

Original

Levels of vaspin and omentin-1 in gingival crevicular fluid as potential markers of inflammation in patients with chronic periodontitis and type 2 diabetes mellitus

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Abstract: The aims of the present study were to determine the levels of vaspin and omentin-1 in gingival crevicular fluid (GCF) in patients with chronic periodontitis (CP) with and without type 2 diabetes mellitus (T2DM), and to evaluate GCF vaspin and omentin-1 levels after non-surgical periodontal therapy. The study included 60 subjects: 15 systemically and periodontally healthy individuals, 15 periodontally healthy patients with T2DM, 15 systemically healthy patients with CP, and 15 patients with both CP and T2DM. GCF and clinical periodontal parameters were examined at the baseline and 6 weeks after periodontal therapy. Levels of vaspin, omentin-1 and tumor necrosis factor-alpha (TNF- α) were measured by ELISA, and their relative ratios were calculated. GCF vaspin and TNF- α levels were significantly higher in the CP groups than in the periodontally healthy groups ($P < 0.008$) and decreased after therapy in the former ($P < 0.025$). GCF omentin-1 levels were significantly lower in the CP groups than in the periodontally healthy groups ($P < 0.008$) and increased after therapy in the former ($P < 0.05$). Statistically significant positive correlations were found between the total amount of vaspin

and TNF- α , glycated hemoglobin (HbA1c), clinical attachment level and gingival index, whereas the level of omentin-1 was negatively correlated with these parameters in all groups ($P < 0.05$). We found that non-surgical periodontal therapy influenced the GCF levels of both vaspin and omentin-1 in the CP groups. Our results suggest that the levels of vaspin and omentin-1 in GCF could have potential application as inflammatory markers of diabetes, periodontal disease and treatment outcome.

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Keywords: vaspin; omentin; periodontitis; diabetes mellitus; gingival crevicular fluid.

Introduction

Chronic periodontitis (CP) is an infectious condition that degrades and deteriorates the vital periodontal tissues around the teeth (1). The deterioration of soft and hard tissues resulting from periodontitis is due to stimulation of the host immunoinflammatory reaction to pathogens in the mouth (2). The host response to these infectious pathogens leads to dispersal of inflammatory mediators, including the pro-inflammatory cytokines interleukin-1 (IL-1), IL-6, tumor necrosis factor alpha (TNF- α), and prostaglandins (PGE₂), the latter exacerbating deterioration of the periodontium. The increased cytokine levels present in periodontitis are also likely to aggravate existing conditions, such as atherosclerosis, diabetes, and rheumatoid arthritis (3).

A number of theories have been offered to explain the

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increased likelihood and severity of periodontal disease in individuals with diabetes mellitus (DM) (4). It has been suggested that periodontitis could have a detrimental effect on DM control, as it has been noted that periodontal treatment can improve glycemic status. Periodontitis and DM also seem to share a number of pathogenic features; both exhibit increased immunoinflammatory reactions involving similar types of biological mediator (5), and DM can increase the activity of some immunoinflammatory mediators within areas affected by periodontal disease (6,7).

Adipose tissue secretes and disseminates a range of inflammatory products, including cytokines, hormones, and various proteins, which are referred to as adipokines (8,9). These molecular species affect insulin resistance, and are also believed to have an impact on inflammation and immune reactions (10). Abnormal adipokine dissemination is one of the clearest signs of adipose tissue breakdown, and as a component of type 2 diabetes mellitus (T2DM) it might also impact other parts of the body through the release of adipokines, TNF- α , IL-6 and a number of additional pro-inflammatory cytokines (11,12). Therefore, monitoring of persistent low-level inflammation, in terms of cytokine and adipokine activity, could have potential application for assessing the likelihood of T2DM and periodontitis development.

Vaspin (visceral adipose tissue-derived serine protease inhibitor, serpinA12), a recently discovered adipokine, is present in a number of different tissues within the body. It is contained in white adipose tissue cells, which influence the liver and skeletal muscle (13) and can also be found in the skin, hypothalamus, pancreatic islets, and stomach (14). In the context of T2DM, vaspin was first studied in the visceral adipose tissue of Otsuka Long-Evans Tokushima fatty (OLETF) rats, and was found to exert an insulin-sensitising effect (15). In humans, expression of vaspin mRNA or the level of vaspin in serum is believed to be related to blood glucose levels and insulin regulation (16). Teshigawara et al. (17) have shown that serum vaspin levels are notably higher in T2DM patients than in healthy individuals, whereas another study has found that women with diabetes, but good glycemic control, have a lower amount of vaspin than diabetic women with poor glycemic control. This suggests that the serum level of vaspin may fluctuate according to the severity of diabetes (16). Li et al. (14) have also reported that vaspin levels are reduced after temporary subcutaneous insulin infusion. Taken together, these findings indicate that vaspin might have a positive impact on insulin resistance and T2DM.

Omentin (intelectin) is primarily secreted by adipose tissue stromal cells (18). It is also an adipokine, and was

discovered by examination of a visceral adipose tissue cDNA library. Humans have a pair of significantly homologous omentin isoforms: omentin-1 and omentin-2. Omentin-1 is the key form circulating in human blood, and it has been shown that the circulating levels of the protein and mRNA are notably lower in patients with degraded glucose tolerance and T2DM (19). Omentin exhibits anti-inflammatory, anti-atherogenic and anti-diabetic characteristics. However, it also stimulates vasodilation of blood vessels and reduces the impact of angiogenesis (20). While omentin is likely to enhance insulin regulation, its exact role in diabetes patients remains unclear.

