels following SRP ($P < 0.025$). Additionally, there was significant difference in TNF-α levels between CP groups following SRP ($P < 0.05$).

Correlations

Correlation coefficients are presented in Tables 4 and 5. Total quantities of vaspin and omentin-1 were negatively correlated in all groups. Combined analysis of all clinical groups revealed that the total quantity of vaspin correlated positively with TNF-α, BMI, WHR, CAL, and GI, ($P < 0.05$), whereas the total quantity of omentin-1 correlated negatively with vaspin, TNF-α, BMI, WHR, CAL and GI ($P < 0.05$).

Furthermore, vaspin, TNF-α, and BMI correlated positively with the number of $4 \leq PPD \leq 5$ mm and PPD ≥ 6 mm sites. However, in the two CP groups, a negative correlation was observed between the total quantity of omentin-1 and the number of $4 \leq PPD \leq 5$ mm and PPD ≥ 6 mm sites ($P < 0.05$). Meanwhile, WHR correlated positively with the number of $4 \leq PPD \leq 5$ mm and PPD ≥ 6 mm sites in the O-CP group ($P < 0.05$).

Discussion

In this study, the influence of age on adipokine levels was minimized by selecting participants within an age range of 30-49 years. To minimize the influence of gender, gender was matched between groups. Moreover, HbA1c and FPG were evaluated to ensure no confounding effects of glucose metabolism on adipokines.

GCF constituents depict the local cellular response to infection within gingival tissue. Analyzing GCF is a non-invasive method for evaluating both periodontal disease status and the outcome of therapy (32). In our study, chemerin and IL-6 levels were evaluated in GCF, which was sampled using filter strips by an intracrevicular method. This method is quick, easy, and largely non-invasive (31). Expressing protein quantities as a total amount within GCF as opposed to concentrations was found to be more useful in determining the association of GCF constituents with periodontal disease (33,34). By increasing GCF volume in diseased areas, GCF elements would be diluted when locally secreted in the GCF (31,34). The GCF volume whereby concentration is affected directly is a dependent variance, as such, data working in concentration values shift to dependent variables in quantifying a GCF element (34). This suggests that quantifying biological indicators within GCF represents a better, valid, and reliable diagnostic and prognostic approach. For this reason, we have reported total protein amounts in this study.

Obesity is defined as a low-grade chronic systemic inflammatory disease (35). It is characterized by both expansion and inflammation of adipose tissue with elevated levels of circulating inflammatory markers and increased production of inflammation-related adipokines (36,37). TNF-α is a pro-inflammatory and immunomodulatory adipocytokine that contributes to a variety of inflammatory diseases such as obesity and periodontitis (13,37). In our study, TNF-α levels were significantly elevated in CP individuals and obese patients. After periodontal treatment, TNF-α levels decreased in CP groups.

Vaspin is a member of the serine protease inhibitor

family (15). Changes in vaspin levels have been investigated in many diseases (38-42). Vaspin was associated with obesity, type 2 diabetes mellitus, rheumatoid arthritis, and systemic inflammation (39,43). In addition, the levels of vaspin elevated in patients with acute ischemic stroke, but did not correlate with the severity of coronary stenosis (42). However, lower vaspin levels were reported in individuals with coronary artery disease and these correlated with disease severity (40). Vaspin levels increased in overweight individuals and associated positively with age, female gender, and BMI. Moreover, higher vaspin and TNF-α levels were found in obese compared to non-obese individuals. In addition, a significant correlation was observed between waist circumference (WC) and higher vaspin levels (41). This is consistent with our finding that GCF vaspin and TNF-α increased with obesity. Conversely, Youn et al. reported that serum vaspin concentrations were not different in obese individuals with type 2 diabetes compared with controls. Furthermore, circulating vaspin correlated with BMI and insulin sensitivity only in control individuals (44). El-Mesallamy et al. reported a positive correlation between vaspin and BMI, WHR, and FPG, which is consistent with our findings (39). In addition, BMI and WHR correlated positively with the number of PPD with 4-5 mm and with 6 mm or more. These findings could indicate that body weight has a detrimental effect on the periodontal status.

