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Original

Visfatin levels in gingival crevicular fluid and serum before and after non-surgical treatment for periodontal diseases

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Abstract: The purpose of this study was to evaluate visfatin levels at different stages of periodontal disease and in healthy tissues. In addition, the effect of non-surgical periodontal therapy on visfatin levels in gingival crevicular fluid and serum was investigated. Forty-five patients were divided into three groups based on clinical and radiographical findings. Group 1 comprised periodontally healthy individuals (n = 15); group 2 comprised patients with gingivitis (n = 15); and group 3 was composed of patients with generalized chronic periodontitis (n = 15). Gingival crevicular fluid and serum samples were collected before treatment and at 1, 3, and 6 months after treatment. Visfatin levels were measured by enzyme-linked immunosorbent assays. Gingival crevicular fluid and serum visfatin levels were higher in patients with chronic periodontitis than those with gingivitis or healthy controls ($P < 0.016$). In addition, visfatin levels were higher in the gingivitis group than in healthy controls ($P < 0.016$). Non-surgical periodontal treatment resulted in a significant reduction in gingival crevicular fluid and serum visfatin levels. Furthermore, visfatin levels increased with inflammation and decreased following periodontal treatment. Our findings suggest that visfatin is an inflammatory biomarker of periodontal disease.
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Keywords: visfatin; periodontal disease; gingival crevicular fluid; serum; post-treatment.

Introduction

Periodontal disease is a complex biological process involving interactions between microorganisms and the immune/inflammatory response of the host (1). Periodontal destruction is mediated by locally produced proinflammatory cytokines in response to bacterial infection (2). Inappropriate inflammatory responses to pathogenic infection result in loss of connective tissue attachment and alveolar bone breakdown (3). Gingivitis is a gingival inflammation without the loss of supporting periodontal tissues, while periodontitis is characterized by impaired attachment and loss of bone (4). Inflammatory biomarkers can provide additional information to disease progression beyond standard clinical and radiographic examinations (5).

The genetic, microbiological, environmental, and immunological factors involved in the transition from gingivitis to periodontitis have not been defined. Several cytokines are released in response to periodontal infection (5), and cytokine release measurements are used to determine the local responses to bacterial infection. Many cytokines in the gingival crevicular fluid (GCF) have been suggested as potential markers of periodontal destruction (6). Proinflammatory cytokines, such as IL-6 and TNF-α, are considered to be associated with periodontal inflammation (7).

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Adipose tissue produces a variety of cytokines and inflammatory molecules, commonly referred to as adipo(cyto)kines, which regulate different inflammatory processes and are involved in the pathophysiology of periodontitis. Proinflammatory cytokines, such as TNF-α or IL-6, are overexpressed in the adipose tissue of obese patients and contribute to insulin resistance (8,9). Moreover, adipocyte-derived cytokines interfere with immune processes (10). Levels of these cytokines decrease after weight loss (11).

Visfatin is a 52-kDa molecule secreted primarily by visceral white adipose tissue. It was previously described as pre-B-cell colony-enhancing factor (PBEF) and upregulated the production of the pro- and anti-inflammatory cytokines IL-1β, IL-1Ra, IL-6, IL-10, and TNF-α in human monocytes (10). Visfatin was identified in human bone marrow as a cytokine-like molecule that is secreted by activated lymphocytes and stimulates the early stages of B cell formation (12). It is also secreted by neutrophils in response to pathogens and stimulates monocytes to produce inflammatory mediators (10). Visfatin has been associated with several chronic inflammatory diseases, including diabetes mellitus (13), inflammatory bowel disease (10), rheumatoid arthritis (14), acute lung disease (15), and sepsis (16).

Periodontal inflammation increases proinflammatory cytokine production, which may influence visfatin expression in periodontal tissues. This led us to hypothesize that visfatin is a potential biomarker for periodontal diseases.

Non-surgical periodontal therapy is an anti-infective therapy with mechanical and chemotherapeutic approaches to minimize or eliminate the microbial biofilm, which is the primary etiology of gingivitis and periodontitis. Mechanical therapies, such as supragingival and subgingival scaling and root planing, use hand or power-driven scalers and curets (17).