Against the background of previous research outlined above, the present study was conducted to gain more insight into the role of adipokines in the pathogenetic processes connecting DM and periodontal disease, theorizing that vaspin and omentin-1 are inflammatory adipokines associated with long-term inflammation, T2DM, and CP. This research marks the first attempt to investigate levels of vaspin and omentin in gingival crevicular fluid (GCF) of CP patients with T2DM in relation to adipokine levels following non-surgical periodontal treatment. Our objectives were: 1) to identify the roles of vaspin and omentin-1 in the pathogenesis of periodontal disease in relation to the GCF level of TNF- α , which exhibits a recognized pro-inflammatory influence in this context, 2) to assess the impact of diabetes on GCF vaspin and omentin-1 levels in healthy individuals and patients with periodontal problems, and 3) to explore the impact of non-surgical periodontal treatment on GCF vaspin and omentin-1 levels in CP sufferers both with and without T2DM.

Materials and Methods

Study population and design

This study involved a total of 60 participants: 15 T2DM patients with CP (DM-CP group), 15 CP patients who were systemically healthy (CP group), 15 T2DM patients who were periodontally healthy (DM-CTRL group), and 15 individuals who were both systemically and periodontally healthy (CTRL group). The participants were all selected from among individuals scheduled to undergo either a dental procedure or a dental review at the Department of Periodontology, Faculty of Dentistry, Bülent Ecevit University, Zonguldak, Turkey, between July 2014 and May 2015. The research design was assessed and verified by the Faculty Ethics Committee, in accordance with the Helsinki Declaration of 1975, as revised in 2002 (Protocol ID:2014-116-17/06, Clinical Trial. org-NCT02544347). The participants were made

aware of the design and purpose of the research, and all provided written approval for their involvement.

The diabetic patients had been diagnosed as having T2DM, but did not have any other diagnosed or systemic diseases. The glycemic condition of the participants with T2DM was verified by testing of glycated hemoglobin A1c (HbA1c) levels. Participants with HbA1c levels of $<8\%$ and $\geq 6.5\%$ (well controlled) (21) and a diagnosis of T2DM aged over 12 months were selected to take part in the research. The diabetic individuals were prescribed balanced amounts of oral antidiabetic agents. It was determined that none had been given a change in prescription over the three months preceding the study. HbA1c levels were also tested among the non-diabetic individuals, in order to confirm their non-diabetic condition (HbA1c $<6.5\%$). Anthropometric measurements included the weight (kg) and height (m) of the participants, in order to determine their BMI (weight divided by the square of height, kg/m^2) (22). The diabetic condition and BMI values of all the involved participants were analysed by a specialist (F.K.) at the Department of Endocrinology and Metabolism, Faculty of Medicine, Bülent Ecevit University.

Periodontal condition was verified on the basis of clinical and radiographic guidelines from the 1999 definition of periodontal disease (23). The CP category exhibited radiographic signs of bone loss and clinical attachment loss (AL), with at least 6 teeth presenting a pocket probing depth (PD) of ≥ 5 mm. These teeth showed signs of bleeding on probing (BOP) across a minimum of 2 separate quadrants, and had a gingival index (GI) score of >1 . The inclusion criteria for all participants were the presence of a minimum of 20 teeth, and age of ≥ 35 years. Patients in the control (DM-CTRL and CTRL) groups were judged to be healthy if the full-mouth PD was ≤ 3 mm, GI = 0 (absence of clinical inflammation), and there was no indication of AL or radiographic evidence of alveolar bone loss (i.e., the gap between the cemento-enamel junction [CEJ] and bone crest was <3 mm at $>95\%$ of the proximal tooth sites).

Exclusion guidelines included diagnoses of unrelated systematic conditions that might affect the progression of periodontal disease, pregnancy, lactation, current and ex-smoking habit, prescription of non-steroidal, anti-inflammatory medications or antibiotics within the preceding six months, a need for antibiotic prophylaxis related to dental therapy, and having undergone non-surgical periodontal therapy within the preceding six months or surgical periodontal therapy within the preceding year. Individuals with a BMI of >24.9 kg/m^2 were deemed ineligible.

Clinical measurements and intra-examiner reproducibility

Before the actual readings were taken, 10 participants were randomly chosen and used to calibrate the investigator. The investigator took each clinical measurement on two distinct dates, set 48 h apart. Calibration of the clinician was deemed complete once two sets of readings were $>90\%$ similar at the millimeter level (24).

The participants were clinically assessed in accordance with the following criteria: plaque index (PI) (25), GI (26), PD, clinical attachment level (CAL) and BOP (27) (judged positive if it developed within 15 s following contact). Clinical readings were taken by a single calibrated examiner at a total of six locations per tooth, from the full-mouth teeth (not counting third molars) using a Williams periodontal probe (Hu-Friedy, Chicago, IL, USA). The readings were all taken in millimeters. The examiner was blinded to the diabetic condition of each participant.

