Correlation of salivary visfatin levels in obese and NON-OBESE population with periodontal status

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ABSTRACT

Background: Visfatin is an inflammatory adipokine that has been linked to obesity and plays a key function in immune response modulation. It’s also been proposed that it’s a pro-inflammatory marker for periodontitis. Hence, the aim of the present study was to assess the salivary Visfatin levels in obese and non-obese population and correlate with the periodontal status.

Materials and methods: A total of 40 individuals aged between 21 and 35 years (Group 1: n = 20, non-obese and Group 2: n = 20, obese) were enrolled in this case-control study. Clinical parameters such as periodontal probing depth (PPD) and clinical attachment level (CAL) were recorded. Body Mass Index (BMI) was recorded. Unstimulated salivary samples were collected and the samples were assayed for Visfatin using human Visfatin ELISA kit. The results were analysed using SPSS Software, Version 23.0. Unpaired t-test and Mann-Whitney U test was used to compare the clinical parameters and salivary Visfatin level between both the groups. Also, Spearman’s rank correlation test was done for correlation of BMI with salivary Visfatin levels and clinical parameters.

Results: The salivary Visfatin level was higher in group 2 (33.107 ± 0.81 ng/mL) as compared to group 1 (22.231 ± 1.33 ng/mL). Moderate positive statistically significant association was found between BMI and Visfatin (r = 0.738, p = 0.000). Also when Visfatin and PPD and CAL were correlated, moderate positive statistically significant association was found (r = 0.625, p = 0.000 and r = 0.630, p = 0.000).

Conclusion: The present study suggests that there exists a positive correlation between BMI and salivary Visfatin and BMI and periodontal parameters including probing depth and clinical attachment level. This illustrates that salivary Visfatin may be a potential marker for periodontal inflammation in obese population.

1. Introduction

Periodontitis is a condition that affects both the soft and hard tissues that support and surround the teeth. Despite the fact that bacterial plaque is the major cause of periodontal disease, there are a number of risk factors that might exacerbate the condition. Systemic disorders, smoking, stress, hormones, heredity, age, gender and socioeconomic status are few risk factors. Obesity has recently been identified as a risk factor for periodontitis. Obesity is defined as an excess of body fat relative to lean body mass that compromises one’s health. Both obesity and periodontitis are chronic inflammatory diseases. Adipose tissue dysfunction has been demonstrated to impact the immunological response in obese people, causing endotoxemia and a higher risk of periodontitis. Adipose tissue is a metabolically active and complex endocrine organ that regulates metabolic and vascular function and secretes a number of immunomodulatory mediators. Adipokines include leptin, visfatin, adiponectin, retinol binding protein-4, resistin, tumour necrosis factor-α (TNF-α) and interleukin-6 (IL-6), which are polypeptides released by adipocytes, preadipocytes, inflammatory cells like macrophages, and other cells. Some adipokines including TNF-α and IL-6 act locally, while others including leptin, visfatin, adiponectin, retinol binding protein-4 and resistin are released into the bloodstream and act as signalling molecules for the liver, muscle, and endothelium. Because of alterations in adipokine synthesis, obesity induces an increase in the expression of pro-inflammatory cytokines like IL-1, IL-6, TNF-α. These proinflammatory mediators hasten the progression of periodontal disease.

Literature search reveals adipokines such as leptin, resistin, adiponectin, visfatin are powerful markers of chronic periodontitis. Visfatin is also called pre-B-cell colony enhancing factor. It is secreted by lymphocytes, macrophages, monocytes, dendritic cells, periodontal ligament cells, bone marrow cells and liver cells. It acts as a growth factor, cytokine, proinflammatory mediator and plays role in proliferation of cells, angiogenesis. In addition, it modulates the host response by...
inhibiting neutrophil apoptosis during inflammation and increases the production of TNF-α, IL-1β, and IL-6.11

There are studies correlating various serum or gingival crevicular fluid (GCF) adipokine levels with periodontal status.12,13 However, literature search reveals only few studies correlating salivary Visfatin in obese and non-obese population with periodontal status.14,15 Therefore the present study intended to assess the salivary Visfatin levels in obese and non-obese population and correlate with the periodontal status.

2. Materials and methods

This case-control, cross-sectional study was conducted among 40 outpatient who reported to Department of Periodontics, Saveetha Dental College and Hospitals, Chennai. The study was performed from April 2021 to December 2021 after getting approval from Institutional Ethical committee. The patients were explained the whole study protocol and were asked to submit a duly signed written informed consent.

A total of 40 participants (22 males and 18 females) were enrolled. Group 1 consisted of 20 non-obese individuals (11 males and 9 females, aged between 21 and 35 years) and group 2 consisted of 20 obese individuals (11 males and 9 females, aged between 21 and 35 years). The individuals were categorised as obese and non-obese based on the definition of obesity using body mass index (BMI) developed by the National Institutes of Health. Based on this criteria, individuals with BMI of 18.5–24.9 were considered as non-obese (group 1) and individuals with BMI of >29.9 were considered as obese (group 2). Using an unpaired t-test with a two-sided significance level of 0.05, the sample size was calculated to be 40, with 80% power.