Proinflammatory cytokines associated with periodontal inflammation such as IL-6 and IL-1β may be related to visfatin expression in periodontal tissues (18). Visfatin has been associated with several chronic inflammatory diseases; one such example is rheumatoid arthritis. Periodontitis and rheumatoid arthritis are chronic inflammatory conditions with similar pathological features (19). We evaluated visfatin levels in GCF and serum in periodontally healthy tissue and at different stages of periodontal disease to test the hypothesis that visfatin is as a diagnostic biomarker and increases as periodontal disease progresses. In addition, we quantified the effect of non-surgical periodontal therapy on visfatin levels in GCF and serum 1, 3, and 6 months after treatment. Furthermore, we tested the correlations between visfatin levels and clinical parameters.

Materials and Methods

Study population

This study investigated a population of individuals who came to the Department of Periodontology at Bulent Ecevit University. Forty-five participants were recruited and included 22 women and 23 men aged 25-45. The study was designed in accordance with the rulings of the declaration of Helsinki and the Ethics Committee of Ondokuz Mayıs University (2012/568). Informed written consent was provided by every participant.

Inclusion criteria

The participants were separated into three groups depending on the periodontal parameters as follows: Group 1 contained individuals with a clinically healthy periodontium ($n = 15$), group 2 was composed of patients with gingivitis ($n = 15$), and group 3 contained patients with generalized chronic periodontitis ($n = 15$). A classification system published in 1999 was utilized for disease characterization (4).

A clinical full-mouth examination and a radiographic periodontal examination were performed before participation in the study. The plaque index (PI) (20), bleeding on probing (BOP) (21), gingival index (GI) (22), and probing pocket depth (PPD) were measured during this examination. All participants had at least 20 teeth, not including their third molars. According to the examination, group 1 had no indications of inflammation, no loss of attachment, or loss of bone (GI = 0, PPD ≤ 3 mm, CAL ≤ 3 mm). Individuals in group 2 showed signs of inflammation, such as redness, higher BOP levels, and edema. However, no attachment loss or alveolar bone loss were observed (GI ≥ 1, PPD and CAL ≤ 3 mm). Individuals in group 3 showed clinical signs of inflammation, GI > 1, PPD ≥ 5 mm, CAL ≥ 5 mm, and alveolar bone loss affecting >30% of the existing teeth. A Williams periodontal probe was used to take all the measurements (Hu-Friedy, Chicago, IL, USA) and all measurements were made by one person. Ten people were chosen at random to calibrate the measurements beforehand. These people were assessed on two separate occasions, with two days separating each of the measurements. These findings were satisfactorily reproducible, the baseline measurements and measurements taken after 48 h were within 10% of each other on the mm scale (23).

Exclusion criteria

Individuals with diseases that could influence visfatin

expression such as rheumatoid arthritis, diabetes mellitus, cancer, or obesity, were excluded. Body mass index (BMI) was used to determine obesity and was calculated in kilograms divided by height in meters squared. Participants with a BMI greater than 24.9 were excluded. Further exclusion criteria were pregnancy, lactation, history of medications that could impact periodontal status, aggressive periodontitis, smoking, tobacco use, and periodontal care within the last half year. Each participant was questioned directly about tobacco use.

Collecting GCF and site selection

To prevent contamination of samples with blood, the samples were collected by a single researcher (CCT) a day after the measurements were taken. A single sampling site was set for each individual, and GCF samples were collected from sites with no clinical inflammation in group 1. In group 2, GCF samples were collected from the main site of inflammation displaying redness, edema and BOP without CAL. GCF samples were taken from sites with the most significant inflammation and CAL in group 3.

Supragingival plaque was managed at baseline, 1 month, 3 months, and 6 months after periodontal therapy if needed. To prevent contamination from saliva, samples were collected with the use of cotton rolls, and GCF samples were taken after the site had been carefully dried using the intracrevicular method with filter paper (Periopaper, ProFlow, Inc., Amityville, NY, USA) (24). The strips were inserted into the crevice until resistance was felt and put to one side in stasis for 30 s. Electronic impedance was used to determine the GCF volume of every strip (Periotron 8000, ProFlow Inc., Hewlett, NY, USA). Strips with traces of blood or saliva were discarded. All strips were placed into an Eppendorf tube and stored at −40°C before assaying.