Periodontal therapy

The participants were given oral hygiene advice. The periodontitis patients underwent scaling and root planing (SRP) using standard scalers and curettes (Hu-Friedy) and a local anesthetic, but no accompanying treatments were needed. The treatment was finished within a 2 weeks of the start of the research. The participants attended twice a week for two weeks, and each session lasted around 45-60 min. Six weeks after the periodontal treatment, clinical measurements and GCF sampling were carried out a second time for the CP groups. Periodontal treatment was conducted by the same investigator.

Site selection and GCF sampling

The clinical and radiological assessments and sampling site selections were carried out by a single examiner. The samples were gathered on the day following the clinical evaluations of the participants, in order to avoid contaminating the GCF with blood caused by contact with areas of inflammation. The deepest two pocket sites of single-rooted teeth (from the mesiobuccal or distobuccal surfaces) were chosen for collection of GCF, across both of the periodontitis groups. Two pocket locations showing a lack of clinical inflammation were also tested to guarantee collection of a sufficient volume of GCF across the control groups. The numbers of locations for GCF sampling were determined according to an earlier study (5), which had investigated the levels of TNF- α in GCF. For participants from the CP and DM-CP groups, locations exhibiting the most PD, symptoms of clinical inflammation (the highest GI readings with BOP), and

radiographic verification of bone loss, were tested. GCF samples were gathered at the baseline, and again 6 weeks after periodontal treatment, in both periodontitis groups. Samples were collected only at the baseline for control participants. To prevent contamination from saliva, the locations to be tested were cleaned with water, isolated using cotton balls, and carefully dried. Paper strips (Periopaper; Oraflow Inc., Smithtown, NY, USA) were delicately positioned in the crevice until a small amount of pressure was registered (intracrevicular method). They were then left in the crevice for 30 s (28). The examiner took care not to cause manual damage to the gingival tissues. Strips contaminated with blood and saliva were destroyed. The two usable samples gathered from each participant were combined to make a single sample, and placed immediately into labeled plastic Eppendorf tubes. The tubes were then stored at -80°C until further evaluation.

Biochemical analysis

For assay, 300 μL of phosphate-buffered saline (pH 7.4) was added to each of the tubes holding the test strips. The tubes were vortexed and the contents homogenized for 60 s, followed by centrifugation at $3,000 \times g$ for a further 15 min at 4°C . The supernatants were then collected, and the total amounts of vaspin (Hangzhou Eastbiopharm Co., Ltd., Hangzhou, China), omentin-1 (Hangzhou Eastbiopharm Co., Ltd.) and TNF- α (Boster Biological Technology Co., Ltd., Pleasanton, CA, USA) contained in the samples were determined by sandwich enzyme-linked immunosorbent assay employing standard commercial equipment. The samples were all assayed more than once, as advised by the manufacturer.

Total values were recorded in ng for vaspin and pg for omentin-1 and TNF- α . The recommended detection parameters for the vaspin, omentin-1, and TNF- α assays, as advised by the manufacturer, ranged from a lowermost reading of 0.05 ng/mL, 2 ng/mL, and 7.8 pg/mL, respectively, to an uppermost reading of 10 ng/mL, 600 pg/mL, and 500 pg/mL, respectively. The minimum detection parameters (sensitivity) of the assay were <0.01 ng/mL for vaspin, <1.03 pg/mL for omentin, and <1 pg/mL for TNF- α . The intra-assay and inter-assay coefficients of variation were 10% and 12% for vaspin, 10% and 12% for omentin, and 5.5% and 7.5% for TNF- α , respectively. The intensity of the color was recorded at 450 nm, and the outcomes were determined using the recommended curves provided with the assay kits. GCF vaspin, omentin-1, and TNF- α concentrations were determined by dividing the total amount of vaspin (ng), omentin (pg), and TNF- α (pg) by the volume of GCF (μL). Concentra-

tions are presented as ng/ μL or pg/ μL .

Statistical analysis

The primary outcome variable (GCF vaspin and omentin-1 levels) was employed to determine the sample size calculation and the scope and impact of the research. Unfortunately, no sample size calculation could be carried out, as there had been no clear data relating to GCF vaspin and omentin levels prior to the study. Therefore we had to perform a pilot study as a guide to the estimated readings. This pilot study involved only 10 participants in every category. It was estimated that a sample size of 14 participants (in line with omentin levels) and 10 participants (in line with vaspin levels) in each category would lead to a Type II error level of $\beta = 0.20$ (80% power) and a Type I error level of $\alpha = 0.05$ (5% probability). To consider potential 'no show' participants, 15 people were therefore included in every category. While no sample size calculation could be conducted before the research began, a retrospective calculation was made afterwards. *A posteriori* power calculation yielded a power of 86% for the ability to identify notable dissimilarities in results, before and after the therapy.