Inclusion criteria for all patients in both groups were aged between 21 and 35 years, having atleast 18 natural teeth excluding third molars and systemically healthy. Exclusion criteria considered were: patients with systemic diseases like diabetes mellitus, cardiovascular diseases, hypertension, rheumatoid arthritis, patients who had undergone periodontal therapy, pregnant and lactating women, smokers, patients under long term medications.

2.1. Clinical examination

Full mouth probing pocket depth (PPD) and clinical attachment level (CAL) were measured at six sites around the teeth (mesial, mid and distal on both buccal and lingual surfaces) using a UNC-15 periodontal probe and the average was recorded. BMI was calculated based on each subject’s weight in kilograms divided by the square of his height in meters (kg/m²).

2.2. Saliva collection

Before saliva collection, participants were requested to fast for at least 2 h without eating or drinking. The spitting method was used to collect 5 mL of unstimulated whole saliva from each subject between 11:00 a.m. and 12:00 p.m. Saliva was collected in sterile tubes and immediately frozen at 80°C until the experiment began. Within 6 months of collection, samples were defrosted and examined.

Each 5 mL saliva sample was pipetted into a clean microcap tube and centrifuged at 10,000 rpm for 1 min to clarify it. The supernatant was immediately transferred to clean microcap tubes and used for an enzyme-linked immunosorbent assay (ELISA). Concentrations of Visfatin were determined using Elabscience Human VF (Visfatin) ELISA kit, USA, according to the manufacturer’s instructions. The results of Visfatin assay were expressed as ng/mL. The minimum detectable dose of Visfatin using this kit was determined to be 0.19 ng/mL.

3. Results

In the present study, 20 non-obese individuals in group 1 and 20 obese individuals in group 2 with mean age of 28.30 ± 4.86 and 28.55 ± 5.20 respectively were enrolled. Demographic details of the study participants are given in Table 1. The results were evaluated using the Kolmogorov-Smirnov test and the Shapiro-Wilk test of normality. According to the data, the findings followed a non-parametric distribution. The clinical parameters and salivary Visfatin levels were compared using an unpaired t-test and a Mann-Whitney U test. The data was analysed using the Statistical Package for Social Sciences (SPSS Software, Version 23.0; IBM Corp., Armonk, NY, USA). The results were considered statistically significant when the p-value was less than 0.05.

Table 2 summarizes the intergroup comparison of salivary Visfatin levels and clinical parameters using the Mann-Whitney U test. The salivary Visfatin level was higher in group 2 (33.1070 ± 0.81 ng/mL) as compared to group 1 (22.2310 ± 1.33 ng/mL). A statistically significant difference in Visfatin levels in the saliva was found when Group 1 was compared with Group 2 (p < 0.05). Also, statistically significant differences were observed when PPD and CAL were compared between the groups (p < 0.05).

Table 3 shows the Spearman’s rank correlation of BMI with salivary Visfatin levels and clinical parameters. The correlation between BMI and Visfatin was moderate positive and statistically significant (r = 0.738, p = 0.000) (Graph 1). The correlation between Visfatin and PPD was moderate positive and statistically significant (r = 0.625, p = 0.000) (Graph 2). Similarly, the correlation between Visfatin and CAL was moderate positive and statistically significant (r = 0.630, p = 0.000) (Graph 3).

4. Discussion

The present study correlated the salivary Visfatin levels in obese and non-obese population with their periodontal status.

A perfect and confident smile is highly dependent on periodontal health. Apart from clinical manifestations of periodontal disease including bleeding gums, mobility, sensitivity, from the patient’s perspective, periodontitis negatively impacts the quality of life. In most cases, periodontitis is left unnoticed as it is asymptomatic in its early stage. Early detection of disease plays a crucial role in successful therapy. The clinical and radiographic methods of evaluating periodontal disease will only assess the current state of the disease. Those methods do not really help when it comes to predicting disease activity. To address this obstacle, researchers are working to discover biomarkers, which can identify the disease before the disease becomes complicated. Saliva and GCF comprised of variety of inflammatory biomarkers. Collecting saliva as a diagnostic fluid in analyzing periodontal disease is a convenient and non-invasive technique as compared to GCF. To the best of our knowledge, the present study is the first of its kind to correlate the salivary Visfatin levels in obese and non-obese population with their periodontal status.