Serum collection

Peripheral blood samples were collected (2 mL) from the antecubital fossa via venipuncture using a sterile syringe, and the serum component was separated by centrifugation for 5 min at 3,000 g. Serum was stored at −40°C.

Periodontal treatment

The same investigator (CCT) conducted all non-surgical periodontal treatments. Patients in group 3 received non-surgical periodontal therapy along with oral hygiene guidelines once the baseline GCF and the serum samples were collected. Non-surgical periodontal therapy included scaling and root planing (SRP) with manual scalers and curets (Hu-Friedy). SRP was applied quadrant-by-quadrant over 2 months. A local anesthetic was applied where necessary. Patients in group 2 received non-surgical periodontal treatment for 4 weeks involving scaling along with oral hygiene guidelines. Oral hygiene instructions were provided to group 1 with no additional treatment. All oral hygiene guidelines were in accordance with the modified Bass method and included brushing and dental flossing repeated every 2 weeks. After periodontal therapy, patients were reassessed and serum and GCF samples were collected 1 month, 3 months, and 6 months after treatment. All patients were present for the subsequent assessment. A follow-up examination was performed on all participants and no participant reported the use of antibiotics during the study period.

Assaying visfatin levels

Sample strip tubes were combined with 100 µL phosphate-buffered saline (pH 7.4) and homogenized for 1 min. Samples were vortexed, then centrifuged for 15 min at 4°C at 3,000 g. Total visfatin levels were measured in the supernatants using a sandwich enzyme-linked immunosorbent assay, using commercially available kits (Hangzhou Eastbiopharm Co. Ltd, Hangzhou, China). All samples were assayed in replicate according to the manufacturer's instructions. The amount of concentration GCF was calculated by dividing the total amount (ng) (the visfatin level in GCF analyzed by ELISA using commercially available kits) by the volume of the GCF (µL).

Statistical analysis

We used the primary outcome variable (the difference in the GCF visfatin levels post-treatment) to calculate the sample size. Predictions and estimations were based on the pilot research study, and ten individuals were used per group. We speculated that a sample population of 11 individuals within all groups would allow a type I error level of $\alpha = 0.05$ (5% probability) and a type II error level of $\beta = 0.20$ (80% power). To allow for possible dropouts, we included 15 patients in each group.

To determine normal distribution, the Shapiro-West test was used. Once normality was rejected ($P < 0.05$), the inter-group comparisons were analyzed by nonparametric Kruskal-Wallis test, after which the Mann-Whitney *U* (Bonferroni-adjusted) test was utilized for a post-hoc comparison ($\alpha = 0.05/3 = 0.016$ was considered statistically significant). Inter-group differences in clinical and biochemical parameters at 1, 3 and 6 months post-treatment were assessed using the Mann-Whitney *U* test. The paired clinical limitations (between 6 months and baseline) and intra-group comparisons

Table 1 Clinical parameters at baseline and 6 months (periodontal examination of full-mouth and sampled sites) in the different groups

