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The Influence of Periodontal Treatment on Salivary Visfatin Biomarker levels in Non-obese Indian Population with Different forms of Periodontal Disease - A Clinico - Biochemical Study

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Abstract

The ability to correctly diagnose and institute effective periodontal therapy is essential to control periodontal diseases. Rapid advances in diagnostic research are moving towards methods whereby periodontal disease risk can be identified and quantified by measuring biomarkers. This study investigated the effect of Non-Surgical Periodontal Treatment (NSPT) on clinical indices and salivary levels of visfatin in subjects with increasing severity of the periodontal disease. This interventional clinical trial was performed on 60 systemically healthy male and female subjects (20 to 50 years) who were categorised into Group-1, Twenty subjects with healthy periodontium, Group-2, Twenty subjects with generalized moderate gingivitis, and Group-3 (20 subjects with moderate to severe periodontitis (Stage III according to the new classification of periodontal diseases). The visfatin levels were measured in unstimulated saliva by using standard Enzyme-Linked Immunosorbent Assay (ELISA) technique at baseline and six weeks after NSPT. The salivary visfatin levels were highest in Group-3 (38.22±3.38 ng/ml) followed by Group - 2 (26.66±2.24 ng/ml) and Group-1 (25.60±2.19 ng/ml) at baseline. After NSPT statistically significant reduction in salivary visfatin levels ($p<0.001$) in Groups -2 and 3 were seen. Visfatin levels at baseline were almost equivocal in normal weight and overweight subjects, irrespective of body mass index and showed a statistically significant reduction in salivary visfatin levels in both groups six weeks after NSPT ($p<0.001$). The present study suggests that salivary visfatin is a strong contributor in the pathology of periodontal disease and can be used as its diagnostic/therapeutic biomarker.

Keywords: Adipokines, Periodontitis, Non-surgical periodontal therapy, Saliva, Visfatin.

INTRODUCTION

Despite Gingivitis and periodontitis are the most common forms of inflammatory periodontal disease. The ability to correctly diagnose and provide effective periodontal therapy is critical to control periodontal diseases. Traditional clinical diagnostic aids (probing pocket depth, bleeding on probing, clinical attachment loss, plaque index, radiographs) used for the diagnosis of periodontal disease are often not precise. They are of limited usefulness because they indicate past periodontal disease rather than the current disease activity. Newer advances in diagnostic research is moving towards techniques whereby periodontal disease risk can be identified and quantified by objective measures such as biomarkers.

A Biomarker can be defined as "a characteristic that is an objective measure and is evaluated as an indicator of normal biological processes, pathogenic processes or pharmacological responses to a therapeutic intervention [1]. In short, these biomarkers tell how the body is doing or functioning. Saliva, when considered as a biomarker, is an important physiologic fluid containing a highly complex mixture of substances, has been proven to be an effective non-invasive diagnostic tool [2].

Since, the past two decades, the term referred to as adipo(cyto)kines such as adiponectin, leptin, visfatin, IL (interleukin)-6, leptin, MCP-1 (monocyte chemoattractant protein-1), resistin, TNF-α (tumour necrosis factor-α), vaspinhave been studies in great detail. These are nothing but the factors/cytokines which are produced by the adipocytes in the adipose tissue. These factors have been hypothesized to be involved in the pathophysiology of periodontitis [3].

Visfatin, an adipcytokine molecule, was first described by Fukuhara *et al*. in a seminal paper, which was

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initially termed as Pre B-cell colony Enhancing Factor [4]. He described it as an adipokine that lowers plasma glucose due to its ability to bind and stimulate the insulin receptor. Expression of visfatin is found to be positively regulated in response to microbial stimulation by B cells, T cells, monocytes, macrophages and neutrophils. Long-standing disease and progressive deterioration of beta cells appear to be associated with more pronounced PBEF/visfatin increase [5-11]. Some cytokines such as IL-1β, TNF-α and IL-6 and lipopolysaccharides regulate visfatin synthesis [12]. In diseases with altered polyclonal immune responses, visfatin was found to be increased by lymphocytes [13].