The results of the present study revealed that the mean salivary Visfatin level was higher in obese individuals as compared to non-obese individuals. This is owing to the fact that the release of Visfatin in fat cells is linked to obesity and the abundance of adipose tissue in obese individuals is responsible for elevated levels of Visfatin.16,17 Obesity is also linked to higher levels of Visfatin, which has an insulin-like effect when it binds to the insulin receptor-1 and hypoglycemia is acquired through pathways including glycogenolysis inhibition and glucose utilization stimulation.18,19 Our findings are in accordance with the previous studies. Choi KM et al., suggested that the Visfatin levels were lower in obese and higher in non-obese individuals. This is owing to the fact that the release of Visfatin in fat cells is linked to obesity and the abundance of adipose tissue in obese individuals is responsible for elevated levels of Visfatin.16,17

Table 1

| Parameter | Group 1 (n = 20) | Group 2 (n = 20) |
|-----------|----------------|-----------------|
| Age (years) | 28.30 ± 4.86 | 28.55 ± 5.20 |
| Sex (males/females) (%) | 11/9 (55/45) | 11/9 (55/45) |
resulted in significant reduction of concentration of Visfatin. Similarly, Geitner D et al., observed that there was a significant increase in Visfatin levels as the BMI increases.

Also in our study, there exists a positive correlation between the BMI and Visfatin and BMI and periodontal status. An imbalance between reactive oxygen species (ROS) and antioxidants may be connected to obesity and periodontitis, resulting in a rise in Visfatin levels in periodontal disease patients. Though the molecular processes between obesity and periodontal disorders are still being unravelled, adipokines, a kind of cytokine produced by adipose tissue, may play a key role in causing periodontal tissue damage.

According to a recent study, BMI correlated positively with adipocytokines in GCF in periodontitis patients with BMI of more than 40 and adipocytokines in GCF may be impacted by obesity via a systemic effect.

Our findings are in agreement with studies by Tabari ZA et al., and Pradeep AR et al., Tabari ZA et al., assessed the salivary Visfatin level in periodontally healthy and compromised patients and found that the salivary Visfatin concentrations were significantly higher in the periodontitis group. In addition, a statistically significant association was found between salivary Visfatin levels and CAL in the periodontitis group.

Another study compared serum Visfatin levels in patients with periodontitis and healthy individuals and documented that Visfatin concentrations were significantly lower among periodontally healthy individuals than in patients with periodontitis.

Similarly, Kumar V et al., indicated that the salivary level of Visfatin was higher in obese patients with and without chronic periodontitis. Also, Kongstad J et al., demonstrated a positive association between obesity and periodontitis. In another study, it was demonstrated that the Visfatin levels were higher in obese patients with periodontitis and were reduced after periodontal therapy and the authors concluded that there might be an association between Visfatin level and periodontitis.

Also, Visfatin levels were significantly elevated in chronic periodontitis patients as compared to healthy controls in studies by Abolfazli N et al., and Ozcan et al., which is consistent with the current study. Since the findings of the current study are similar with those of studies that looked at serum or GCF Visfatin concentrations in periodontitis, it appears that assessing Visfatin concentration in saliva could be an useful and convenient alternative in the diagnosis of periodontitis. However, there are certain limitations, such as the cross-sectional study design. Prospective multicentre studies with a larger population are to substantiate the findings of the present study. Furthermore, different classes of obesity and the severity of periodontitis were not taken into account in this investigation. Further studies are warranted to correlate different classes of obesity with the severity of periodontitis. Also, due to

| Variable | Mean ± SD | Median | p value |
|----------|-----------|--------|---------|
| Group 1  | Group 2   | Group 1| Group 2 |
| Visfatin (ng/mL) | 22.23 ± 10.33 | 33.10 ± 8.10 | 22.72 ± 10.00 | 33.20 ± 0.81 | .000* |
| PPD      | 1.82 ± 0.15 | 5.17 ± 1.00 | 1.36 ± 0.25 | 5.28 ± 0.81 | .000* |
| CAL      | .02 ± 0.07  | 4.23 ± 1.00 | .0000 0.000 | 4.32 ± 0.25 | .000* |

*Statistically significant at p < 0.05.

| Correlation | Correlation Coefficient (r) | p value |
|-------------|----------------------------|---------|
| BMI vs Visfatin | .738** | .000 |
| BMI vs PPD    | .625** | .000 |
| BMI vs CAL    | .650** | .000 |

**. Correlation is significant at the 0.01 level (2-tailed).
the rigorous inclusion criteria, this study was unable to investigate the influence of ageing on Visfatin concentration.

The present study suggests that the level of salivary Visfatin was higher in obese population as compared to non-obese population. Also, there exists a positive correlation between BMI and salivary Visfatin and BMI and periodontal parameters including probing depth and clinical attachment level. This illustrates that salivary Visfatin may be a potential marker for periodontal inflammation in obese population. However, to analyse the causative association between obesity and periodontal disease and to understand the role of Visfatin in the etiopathogenesis of periodontal disease, further long-term interventional studies are needed.

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