Evaluation of Salivary Levels of Visfatin in Obese Patients with Chronic Periodontitis

Vivek Kumar, Amrita1, Mahendra Pratap2, Sonika Sharma3, Kushal Singh4, Charanjit Singh Saimbi5

Department of Periodontology, Hazaribag College of Dental Sciences and Hospital, Demotand, Hazaribag, Jharkhand, Departments of 1Periodontology and 2Orthodontics and Dentofacial Orthopedics, Uttaranchal Dental and Medical Research Institute, Dehradun, Uttarakhand, Departments of 3Orthodontics and 4Dentofacial Orthopedics and Prosthodontics and Crown and Bridge, Teerthanker Mahaveer Dental College and Research Centre, Moradabad, Uttar Pradesh, India, 5Department of Periodontology, Universal College of Medical Sciences, Bhairahwa, Nepal

Abstract

Background: Visfatin, also known as nicotinamide phosphoribosyltransferase, is an adipokine implicated in obesity and plays an important role in regulating the defense and immune functions and is also suggested as a pro-inflammatory and immunomodulating marker for periodontitis. Hence, the aim of the present study was to assess the salivary levels of visfatin in obese patients with and without chronic periodontitis and nonobese healthy patients. Materials and Methods: A total of 45 patients were divided into: nonobese healthy (Group 1, n = 15, body mass index [BMI] ≤22.9 and waist circumference [WC] <90 for male patients and <80 for female patients, pocket probing depth [PPD] ≤3 mm, gingival index [GI] <1, and clinical attachment level [CAL] = 0), obese without chronic periodontitis (Group 2, n = 15, BMI ≥25.0 and WC ≥90 for male patients and ≥80 for female patients, PPD ≤3 mm, GI <1, and CAL = 0), and obese with chronic periodontitis (Group 3, n = 15, BMI ≥25.0 and WC ≥90 for male participants and ≥80 for female participants, PPD ≥5 mm, GI ≥1, and CAL ≥3 mm). Whole saliva samples were collected, and visfatin levels were evaluated using standard enzyme-linked immunosorbent assay. The results were analyzed using SPSS and Mann–Whitney analysis. Results: The highest visfatin levels from saliva were detected in Group 3, while the lowest levels were detected in Group 1. Significant differences were found in salivary visfatin concentrations among the three groups. Conclusion: The salivary level of visfatin was higher in obese patients with and without chronic periodontitis. Visfatin may be used as an inflammatory marker for the detection of obesity and periodontal disease.

Keywords: Obesity, periodontitis, saliva, visfatin

INTRODUCTION

Periodontal disease is a chronic disease of the oral cavity comprising a group of inflammatory conditions, affecting the supporting structures of the dentition.[1] It is characterized by overproduction of inflammatory mediators and tissue-destructive molecules against microbial pathogens.[2] It seems that patients with periodontitis are linked to systemic conditions, including heart diseases, diabetes, obesity, and metabolic syndrome. The association between periodontal disease and these systemic conditions appears to be attributable to a low-grade inflammatory burden that links them through a common pathophysiologic mechanism.[3]

The World Health Organization has recognized obesity as a predisposing factor for major chronic diseases ranging from cardiovascular disease to cancer.[4] Obesity is characterized by the presence of chronic subclinical inflammation with increased concentration of pro-inflammatory mediators.[5] In 1998, Saito et al. for the first time showed an association between obesity and periodontal disease in humans by analyzing 241 healthy Japanese individuals.[6] The prevalence of periodontitis was significantly more in obese than in nonobese individuals, and they concluded that strong correlation exists between obesity and periodontitis.[7,8]

Saliva is used as a diagnostic tool to evaluate various biomarkers associated with periodontal disease.[9] It is...
an important biological fluid containing both local and systemically derived biochemical substances used for detecting periodontal disorders.\textsuperscript{[10]} Identifying biomarkers related to inflammatory conditions may help determine the presence as well as risk and progression of periodontal diseases.\textsuperscript{[11]} Inflammatory biomarkers can easily be assessed in the saliva. Therefore, salivary samples provide a simple, fast, and noninvasive diagnostic test to evaluate periodontal diseases.\textsuperscript{[12]}