	Baseline					6 months				
	PPD (mm)	CAL (mm)	GI	PI	BOP (%)	PPD (mm)	CAL (mm)	GI[c]	PI[c]	BOP[c] (%)
Full-mouth group 1	2.07 ± 0.39 (1.96)	2.07 ± 0.39 (1.96)	0.00 ± 0.00 (0.00)	0.41 ± 0.14 (0.43)	0.00 ± 0.00 (0.00)					
Sampled sites group 1	1.80 ± 0.41 (2.00)	1.80 ± 0.41 (2.00)	0.00 ± 0.00 (0.00)	0.00 ± 0.00 (0.00)	0.00 ± 0.00 (0.00)					
Full-mouth group 2	2.11 ± 0.25 (2.16)	2.11 ± 0.25 (2.16)	1.64 ± 0.05[a] (1.64)	1.05 ± 0.07[a] (1.03)	77.79 ± 7.32[a] (78.12)	2.03 ± 0.30 (2.03)	2.03 ± 0.30 (2.03)	0.23 ± 0.16 (0.24)	0.35 ± 0.14 (0.35)	11.19 ± 2.05 (10.58)
Sampled sites group 2	2.00 ± 0.76 (2.00)	2.00 ± 0.76 (2.00)	2.40 ± 0.51[a] (2.00)	2.47 ± 0.52[a] (2.00)	100.00 ± 0.00[a] (100.00)	1.60 ± 0.51 (2.00)	1.60 ± 0.51 (2.00)	0.33 ± 0.49 (0.00)	0.13 ± 0.35 (0.00)	1.33 ± 0.49 (1.00)
Full-mouth group 3	4.56 ± 0.43[ab] (4.44)	5.80 ± 0.34[ab] (5.78)	2.53 ± 0.18[ab] (2.63)	2.28 ± 0.20[ab] (2.26)	88.80 ± 3.58[ab] (88.61)	2.50 ± 0.22[bc] (2.51)	3.30 ± 0.52[bc] (3.33)	0.55 ± 0.20[b] (0.56)	0.46 ± 0.21 (0.47)	11.32 ± 2.65 (11.26)
Sampled sites group 3	6.20 ± 0.77[ab] (6.00)	7.13 ± 0.83[ab] (7.00)	2.53 ± 0.52[a] (3.00)	2.13 ± 0.35[a] (2.00)	100.00 ± 0.00[a] (100.00)	2.67 ± 0.49[bc] (3.00)	3.60 ± 0.51[bc] (4.00)	0.40 ± 0.51 (0.00)	0.33 ± 0.49 (0.00)	1.13 ± 0.35 (1.00)

Data are expressed as the mean ± standard deviation. [a]Statistically significant difference from group 1 ($P < 0.016$); [b]Statistically significant difference from group 2 ($P < 0.016$); [c]Statistically significant difference from baseline ($P < 0.05$). Kruskal-Wallis/Bonferroni-adjusted Mann-Whitney *U* (Bonferroni correction $\alpha = 0.05/3 = 0.016$) Wilcoxon signed-rank test.

were analyzed using the Wilcoxon signed-rank test for the periodontitis and gingivitis groups. The Freidman test was used to determine significant differences in biochemical parameters in group 2 and group 3 at baseline, 1, 3, and 6 months. To assess the significance of pairwise differentiation, the Wilcoxon signed-rank test was used with Bonferroni correction to account for multiple comparisons. For comparison of paired periodontal parameters (baseline and 6 month), $\alpha = 0.05$ was applied to determine statistical significance, and for comparison of paired biochemical parameters (at baseline and 1, 3, and 6 months) $P = 0.05/6 = 0.008$ was applied to determine statistical significance. χ^2 analysis determined the percentage of genders among the groups and the BOP percentage. Spearman's rank correlation test was used to test that the relationship between the total volume of visfatin and the visfatin serum levels were within clinical periodontal limits. All tests were performed using SPSS, and $P < 0.05$ indicated statistical significance (SPSS Inc., version 19.0, Chicago, IL, USA).

Results

Clinical results

Table 1 summarizes the clinical findings. PPD, BOP, GI, PI, and CAL are provided. All clinical parameters were significantly greater in group 2 and group 3 compared with group 1 (Kruskal-Wallis/Bonferroni-adjusted test, Mann-Whitney *U*, $P < 0.016$). There were no significant differences in sex, age (Kruskal-Wallis, Chi-square test, $P > 0.05$), or BMI (Kruskal-Wallis, $P > 0.05$) of participants between groups (Table 2). Six months after SRP treatment finished, the clinical parameters were significantly reduced with regard to full-mouth and sample site examinations (Mann-Whitney *U*, $P < 0.05$).