Recently, various studies have been reported that visfatin level increases in serum and Gingival crevicular fluid (GCF) [14, 15]. saliva [16, 17] and periodontal tissue [18] in patients with periodontitis and may have a role in the pathogenesis of the periodontal disease**.** However, salivary visfatin levels in individuals with an aggressive form of periodontitis (according to the American Academy of Periodontology (AAP) 1999, classification of gingival and periodontal diseases [19] has not been explored extensively. Hence, the aim of the present study was to evaluate the effect of Non-surgical periodontal therapy (NSPT) on salivary visfatin levels in non-obese Indian population with periodontal disease.

MATERIALS AND METHOD

Study design and samples

Sixty systemically healthy patients (20 to 50years) were randomly selected from the outpatient division of the Department of Periodontology, The Oxford Dental College, Bangalore. These subjects (with at least 20 natural teeth with a Body Mass Index (BMI) of 18.5 to 29.9 kg/^{2}) [20] were recruited according to the new and recent 2017 World Workshop Classification of Periodontal Diseases and Peri-Implant Diseases and Conditions [21]. Group-1 (27±3.92years); twenty periodontally healthy subjects (no clinical attachment loss and probing depths ≤ 3mm), Group-2 (33±4.90years); twenty generalized moderate gingivitis (with a generalized probing depths of ≤3mm, no clinical attachment loss and generalized bleeding on probing) and Group-3 (35±8.89years); twenty generalized periodontitis (pocket depths of ≥ 5mm and with alveolar bone loss extending to the middle third of root and beyond).

Ethical committee approval of research

This study was approved by the Institutional Ethics Committee of The Oxford Dental College, Bangalore (IEC No.433/2015-16) and the research protocol was performed as per Helsinki's declaration for experiments involving human subjects. The study was explained to all the participants, and written consent was obtained.

Clinical examination and sample collection

A calibrated examiner performed periodontal examinations with a UNC probe (Hu-Friedy® Manufacturing Inc.). The measurements were recorded by a calibrated periodontist, in four points per tooth for all the teeth except the third molars. The diagnosis of periodontitis was established based on the clinical parameters of the gingival index (GI), plaque index (PI) and probing pocket depth (PPD) and clinical attachment level (CAL). Parallel periapical radiographs and Orthopantomograph (OPG) were used to determine the presence of bone resorption, and the BMI was recorded. Clinical parameters were recorded a day earlier to the saliva sample collection. Unstimulated whole saliva was collected according to the drooling method demonstrated by Navazesh [22]. The samples (baseline & 6-weeks recall) were immediately analyzed for visfatin levels using ELISA.

Non-Surgical Periodontal Treatment (NSPT)

After the collection of saliva samples at baseline, scaling and root planing (SRP) with ultrasonic and hand devices (Gracey curettes, Hu-Friedy® Manufacturing Inc. USA) were carried out for Groups 2 and 3 patients. The treatment was completed in two sessions within two weeks from the patient's initial visit. Patients were put on oral hygiene maintenance, which included strict oral hygiene instructions, interdental cleaning aids (e.g., dental floss and proximal brushes), and recalled after every 2 weeks for 6 weeks.

Patients requiring periodontal surgical therapy after NSPT were refrained from treatment for 6 weeks as per Helsinki's declaration for the purpose of the study. Following NSPT, at the end of six weeks, saliva samples were collected from group 2, and group 3 patients and all the clinical parameters were recorded in the same manner as mentioned above.

Biomarker Analysis

The saliva samples were cleared by centrifugation at 10000g for five minutes. The supernatants were transferred to ELISA kits to measure the amount of salivary visfatin according to the manufacturer's protocol (Ray Biotech Life, Georgia, USA). It is based on the principle; microplate in the kit is pre-coated with an anti-rabbit secondary antibody. After a blocking step and incubation of the plate with anti-visfatin antibody, both biotinylated visfatin peptide and targeted peptide in samples interact competitively with the visfatin antibody. A standard curve of visfatin peptide was obtained, and the concentrations of visfatin peptide in the samples were calculated accordingly in ng/ml.