The Shapiro-Wilk test was employed to confirm that the data were appropriately spread. Comparisons of biochemical and clinical parameters were examined using the Kruskal-Wallis nonparametric test and a post-hoc group comparison supported by the Bonferroni-adjusted Mann-Whitney U test, after the data had been deemed irregularly distributed. For the Bonferroni correction, $\acute{\alpha} = 0.05 / 6 = 0.008$ was judged to be statistically significant. The Wilcoxon signed-rank test with the Bonferroni correction (paired observations) was used to contrast the baseline readings with the readings gathered after therapy. For comparison of paired data, $\acute{\alpha} = 0.05 / 2 = 0.025$ was judged to be statistically significant. A χ^2 analysis was conducted to contrast the BOP percentage and the gender split across the categories. The Spearman's rank correlation technique was used to identify interactions between the total amounts of vaspin, omentin, TNF- α and HbA1c with CAL and GI from the sample locations. These tests were all carried out using a statistical software program (SPSS Inc., version 19.0, Chicago, IL, USA). $P < 0.05$ was judged to indicate statistically significant differences.

Results

Clinical findings

There were no notable differences in age, gender, or BMI across the categories or in relation to the progression of diabetes among the diabetic groups. HbA1c was notably higher in participants with T2DM than in

Table 1 Demographic data of the study population

	Gender* (male:female)	Age* (years)	BMI* (kg/m ²)	HbA1c (%)	Duration (years)
CTRL	7:8	49.53 ± 5.15 (52.00)	22.10 ± 1.57 (22.42)	5.01 ± 0.47 (5.10)	–
CP	8:7	48.73 ± 5.24 (48.00)	22.91 ± 1.51 (22.54)	5.09 ± 0.65 (5.30)	–
DM-CTRL	7:8	48.27 ± 6.07 (49.00)	22.82 ± 1.89 (23.21)	7.32 ± 0.36 [†] (7.30)	3.93 ± 0.88* (4.00)
DM-CP	6:9	47.53 ± 5.34 (49.00)	22.96 ± 1.20 (23.20)	7.31 ± 0.46 [†] (7.30)	3.73 ± 1.10* (4.00)

CTRL, systemically and periodontally healthy control group; DM-CTRL, type 2 diabetes with periodontally healthy group; CP, chronic periodontitis with systemically healthy group; DM-CP, chronic periodontitis with type 2 diabetes group; BMI, body mass index; HbA1c, glycosylated haemoglobin A1c. Data are expressed as the mean ± standard deviation (Median). *No significant difference between groups ($P > 0.008$). [†]Significant difference from 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 and sampled sites periodontal examination) in the study groups

	Before treatment					After treatment				
	PD (mm)	CAL (mm)	GI	PI	BOP (%)	PD [‡] (mm)	CAL [‡] (mm)	GI [‡]	PI [‡]	BOP [‡] (%)
Full-mouth CTRL	2.20 ± 0.24 (2.22)	2.33 ± 0.33 (2.36)	0.15 ± 0.07 (0.18)	0.11 ± 0.06 (0.09)	6.95 ± 1.62 (6.82)					
Sampled sites CTRL	2.07 ± 0.42 (2.00)	2.17 ± 0.52 (2.00)	0.00 ± 0.00 (0.00)	0.00 ± 0.00 (0.00)	0.00 ± 0.00 (0.00)					
Full-mouth CP	3.98 ± 0.32 (4.11)	4.63 ± 0.40 (4.69)	2.17 ± 0.41 (2.18)	2.23 ± 0.41 (2.19)	72.96 ± 10.56 (69.23)	2.59 ± 0.33 (2.66)	3.26 ± 0.43 (3.24)	0.81 ± 0.31 (0.84)	0.46 ± 0.34 (0.52)	8.74 ± 2.35 (8.33)
Sampled sites CP	6.00 ± 0.60 (6.00)	6.17 ± 0.67 (6.00)	2.33 ± 0.49 (2.00)	2.27 ± 0.46 (2.00)	100.00 ± 0.00 (100.00)	2.57 ± 0.42 (2.50)	3.03 ± 0.48 (3.00)	0.27 ± 0.46 (0.00)	0.27 ± 0.46 (0.00)	0.33 ± 0.49 (0.00)
Full-mouth DM-CTRL	2.33 ± 0.33* (2.42)	2.51 ± 0.30* (2.62)	0.16 ± 0.05* (0.18)	0.11 ± 0.06* (0.09)	8.00 ± 1.47* (8.02)					
Sampled sites DM-CTRL	2.10 ± 0.57* (2.00)	2.23 ± 0.50* (2.00)	0.00 ± 0.00* (0.00)	0.00 ± 0.00* (0.00)	0.00 ± 0.00* (0.00)					
Full-mouth DM-CP	4.06 ± 0.38 [†] (4.02)	4.75 ± 0.43 [†] (4.79)	2.17 ± 0.41 [†] (2.21)	2.26 ± 0.46 [†] (2.14)	70.00 ± 7.83 [†] (69.14)	2.65 ± 0.33 (2.65)	3.38 ± 0.40 (3.34)	0.82 ± 0.36 (0.96)	0.46 ± 0.32 (0.50)	8.84 ± 2.20 (8.33)
Sampled sites DM-CP	5.97 ± 0.61 [†] (6.00)	6.23 ± 0.62 [†] (6.00)	2.27 ± 0.46 [†] (2.00)	2.27 ± 0.46 [†] (2.00)	100.00 ± 0.00 [†] (100.00)	2.60 ± 0.39 (2.50)	3.10 ± 0.54 (3.00)	0.33 ± 0.49 (0.00)	0.27 ± 0.46 (0.00)	0.40 ± 0.51 (0.00)