Visfatin, also known as pre-B-cell colony-enhancing factor and nicotinamide phosphoribosyltransferase, is a novel adipokine that is preferentially produced by visceral adipose tissue and has insulin-mimetic actions.\textsuperscript{[13]} It has several immunity functions that are secreted by neutrophils in response to inflammatory stimuli and upregulates the production of cytokines such as interleukin-6 (IL-6), IL-1β, and tumor necrosis factor alpha in human monocytes.\textsuperscript{[14]} This mediator was found to be present in a variety of cell types, including macrophages, lymphocytes, peripheral blood monocytes, and dendritic cells.\textsuperscript{[13]} An increase in the levels of pro-inflammatory cytokines in the periodontal tissues can induce visfatin production. Visfatin levels are strongly correlated with amount of visceral fat in humans which is positively correlated with obesity.\textsuperscript{[15,16]}

In light of above facts, the current study aimed to assess the salivary levels of visfatin in obese patients with and without chronic periodontitis and nonobese healthy patients.

**Materials and Methods**

This case–control cross-sectional study was conducted from January 2017 to July 2017 in the Department of Periodontology after obtaining ethical approval from the Institutional Review Board. A total of 45 participants aged between 25 and 50 years participated in the study. Written informed consent was obtained from all the participants before the commencement of the study.

**Anthropometric measurements**

Measurements were taken uniformly according to a standard protocol. Waist circumference (WC) was measured at 2.5 cm above the umbilicus and hip circumference at the level of maximum width of the buttocks with a participant in the standing position. All the measurements were taken twice. A tolerance limit of 1 kg was set for weight and 1 cm for height and circumference measurements. A third measurement was taken only when the difference between first two measurements was greater than the tolerance limit. Body mass index (BMI) was calculated as body weight divided by the square of height (m). The waist–hip ratio (WHR) was calculated as WC (cm) divided by hip circumference (cm).\textsuperscript{[17]}

Participants were categorized into three groups based on the gingival index (GI), pocket probing depths (PPDs), BMI, and WC.

- **Group 1 (healthy)** consisted of 15 nonobese individuals (BMI ≤22.9 and WC <90 for male and ≤80 for female participants) with clinically healthy periodontium, where GI ≤1, PPD ≤3 mm, and clinical attachment level (CAL) =0
- **Group 2 (obese with healthy gingiva)** consisted of 15 obese individuals (BMI ≥25.0 and WC ≥90 for male and ≥80 for female participants) with clinically healthy periodontium, where GI <1, PPD ≤3, mm and CAL = 0
- **Group 3 (obese with chronic periodontitis)** consisted of 15 obese individuals (BMI ≥25.0 and WC ≥90 for male and ≥80 for female participants) and individuals who had signs of clinical inflammation, where GI >1, PPD ≥5 mm, and CAL ≥3 mm.

**Inclusion criteria**

- Patients aged between 25 and 50 years with clinical signs of disease in their respective groups
- No systemic diseases
- Presence of at least 20 natural teeth.

**Exclusion criteria**

- Systemic diseases such as diabetes mellitus, hypertension, and rheumatoid arthritis
- Cigarette smoking, tobacco use, and alcoholism
- Pregnancy
- Systemic bacterial, viral, or fungal infections
- History of antibiotic therapy or the use of anti-inflammatory medications during the past 6 months
- History of periodontal treatment in the past 6 months.

**Sample collection**

Participants were asked to refrain from eating and drinking for at least 2 h before saliva collection. Using the spitting method, unstimulated saliva was collected between 11:00 am and 13:00 pm for 5 min (one spit per minute). The saliva was collected in sterile tubes and immediately frozen at −70°C until the experiment. After thawing, ELISA kit (RD191016100; BioVendor Laboratory Medicine, Brno, Czech Republic) was used to determine the concentration of visfatin. In favorable circumstances, the double-well assay is recommended. For the standard well, 100 µl of horseradish peroxidase (HRP)-streptavidin was added. 100 µl of anti-visfatin antibodies was added to each well and incubated for 1.5 h. The wells were washed five times with 300 µl of wash solution to eliminate the unreacted enzyme. 100 µl of prepared HRP-streptavidin solution was added to each well and incubated for 45 min at room temperature. The solution was discarded, and wells were washed five times with wash solution (200 µl each). 100 µl of TMB One-Step Substrate Reagent was added to each well and incubated for 30 min at 37°C in the dark environment. A total of 50 µl of stop solution was added into each well to stop the reaction (immediately the blue color changed into yellow). Absorbance of the substrate color reaction was read on ELISA plate reader (BioTeK Instruments Inc., Winooski, VT, USA) using 450 nm as primary wavelength carried out within 10 min after incorporation of stop solution. The visfatin concentration in each well was reported as ng/mL.
Data analysis
Statistical analyses were performed using SPSS software version 21 (SPSS Inc., Chicago, IL, USA). Clinical variables and salivary levels of visfatin expression were compared between groups using paired t-test and Mann–Whitney test. 