Statistical evaluation

The Statistical tests were performed using SPSS software version 22.0 (SPSS Inc., Chicago, USA). Student paired t-test was used to compare the mean values of clinical parameters and salivary visfatin levels in Group 2 and Group 3 subjects during pre & post-treatment period. A one-way ANOVA and Tukey's post hoc test were used to evaluate the different clinical parameters and visfatin levels of all the 3 groups at the baseline. The Pearson coefficient correlation assessment was done to evaluate the periodontal parameters, and BMI with Salivary Visfatin levels in all the three study groups during Pre-treatment & Post-treatment period. A P value ≤ 0.05 was considered to be significant.

RESULTS

In this study, the salivary visfatin concentration was evaluated before and after NSPT in gingivitis, periodontitis subjects and compared with a healthy periodontium control group. Visfatin levels were detected in all the collected samples. At baseline, the visfatin levels increased with the severity of periodontal disease; periodontally healthy (25.60±2.19 ng/ml) < gingivitis group (26.66±2.24 ng/ml) < generalized periodontitis (38.22±3.38 ng/ml). There was a statistically significant reduction in the levels of salivary visfatin 6 weeks after non-surgical periodontal therapy (p<0.001) in both the gingivitis group (25.05±2.00ng/ml) and periodontitis group (27.49±2.77ng/ml). (Table 1)

Table 1: Comparison of salivary Visfatin levels between pre-treatment and post-treatment

Group	Pre treatment		Post treatment		T test	p Value
	Mean	SD	Mean	SD		
Group - 1	25.60	2.19				
Group - 2	26.66	2.24	25.05	2.00	4.612	<0.001**
Group - 3	38.22	3.38	27.49	2.77	10.033	<0.001**

SD; Standard Deviation **p<0.001 – highly significant

The visfatin levels (33.14 ng/ml; normal weight, 34.18ng/ml; overweight) subjects were almost equal. After non-surgical periodontal therapy, there was a statistically significant reduction in visfatin levels in both normal weight (25.8ng/ml) and overweight subjects (26.88ng/ml). (p<0.001). (Table 2)

Table 2: Relationship between Body Mass Index (BMI) (kg/m^2) and salivary Visfatin levels pre-treatment and post-treatment

Body Mass Index (BMI) Category			Mean	N	SD	SE (Mean)	Significance
Normal Weight	Pair 1	Visfatin Pre-Treatment	33.148	33	6.7098772	1.3991061	0.000
		VisfatinPost-Treatment	25.827	33	2.7881098	0.5813611	
Over Weight	Pair 1	Visfatin Pre-Treatment	31.487	27	6.2788810	1.5228523	0.001**
		Visfatin Post-Treatment	26.880	27	2.5007749	0.6065270	

N: no of patients, SE: Standard Error, SD; Standard Deviation **p<0.001 – highly significant

The mean levels of visfatin and clinical parameters at baseline were comparatively higher in generalized aggressive periodontitis compared to generalized moderate chronic periodontitis. However, after NSPT, the difference was not statistically significant.

DISCUSSION

Visfatin/NAMPT (Nicotinamidephosphoribosyltransferase) is a protein with several suggested functions. The presence of visfatin in a large variety of white blood cells and tissue-bound macrophage suggests an important role of visfatin in the regulation of immune and defence functions [23, 24]. In 2011, Pradeep *et al*., [15] reported increased levels of visfatin in GCF in patients with periodontitis. Then, Raghavendra *et al*., [14] showed that increased GCF visfatin levels in periodontitis reached similar levels of healthy individuals after the periodontal treatment.

In this study, the PI, GI, BI, BOP, CAL scores were higher at baseline, which could be attributed to improper oral hygiene maintenance, lack of motivation, and ignorance by the patients, thereby increasing local factors. Post-treatment, there was a significant reduction in all the clinical parameters (PI, GI, BI, BOP, CAL) for Group 2 and Group 3, respectively (p<0.001). This reduction can be attributed to the removal of local factors, better oral hygiene maintenance through education, motivation and reinforcement of the oral hygiene instructions at regular intervals and compliance with the treatment protocol [25].