Only one clinical study has explored the association between GCF vaspin levels and periodontal disease (12). In agreement with our findings, vaspin levels were up-regulated in the GCF of CP and/or obese patients in that study. In addition, vaspin levels correlated positively with BMI, CAL and GI in CP patients (12), in agreement with our findings. In the present study, vaspin also correlated with the number of deep sites.

We also examined the effect of nonsurgical periodontal treatment on vaspin levels in GCF. This has not been investigated previously; therefore, a direct comparison with other studies is not possible. We showed that vaspin levels decreased in GCF after nonsurgical periodontal therapy. Taken together, this indicates that vaspin has a pro-inflammatory role in periodontitis pathogenesis, which may involve TNF-α. In contrast to this finding, Phalitakul et al. reported that vaspin has an anti-inflammatory effect on vascular smooth muscle cells (45). It was reported that vaspin inhibited TNF-α signaling by preventing reactive oxygen species production and subsequent activation of NF-κB and protein kinase C. In addition, vaspin was found to increase endothelial nitric oxide synthase activity by reducing asymmetric dimethylarginin levels, thus protecting vascular tone and structure (46).

Omentin is an adipocytokine identified as a modulator of insulin activity (18). An *in vitro* study showed that omentin enhanced insulin-stimulated glucose uptake in adipocytes (47). Omentin has an anti-inflammatory role in vascular endothelial cells by preventing TNF-α-induced COX-2 expression (48). Omentin also induced phosphorylation of AMPK and endothelial nitric oxide synthase in human umbilical vein endothelial cells and promoted migration of human primary mesothelial cells during immune defenses (48). In addition, C reactive protein- and TNF-α-induced NF-κB activation was reduced by omentin-1 in human endothelial cells (49). However, the involvement of omentin in periodontitis or the effect of nonsurgical periodontal treatment on omentin levels has not been investigated so far. In the present study, omentin-1 levels in GCF were down-regulated in both CP and obese patients. Similar to our biochemical findings, circulating omentin concentrations were lower in obese individuals (18,50). This supports our finding that omentin is negatively influenced by inflammatory processes. In agreement with our findings, it was also reported that omentin negatively correlated with BMI, TNF-α and WC (19,51).

We also examined the effect of nonsurgical periodontal treatment on omentin levels in GCF. This has not been investigated so far; therefore, direct comparisons with other studies are not possible. We demonstrated that omentin-1 levels increased after nonsurgical periodontal therapy, while TNF-α levels decreased in CP patients. Taken together, this suggests that omentin-1 may have an anti-inflammatory role in periodontitis.

In contrast to our biochemical findings, we did not find any differences in clinical parameters following periodontal treatment between obese and non-obese individuals. This finding is in agreement with the results of Ongoz Dede et al. and Duzagac et al. (52,53). Gonçalves et al. showed that reduced probing depth was lower in obese patients with CP compared with non-obese individuals with CP six months after SRP treatment; no difference was observed for three months (13,54).

This study showed that vaspin and TNF-α levels are up-regulated, while omentin-1 levels are down-regulated in obesity and/or periodontal disease. Increased vaspin and decreased omentin-1 levels were associated with worsening of periodontal and anthropometric measurements. In addition, nonsurgical periodontal therapy reduced vaspin and TNF-α levels and increased levels of omentin-1. Taken together, these findings suggest that vaspin has a pro-inflammatory role, whereas omentin-1

has an anti-inflammatory role in periodontitis.

Our findings suggest that GCF vaspin and omentin-1 levels represent reliable biomarkers for the diagnosis and prognosis of periodontal disease and treatment outcome. Periodontal treatment improved adipocytokine levels in GCF. Taken together with previous findings, our study suggests that vaspin and omentin-1 may provide promising novel insights into the management of obesity and periodontal disease. However, further prospective large-cohort studies are required to validate our findings and to better understand the pathological mechanisms that link adipokines to obesity and periodontal disease.

Conflict of interest

The authors declare that they have no financial relationships related to any products involved in this study. The study was self-funded by the authors.

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