Results
A total of 45 participants aged between 25 and 50 years participated in the study. Visfatin was detected in the saliva of all patients. The mean values of clinical and biochemical parameters were expressed as mean ± standard deviation [Table 1]. The highest visfatin levels from the saliva were detected in Group 3 (33.85 ± 6.98 ng/mL), while the lowest levels were detected in Group 1 (20.93 ± 4.04 ng/mL) [Graph 1].

Intergroup comparisons of the clinical and biochemical parameters are summarized in Table 2. A significant difference in visfatin levels in the saliva was found when Group 1 was compared with Group 3 (P < 0.0001) and Group 2 was compared with Group 3 (P = 0.0093). Statistically significant differences were observed when the comparison of GI and PPD scores was made with Groups 1 and 2 versus the periodontally diseased Group 3. A significant difference in BMI, WC, and WHR was found when Group 1 was compared with Groups 2 and 3.

Discussion

The potential role of saliva in the diagnosis of oral and systemic health is marked in researches. Salivary biomarkers could be used to screen periodontal health status and disease progression.[18,19] The analysis of saliva is proven and accepted alternative to serum analysis. Saliva is a salient body fluid used for diagnostic purposes, which can be collected and pursued easily and cost-effectively.[20]

Visfatin is responsible for inflammatory reactions in periodontal structures and the consequent bone loss. It has been shown that microbial infection (presence of Fusobacterium nucleatum) has a controlling influence on production of visfatin.[21] The presence of EBV in oral plaque biofilm may be another factor that causes an increase in visfatin levels.[22]

The results of the present study revealed that the mean salivary visfatin levels of Group 3 were higher than that of Groups 1 and 2. This increase in visfatin levels in patients with periodontitis was due to polymorphonuclear leukocytes and macrophages in inflammatory conditions.[23,24] Visfatin has more potent destructive and pro-inflammatory properties and has a key role in the diligence of inflammation through reticence of apoptosis and neutrophils.[25] Visfatin levels can be considered a possible link between periodontal infection and other systemic diseases.[26] The findings in this study are in accordance with the studies conducted by Tabari et al.,[27] Abolfazli et al.,[28] and Özcan et al.,[29] where the visfatin levels were increased in chronic periodontitis patients.

Kadkhodazadeh et al.[30] observed no significant relationships in salivary concentrations of visfatin between peri-implantitis and chronic periodontitis patients and healthy controls.

There is a controversy regarding the relationship of visfatin with BMI in literature.[32] Hence, we considered normal range of BMI as well as high BMI as inclusion criteria to eliminate the confounding effects of this variable. Our study is the first one to evaluate the salivary visfatin levels with respect to BMI since we included patients with high range of BMI in the study. We established that higher salivary visfatin levels were found in obese patients with and without chronic periodontitis compared to normal healthy groups. In the present study, the mean salivary visfatin levels were significant between Groups 1 and 2, which is similar to the study conducted by Sheta et al.[33] Significant elevation of plasma visfatin in obese patient sounds acceptable because visceral fat is the source of visfatin.[34] Accordingly, obese patients reasonably have huge amount of all types of fat, including visceral type and hence