The mean baseline salivary visfatin levels in Group 2 and Group 3 were 26.66±2.24 ng/ml and 38.22±3.38 ng/ml respectively, which were in accordance to the results made by Pradeep AR *et al*., [15] His study demonstrated that visfatin levels in the serum and GCF increased with the severity of periodontal disease. This phenomena can be attributed to the levels of visfatin secreted by the predominant cells in different stages of the disease process[21] as well as changes in microbial [16]. Following non-surgical periodontal therapy, the mean levels of visfatin in Group-2 and Group-3 was 25.05±2.00 ng/ml and 27.49±2.77 ng/ml, respectively. The reduction was statistically highly significant (p<0.001). This reduction in visfatin levels may be because of the reduction in the inflammatory components of periodontal disease mainly, the macrophages, leukocytes, periodontal pathogens and the proinflammatory cytokines like IL-1, tumour necrosis factor-alpha, which are the key sources for the up regulation of visfatin production in the periodontium [25].

The mean levels of salivary visfatin in generalized aggressive periodontitis subjects and generalized moderate chronic periodontitis subjects were 38.12±3.05 ng/ml and 38.32±3.83 ng/ml respectively, at baseline. These findings correlate studies done by Nokhbehsain *et al*. and Özcan E *et al*. demonstrating that visfatin stimulates the production of C-C motif chemokine ligand 2 and matrix metalloproteinase-1 in the periodontal cells; thus leading to inflammation and destruction of periodontal tissues induced by various periodontal pathogens such as Porphyromonas gingivalis, Fusobacterium nucleatum and proinflammatory cytokines such as IL-1β present in the periodontal ligament [26, 27].

Following non-surgical periodontal therapy, the mean levels of salivary visfatin in aggressive and generalized moderate chronic periodontitis was reduced to 28.16±3.10 ng/ml and 26.82±2.35 ng/ml, respectively. Also, in the present study, the mean levels of visfatin and clinical parameters were comparatively higher in aggressive periodontitis compared to generalized moderate chronic periodontitis which is similar to the previous studies [18, 28-29]. Also, the present study has shown that expression of visfatin in the saliva of aggressive periodontitis was more than the generalized moderate chronic periodontitis samples, but this difference was not statistically significant, which may be due to a small number of samples in this group.

In the previous studies, a strong positive correlation between obesity and visfatin levels were elucidated [12]. In the current study, the correlation between salivary visfatin levels in non-obese subjects (BMI less than 30mg/m^2) at baseline and 6 weeks after scaling and root planning was analyzed, which was not compared in the previous studies. Average visfatin levels in the were 3.31482 ng/ml and 3.1487ng/ml in normal weight and overweight patients, respectively. These baseline values show that visfatin levels increase irrespective of the weight of the patient. After scaling and root planing, the visfatin levels in normal-weight subjects was reduced to 2.582ng/ml, and in overweight subjects, to 2.688ng/ml. The reduction in visfatin levels in both normal and overweight patients were statistically significant (p<0.001). This inverse relation between BMI and salivary visfatin levels can be explained due to (a) ethnic heterogeneity and/or (b) a genetic association of the visfatin gene with lipid metabolism which is similar to the study by Jihan *et al*., [28].

Although, the present study tried to eliminate some of the drawbacks in the previous studies, it encountered few limitations. Despite a few limitations in this study (small sample size and inclusion of obese patients with a longer duration of observation) the present study showed that at baseline, salivary levels of visfatin increased in the order; healthy periodontium< gingivitis < generalized moderate chronic periodontitis < aggressive periodontitis subjects, which suggests that visfatin levels were increased during inflammation. After non-surgical periodontal therapy, the levels of salivary visfatin were reduced and comparable with the healthy individuals, which was statistically significant. Therefore, the salivary visfatin levels may have the potential to be both a diagnostic as well as a therapeutic marker in periodontal disease.

CONCLUSION

Salivary visfatin increases with the severity of periodontal disease and decreases on non-surgical periodontal therapy in non-obese subjects. Hence, this biomarker can be used as a diagnostic and/or a therapeutic biomarker in various forms of periodontal disease.

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Conflict of interest

All authors declare no conflicts of interest in this paper.

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