CTRL, systemically and periodontally healthy control group; DM-CTRL, type 2 diabetes with periodontally healthy group; CP, chronic periodontitis without type 2 diabetes group; DM-CP, chronic periodontitis with type 2 diabetes group; PD, pocket probing depth; CAL, clinical attachment level; GI, gingival index; PI, plaque index; BOP, bleeding on probing. Data are expressed as the mean ± standard deviation (Median). *No statistically significant difference from CTRL group ($P > 0.008$). [†]No statistically significant difference from CP group ($P > 0.008$). [‡]No statistically significant difference between groups ($P > 0.008$). Kruskal-Wallis/Bonferroni-adjusted Mann-Whitney. Bonferroni correction $\alpha = 0.05 / 6 = 0.008$.

non-diabetic individuals ($P < 0.05$) (Table 1). The full-mouth and sample sites PD, AL, BOP, PI, and GI were notably greater among the CP groups (CP and DM-CP) than among the periodontally healthy groups (CTRL and DM-CTRL) ($P < 0.05$). The average PD, AL, BOP, PI, GI levels were notably reduced across the CP groups after the participants had undergone nonsurgical periodontal treatment ($P < 0.05$) (Table 2).

Biochemical findings

The total amounts and concentrations of vaspin are presented in Fig. 1. The total amount and concentration of

vaspin in GCF was notably greater among the CP groups than in the periodontally healthy groups ($P < 0.008$). The total amount and concentration of vaspin was notably greater in the DM-CP group than in the CP group, and in the DM-CTRL group than in the CTRL group ($P < 0.008$). The total amount and concentration of vaspin was reduced among the CP groups after SRP ($P < 0.025$).

The total amounts and concentrations of omentin-1 are presented in Fig. 2. The total amount and concentration of omentin-1 was notably smaller among CP groups than the periodontally healthy groups ($P < 0.008$). The total amount and concentration of omentin was notably

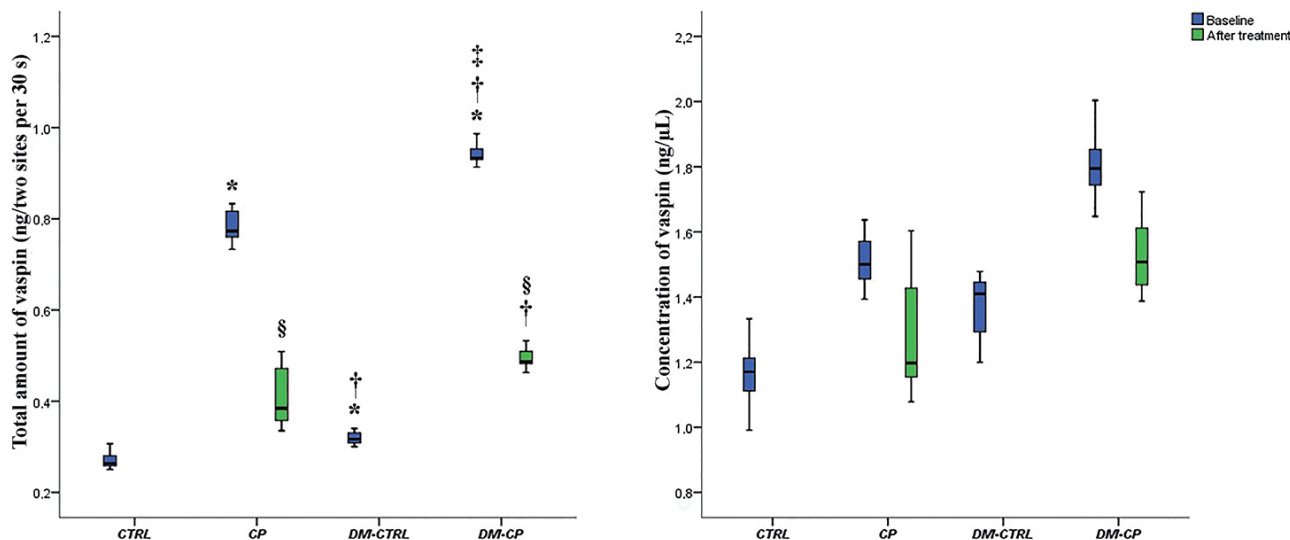


Fig. 1 Total amounts and concentrations of vaspin in GCF among the groups.

*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 DM-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.