**Table 1:** Values (mean±standard deviation) of visfatin, pocket probing depths, gingival index, body mass index, waist circumference, and waisthip ratio in the three groups

| Variable          | Group 1 | Group 2 | Group 3 |
|-------------------|---------|---------|---------|
| Visfatin (ng/mL)  | 20.93±4.04 | 27.58±5.23 | 33.85±6.98 |
| PPD               | 1.47±0.57 | 2.0±0.73 | 6.80±1.21 |
| GI                | 0.28±0.15 | 0.35±0.17 | 2.32±0.49 |
| CAL               | 0       | 0       | 4.54±1.31 |
| BMI               | 21.31±1.68 | 28.70±4.07 | 27.65±5.05 |
| WC                | 73.20±5.84 | 99.47±8.43 | 97.24±7.98 |
| WHR               | 0.76±0.09 | 0.94±0.12 | 0.91±0.15 |

PPD: Pocket probing depth; GI: Gingival index; CAL: Clinical attachment level; BMI: Body mass index; WC: Waist circumference; WHR: Waist-hip ratio
Table 2: Consolidated pairwise comparison (P) among the three groups (P<0.05)

| Variable | Group | Mean difference (95% CI) | t    | P     |
|----------|-------|--------------------------|------|-------|
| Visfatin | Group 1 versus Group 2 | −6.650 (−10.14−−3.15) | −3.897 | 0.006* |
|          | Group 1 versus Group 3 | −12.920 (−17.18−−8.65) | −6.205 | <0.0001* |
|          | Group 2 versus Group 3 | −6.270 (−10.87−−1.65) | −2.784 | 0.0095* |
| PPD      | Group 1 versus Group 2 | −0.530 (−1.01−−0.04) | −2.216 | 0.0350* |
|          | Group 1 versus Group 3 | −5.330 (−6.33−−4.31) | −10.878 | <0.0001* |
|          | Group 2 versus Group 3 | −4.80 (−5.83−−3.75) | −9.525 | <0.0001* |
| GI       | Group 1 versus Group 2 | −0.070 (−0.18−−0.04) | −1.196 | 0.2418 |
|          | Group 1 versus Group 3 | −2.04 (−3.31−−1.75) | −15.418 | <0.0001* |
|          | Group 2 versus Group 3 | −1.970 (−2.24−−1.67) | −14.711 | <0.0001* |
| BMI      | Group 1 versus Group 2 | −7.390 (−9.71−−5.06) | −6.50 | <0.0001* |
|          | Group 1 versus Group 3 | −6.340 (−9.15−−3.51) | −4.614 | 0.0001* |
|          | Group 2 versus Group 3 | 1.050 (4.47−3.38) | 0.627 | 0.5357 |
| WC       | Group 1 versus Group 2 | −26.270 (−31.69−−20.83) | −9.921 | <0.0001* |
|          | Group 1 versus Group 3 | −24.040 (−29.27−−18.79) | −9.415 | <0.0001* |
|          | Group 2 versus Group 3 | 2.230 (8.36−3.89) | 0.744 | 0.4631 |
| WHR      | Group 1 versus Group 2 | −0.180 (−0.26−−0.10) | −0.150 | 0.0025* |
|          | Group 1 versus Group 3 | −0.150 (−0.24−−0.05) | −3.321 | <0.0001* |
|          | Group 2 versus Group 3 | 0.030 (−0.71−−0.13) | 0.605 | 0.5501 |

*Statistically significant at P<0.05, independent sample t-test. CI: Confidence interval, PPD: Pocket probing depth, GI: Gingival index, BMI: Body mass index, WC: Waist circumference, WHR: Waist-hip ratio

Conclusions

At present, there are several biomarkers studied in relation to periodontitis and obesity. However, there are very few studies addressing the relation between chronic periodontitis, obesity, and visfatin. In the current study, the level of visfatin was higher in saliva of obese patients with chronic periodontitis. Visfatin may be used as an inflammatory marker for the detection of periodontal disease and obese individuals and can be used in the future as a potential therapeutic target in the treatment of periodontal disease. The limitation of this study was that we did not have information about the levels of visfatin after the treatment of periodontitis. Such information would be useful to understand the role of visfatin in periodontal regeneration. Further long-term and interventional studies with larger sample sizes are required to assess the efficacy of this biomarker for early detection of periodontal disease and prevention of its progression. Finally, for considering visfatin as a biomarker of inflammation in periodontitis and obese patients, further studies are needed with more sample size in different populations.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

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