Table 2 Age, gender distribution and BMI scores in the different groups

Parameter	Group 1	Group 2	Group 3
Age (years)[a]			
Mean ± SD	34.80 ± 4.5	35.20 ± 4.4	38.73 ± 4.3
Median	36	36	38
Gender[b]			
Males	7	8	8
Females	8	7	7
BMI[a]			
Mean ± SD	22.43 ± 1.5	23.11 ± 1.1	22.39 ± 1.5
Median	22.65	23.06	22.48

Data are expressed as the mean ± standard deviation. [a]Kruskal-Wallis, $P > 0.05$; [b]Chi-square tests, $P > 0.05$.

Biochemical results

Visfatin was detected in all GCF and serum samples. Figures 1 and 2 depict the total amounts and concentration amounts of visfatin in GCF, respectively. In group 3, GCF visfatin levels were statistically higher compared with group 1 and group 2 (Bonferroni-adjusted Mann-Whitney *U* test, $P < 0.016$). Furthermore, GCF visfatin levels were reduced in the healthy participants compared with gingivitis patients. Serum visfatin levels were also significantly reduced in group 1 and group 2 compared with group 3 (Fig. 3). (Bonferroni-adjusted Mann-Whitney *U* test, $P < 0.016$). Serum visfatin levels were significantly higher in group 2 compared group 1 (Bonferroni-adjusted Mann-Whitney *U* test, $P < 0.016$). Serum levels are consistent with visfatin levels detected in GCF. Figure 4 depicts the relationship between visfatin levels and periodontal disease.

Impact of periodontal therapy on visfatin levels

Periodontal treatment with SRP significantly reduced

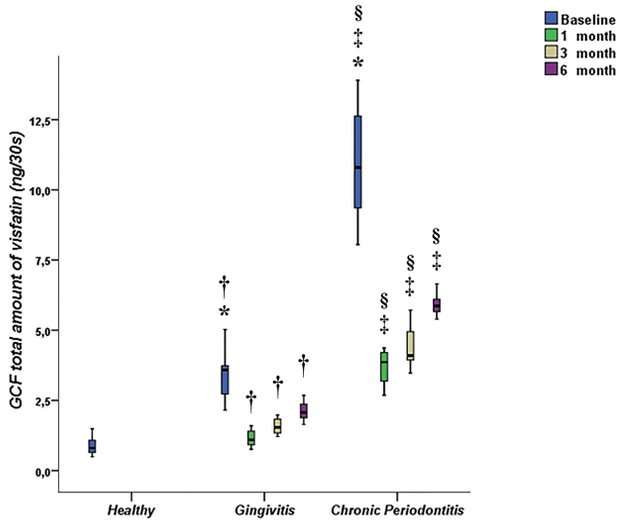

Fig. 1 Total amount of visfatin in GCF in different groups. *Statistically significant difference from healthy (Bonferroni-adjusted Mann-Whitney *U* test); †Statistically significant difference among time frame in gingivitis group (Bonferroni-adjusted Wilcoxon signed-rank test); ‡Statistically significant difference among time frame in chronic periodontitis group (Bonferroni-adjusted Wilcoxon signed-rank test); §Statistically significant difference from gingivitis group (Bonferroni-adjusted Mann-Whitney *U* 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.

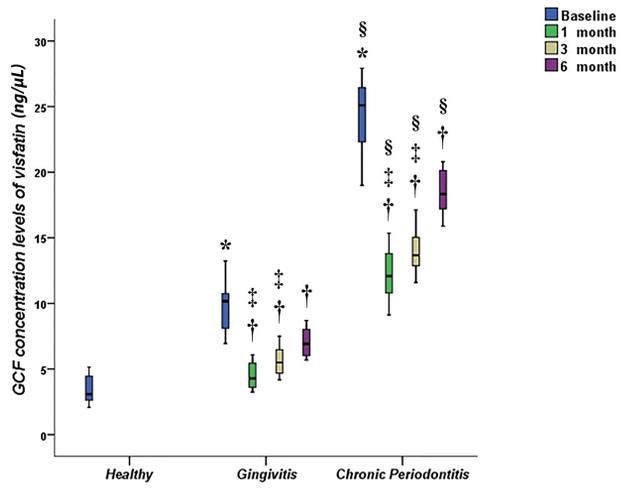

Fig. 2 Concentration levels of visfatin in GCF in different groups. *Statistically significant difference from healthy (Bonferroni-adjusted Mann-Whitney *U* test); †Statistically significant difference from baseline (Bonferroni-adjusted Wilcoxon signed-rank test); ‡Statistically significant difference from 6 month examination (Bonferroni-adjusted Wilcoxon signed-rank test); §Statistically significant difference from gingivitis group (Bonferroni-adjusted Mann-Whitney *U* 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.