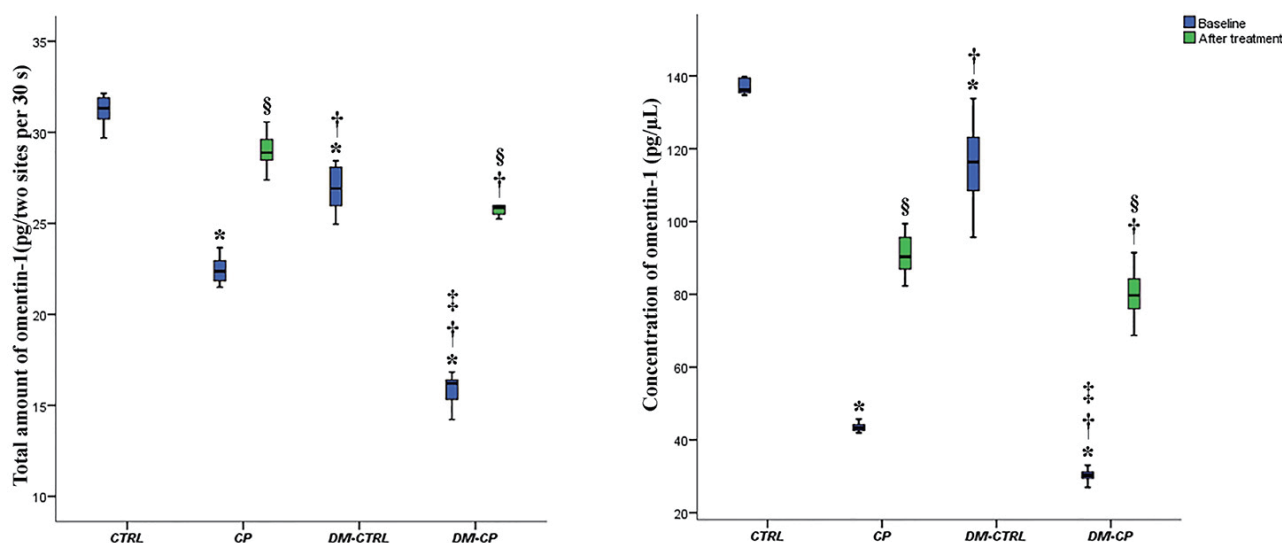


Fig. 2 Total amounts and concentrations of omentin-1 in GCF among the groups.

*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 DM-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.

smaller in the DM-CP group than in the CP group, and in the DM-CTRL group than in the CTRL group ($P < 0.008$). The total amount and concentration of omentin-1 in the CP groups actually rose after SRP ($P < 0.05$).

The total amounts and concentrations of TNF- α were

presented in Fig. 3. The total amount and concentration of TNF- α was notably greater among the CP groups than among the periodontally healthy groups ($P < 0.008$). The total amount of TNF- α was notably greater in the DM-CP group than in the CP group, and in the DM-CTRL group

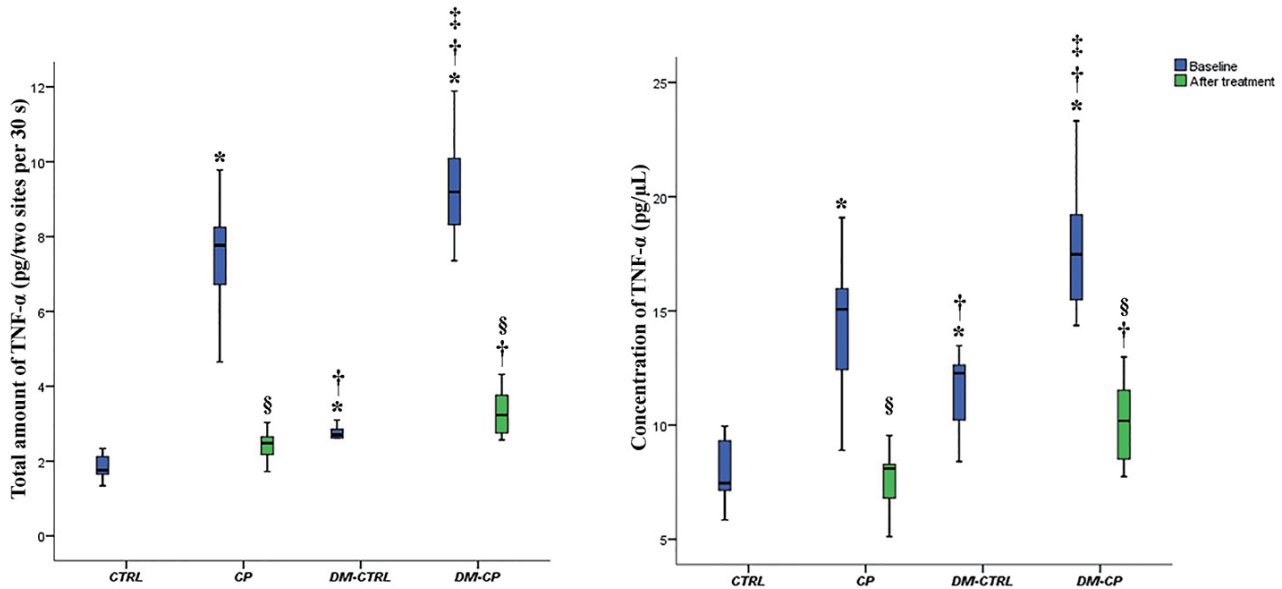


Fig. 3 Total amounts and concentrations of TNF- α in GCF among the groups.