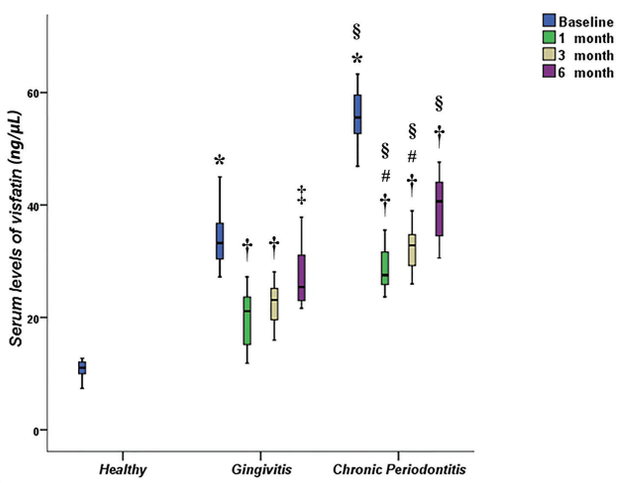

Fig. 3 Serum levels of visfatin in different groups. *Statistically significant difference from healthy (Bonferroni-adjusted Mann-Whitney *U* test); †Statistically significant difference from baseline (Bonferroni-adjusted Wilcoxon signed-rank test); ‡Statistically significant difference from 1 month examination (Bonferroni-adjusted Wilcoxon signed-rank test); #Statistically significant difference from 6 Month examination (Bonferroni-adjusted Wilcoxon signed-rank test); §Statistically significant difference from gingivitis group (Bonferroni-adjusted Mann-Whitney *U* 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.

visfatin levels in GCF and serum (Bonferroni-adjusted Wilcoxon signed-rank test, $P < 0.008$). GCF visfatin levels were statistically significant between group 2 and the group 3 (Bonferroni-adjusted Wilcoxon signed-rank test, $P < 0.008$) (Fig. 1). Following SRP treatment, visfatin levels were significantly different between group 2 and group 3 (Bonferroni-adjusted Wilcoxon signed-rank test, $P < 0.008$) at baseline, 1 and 3 months. A significant reduction in serum visfatin levels were observed 6 months after SRP in group 3. No significant difference was observed in group 2 (Bonferroni-adjusted Wilcoxon signed-rank test, $P > 0.008$) (Fig. 3).

Correlations

Correlation coefficients are presented in Table 3. A significant positive correlation was discovered between the total amount of visfatin and the serum visfatin in group 3 (Spearman's rank correlation, $P < 0.05$). CAL and GI correlated positively with the total amounts of visfatin and serum visfatin levels in group 3 (Spearman's rank correlation, $P < 0.05$). Total visfatin levels and GI correlated positively in group 2 (Spearman's rank correlation, $P < 0.05$). No correlation was observed between serum visfatin levels and CAL or GI in group 1 and group

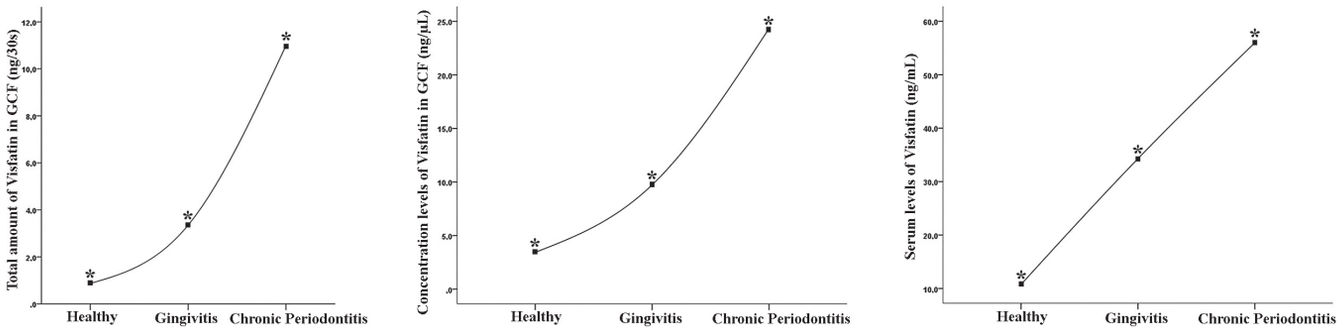

Fig. 4 Visfatin levels in GCF and serum. *Statistically significant difference among all groups (Kruskal-Wallis/Bonferroni-adjusted Mann-Whitney *U*).

Table 3 Spearman's rank correlation (r) among groups with respect to TA-visfatin, S-visfatin, number of deep sites, CAL, and GI

		TA-Visfatin to S-Visfatin	TA-Visfatin to 4 ≤ PPD ≤ 5	TA-Visfatin to PPD ≥ 6	S-Visfatin to 4 ≤ PPD ≤ 5	S-Visfatin to PPD ≥ 6	TA-Visfatin to CAL	S-Visfatin to CAL	TA-Visfatin to GI	S-Visfatin to GI
Group 1	r	0.445	NA	NA	NA	NA	0.424	0.347	NA	NA
	P	0.096	NA	NA	NA	NA	0.115	0.204	NA	NA
Group 2	r	0.493	NA	NA	NA	NA	0.465	0.444	0.535*	0.472
	P	0.062	NA	NA	NA	NA	0.081	0.098	0.040*	0.075
Group 3	r	0.561*	0.549*	0.577*	0.493	0.520*	0.627*	0.522*	0.650*	0.526*
	P	0.030*	0.034*	0.024*	0.062	0.047*	0.012*	0.046*	0.034*	0.044*
All groups	r	0.945*	NA	NA	NA	NA	0.811*	0.802*	NA	NA
	P	0.000*	NA	NA	NA	NA	0.000*	0.000*	NA	NA

TA-Visfatin: Total amount of visfatin; S-Visfatin: Serum levels of visfatin; *Statistically significant ($P < 0.05$); NA: Not applicable.

2 (Spearman's rank correlation, $P > 0.05$). No significant correlation was observed between total visfatin levels and CAL or serum visfatin levels (Spearman's rank correlation, $P > 0.05$).

Significant positive correlations were found between total visfatin levels and the number of areas with $4 \leq$ PPD ≤ 5 mm, the serum visfatin levels and the number of areas with PPD ≥ 6 mm, as well as the total amounts of visfatin and the number of areas with PPD ≥ 6 mm (the Spearman's rank correlation, $P < 0.05$). No correlation was seen between the number of sites with $4 \leq$ PPD $\leq$ 5 mm and serum visfatin levels (the Spearman's rank correlation, $P > 0.05$).

Discussion

We assessed the GCF and serum visfatin levels in periodontal disease and following periodontal treatment. Visfatin levels were lower in healthy fluid compared with periodontally diseased fluid. Furthermore, elevated visfatin levels in the gingivitis group decreased after periodontal treatment.

Increasing evidence suggests that inflammation increases visfatin levels in a number of diseases, including diabetes mellitus (13), inflammatory bowel disease (10), rheumatoid arthritis (14), acute lung disease (15), and sepsis (16). Innate immunity and inflammation is dependent on visfatin (25). Periodontal diseases characterized by chronic inflammation are among the most common conditions in humans worldwide (26).

Periodontal disease has many causes and has been associated with variable systemic diseases and obesity (10,14,15). In this study, participants were selected from a population of healthy, non-obese, non-smoking individuals. Subjects were chosen from a particular age range due to the chronic nature of the disease. To avoid an effect of gender on visfatin levels, an equal number of males and females were tested per group.