*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 DM-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 3 Spearman's rank correlation (r) among groups with respect to vaspin, omentin, TNF- α , HbA1c, and sample site CAL and GI

		Vaspin to omentin	Vaspin to TNF- α	Omentin to TNF- α	Vaspin to HbA1c	Omentin to HbA1c	Vaspin to CAL	Omentin to CAL	Vaspin to GI	Omentin to GI
CTRL	r	-0.720*	0.453	-0.419	0.421	-0.458	0.381	-0.394	NA	NA
	P	0.002*	0.090	0.120	0.118	0.086	0.161	0.146	NA	NA
CP	r	-0.608*	0.554*	-0.518*	0.402	-0.344	0.555*	-0.543*	0.545*	-0.524*
	P	0.016*	0.032*	0.048*	0.135	0.210	0.032*	0.036*	0.035*	0.045*
DM-CTRL	r	-0.544*	0.595*	-0.619*	0.568*	-0.542*	0.460	-0.410	NA	NA
	P	0.036*	0.019*	0.014*	0.027*	0.037*	0.085	0.129	NA	NA
DM-CP	r	-0.650*	0.537*	-0.624*	0.562*	-0.619*	0.536*	-0.633*	0.530*	-0.733*
	P	0.009*	0.039*	0.013*	0.029*	0.014*	0.039*	0.011*	0.042*	0.002*
All groups	r	-0.976*	0.942*	-0.945*	0.449*	-0.451*	0.830*	-0.831*	0.846*	-0.855*
	P	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*

CTRL, systemically and periodontally healthy control group; DM-CTRL, type 2 diabetes with periodontally healthy group; CP, chronic periodontitis without type 2 diabetes group; DM-CP, chronic periodontitis with type 2 diabetes group; TNF- α , tumor necrosis factor-alpha; GI, gingival index; CAL, clinical attachment level; HbA1c, glycated haemoglobin A1c. *Statistically significant ($P < 0.05$). NA: Not applicable.

than in the CTRL group ($P < 0.008$). The total amount of TNF- α in the CP groups was reduced following SRP ($P < 0.025$).

Correlations

The correlation coefficients are presented in Table 3. There was a negative correlation between the total amount of vaspin and that of omentin-1 across all categories. There was a statistically significant positive correlation between the total amount of vaspin and that of TNF- α

or HbA1c, sampled site CAL and GI, despite the total amount of omentin-1 being negatively correlated with that of vaspin, TNF- α or HbA1c, sampled site CAL and GI across all categories, after all clinical categories had been investigated at the same time ($P < 0.05$).

Discussion

Adipokines are thought to act via their impact on insulin sensitivity, which is why fluctuating levels of these hormones are linked to T2DM (29). Recently, Ogawa et

al. (30) reported that adipokines had an impact on the pathogenesis of T2DM and periodontitis. Some adipokines (leptin) have an anti-inflammatory influence on tissue fortification whereas others (resistin and visfatin) have a pro-inflammatory influence. There are also adipokines (progranulin) that have both pro-inflammatory and anti-inflammatory characteristics (31,32). The importance of higher concentrations of resistin, leptin, IL-6 and TNF- α or reduced levels of adiponectin and visfatin in the pathogenesis of T2DM and periodontal disease is widely acknowledged and clearly understood (3,5,9,12,22). Of the recently identified adipokines, vaspin and omentin are those that need to be investigated more fully, as they are linked to T2DM and periodontal disease in humans. Vaspin and omentin are thought to be insulin-sensitising factors, but their impact on serum insulin concentrations in T2DM is unclear. Nevertheless, vaspin and omentin-1 are currently being assessed for their therapeutic applicability in the context of periodontal disease. Molecular research to explore the impact of these adipokines on inflammatory pathways would be desirable, as this could yield new techniques for pharmaceutical therapy combined with host modulation strategies. Furthermore, investigation of GCF vaspin and omentin-1 levels could throw light on the biological connection between DM and periodontitis. Against the above background, the aim of the present study was to identify the impact of vaspin and omentin-1 levels in GCF on periodontal health and dysfunction, in both the presence and absence of T2DM, and to assess GCF vaspin and omentin-1 levels before and after treatment. The impact of age and gender on vaspin and omentin-1 levels was controlled for and reduced by incorporating the same number of men and women in each category, and by choosing participants who fell within a specific age range (35-60 years).

There is substantial support for the theory that adipokines influence a broad range of different physiologic and pathologic functions, such as immunity and inflammation (9). Vaspin has been demonstrated to play roles in insulin resistance, metabolic syndrome and T2DM (33). However, the overall impact of vaspin on glucose dysregulation in humans is not fully known. The serum vaspin levels in participants with T2DM were higher in those with normal glucose tolerance (34,35). Pradeep et al. (36) have explored the levels of vaspin in GCF and tear fluid in patients with CP, both with and without obesity. This was the first attempt to estimate the levels of vaspin in GCF of patients with CP, and revealed that the average vaspin concentration, in both GCF and tear fluid, was higher in overweight individuals with CP group, followed in order by healthy obese individuals, those

with CP, and non-obese healthy individuals. Average vaspin levels were correlated with BMI, CAL, and PD (36). It is important to note that this study differed from ours because our study did not involve obese participants with a BMI of >24.9 kg/m². With respect to BMI, no notable differences emerged between the categories. The present study investigated the impact of vaspin and omentin-1 on the connection between two inflammatory conditions, periodontitis and DM.