GCF may contain potential biomarkers for periodontal and systematic diseases (27,28). PD activity can be determined by inflammatory molecules within GCF (29). We used paper strips to collect GCF, which is inexpensive and can be used for specific locations. Furthermore, this approach produced minimal discomfort for the participant (24). During the collection of GCF, particular care was taken to safeguard the sulcular epithelium.

The amount of concentration GCF was calculated by dividing the total amount (ng) (the visfatin level in GCF analyzed by ELISA using commercially available kits) by the volume of the GCF (µL). Calculating the total amount in GCF rather than a concentration is

more relevant to the connection between periodontal disease and GCF components (30,31). Additionally, total cytokines better indicate the constituent activity of GCF than the concentration because the sample volume directly impacts the concentration (32). Herein, data are considered on the basis of total amounts.

Serum components indicate the level of inflammatory response to periodontal pathogens (33). We investigated serum visfatin levels in the present study before and after periodontal treatment.

Pradeep et al. reported that GCF and serum visfatin concentrations increase with severity of periodontal disease and visfatin was described as an inflammatory marker in periodontal disease (18). Mohamed et al. reported a positive correlation between PD and GCF visfatin levels (34). Visfatin levels were also increased in the saliva following periodontal inflammation (35,36). These findings support our results that visfatin levels correlated positively with the severity of periodontitis. In addition, we evaluated visfatin levels after non-surgical periodontal treatment. A previous study showed that visfatin levels decreased 8 weeks after periodontal treatment (37). Wu et al. (38) evaluated the role of visfatin in patients with T2DM and CP before and 3 and 6 months after non-surgical periodontal treatment. They reported that non-surgical periodontal treatment reduced visfatin and HbA1c levels in periodontitis patients with T2DM. The serum and salivary levels of visfatin were also shown to decrease significantly after non-surgical periodontal treatment (39). In the present study, a significant decrease in visfatin levels was observed 1 month after non-surgical periodontal treatment in gingivitis and CP individuals. This reduction was smaller after 3 and 6 months. Özcan et al. (40) showed that *P. gingivalis* infection in the periodontal pockets and EBV in plaque increase visfatin secretion. We evaluated the correlation between visfatin levels and periodontal pocket depth and found that these were positively correlated. Similarly, Mohamed et al. reported that visfatin increased significantly as the number of diseased sites increased.

This study has demonstrated that visfatin levels were greater in CP and gingivitis patients compared with controls and were higher in CP patients than gingivitis patients. This indicates that visfatin may be secreted in response to inflammation. One month after treatment with SRP, a significant reduction in visfatin levels was seen in group 2 and group 3. This study demonstrated that visfatin levels are reduced by periodontal treatment and that this reduced inflammation. Reduced inflammation may be associated with altered visfatin levels. A small increase in visfatin was found 3 and 6 months after SRP in gingivitis and CP patients. This may be explained by insufficient oral hygiene to control periodontal inflammation. These findings suggested that visfatin levels can increase in response to uncontrolled periodontal inflammation, even after successful non-surgical periodontal treatment.

We also examined the correlation between visfatin levels and two different periodontal pocket depth ranges. We found a positive and significant correlation between visfatin levels in GCF with the number of sites $4 \leq PPD \leq 5$ mm and $PPD \geq 6$ mm. No correlations were found between serum visfatin levels with the number of sites $4 \leq PPD \leq 5$ mm. Thus, may be due to the localized secretion from the cells response to periodontal inflammation.

In conclusion, visfatin levels significantly increased with the severity of periodontal disease. This suggests that visfatin is a potential inflammatory biomarker of periodontal disease in GCF and serum. Furthermore, visfatin levels decreased after periodontal treatment. This supports the hypothesis that visfatin plays a role in periodontal inflammation. Taken together, our findings show that visfatin may represent a biomarker for periodontal disease susceptibility. Multicenter studies with larger populations are needed to validate these findings.

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Conflict of interest

No potential conflict of interest relevant to this article was reported.

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