Omentin is also a recently discovered adipokine, which increases the insulin sensitivity of human adipocytes (37). Wurm et al. (38) recorded no notable fluctuations in rotating omentin levels before and two h after glucose intake. Zhang et al. (39) calculated the levels of serum omentin-1 in healthy individuals and T2DM patients of normal weight, and found that the levels were notably lower in the T2DM patients. The reduced serum omentin-1 levels identified in T2DM might lead to a decrease of insulin-generated glucose uptake by visceral and subcutaneous adipocytes or additional insulin-sensitive tissues. This could have an impact on insulin resistance recorded in T2DM (19). Insulin and inflammation are closely linked (40). As such, omentin levels are likely linked to inflammation, because the levels were lower in individuals with chronic inflammatory bowel disease (41), in the synovial fluid of rheumatoid arthritis patients (42), and in T2DM patients (39). Omentin probably plays an important part in the pathogenesis of inflammation in these conditions.

The results of this study show that participants in the DM-CP group had higher levels of vaspin and TNF- α and reduced levels of omentin-1 in their GCF. There were significant differences between the DM-CP group and the CP group. This might be because of fluctuation in the release of these molecules under local inflammatory conditions in the periodontium, in addition to the systemic inflammatory status related to hyperglycemia. The inflammatory reaction in CP is defined by localized stimulation of inflammatory mediators. DM changes the immunologically active molecules by raising the amounts of cytokines present in periodontal tissues, and in turn this aggravates the development of the disease (43).

Vaspin and TNF- α levels were notably greater and omentin-1 levels were notably lower in the CP groups than in the DM group. These molecules can be identified in GCF, because the latter lies close to periodontal tissues, where periodontal problems are initiated. Thus, sampling of GCF could be used to assess the severity of local inflammatory conditions within the periodontium. The content of GCF is determined by systemic circulation. The serum levels of these molecules under inflammatory conditions might be comparable between CP and DM

patients. However, this study failed to assess the serum levels of these indicators, and this was one of the main restrictions of our research.

Participants in the CTRL and DM-CTRL categories demonstrated statistically significant differences in their vaspin, omentin, and TNF- α levels, even though the clinical parameters in the two categories remained the same. The notable dissimilarities in the levels of vaspin, omentin-1 and TNF- α might be explained by the presence of a systemic inflammatory condition. DM-induced inflammation might also be attributable to polymorphism within the promotor areas of cytokine genes or the activities of advanced glycation end products with monocyte receptors, inducing activation of nuclear factor-Kb (44).

In periodontitis, the pro-inflammatory cytokine TNF- α is a powerful mediator of tissue degradation, stimulating deterioration of connective tissue and resorption of alveolar bone (45). Also, because TNF- α is such an effective disruptor of tyrosine kinase activity within the insulin receptor, it is considered to be an etiologic factor in the emergence of insulin resistance (46). A number of researchers have reported an increased concentration of TNF- α in GCF in activated periodontal locations, and decreased concentrations following periodontal treatment. It has also been found that periodontal treatment is followed by a notable decrease in HbA1c levels in T2DM patients (46,47). In this study, we examined the correlation of GCF vaspin and omentin 1 levels with the GCF levels of TNF- α , because TNF- α has a recognizable pro-inflammatory impact on CP and T2DM. In line with earlier research, GCF TNF- α levels were notably greater in the CP and T2DM groups than in the control groups. Our findings demonstrate that periodontal treatment can significantly lower the levels of vaspin and TNF- α , and also boost omentin-1 levels, in GCF. We demonstrated a notable difference in all clinical periodontal parameters across both CP groups following treatment. The CP groups showed a substantial dip in PD, AL, BOP, PI, GI levels after periodontal therapy. The outcomes of the study indicate that non-surgical periodontal treatment has the potential to significantly ameliorate periodontal inflammation. Moreover, a statistically significant positive correlation was demonstrated between the total amount of vaspin and levels of TNF- α , HbA1c, CAL and GI, despite the fact that the total amount omentin-1 was negatively correlated with these parameters across all categories once the clinical groups were all investigated at the same time.

These findings allow us to suggest that vaspin and omentin-1 levels might indicate chronic inflammation in periodontally healthy individuals and that periodontitis

may impact vaspin and omentin-1 release in T2DM patients. They also indicate that vaspin probably plays an important pro-inflammatory role. However, omentin might also play an important anti-inflammatory role in CP and T2DM patients. This research is the first of its kind, involved a fairly small sample size, and was carried out over a short time frame. Future research would need to incorporate more samples and take into account the increase in vaspin levels and the reduction in omentin-1 levels as hazard-based variables of periodontal disease and T2DM.

The levels of vaspin and omentin-1 in GCF were correlated with periodontal disease progression and hyperglycemic condition. Within the constraints of the study, it can be stated that higher vaspin and reduced omentin-1 levels were identified in GCF of CP patients, both with and without diabetes, and that non-surgical periodontal treatment was advantageous for reducing levels of both. Control of vaspin and omentin 1-levels via the use of periodontal treatment could enhance insulin sensitivity and glucose tolerance in TD2M patients. Our findings suggest that GCF vaspin and omentin-1 levels could have potential as diagnostic and prognostic markers of diabetes, periodontal disease, and response to therapy. However, a larger, more comprehensive study will be needed to confirm these outcomes and to shed more light on the pathological functions that connect adipokines to T2DM and periodontal disease.

Conflict of interest

The authors declare that they have no financial relationships related to any products involved in this study. This study was supported by all the authors.

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