

Salivary Visfatin Concentration in Response to Non-surgical Periodontal Therapy

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ABSTRACT

Introduction: Visfatin is a pro-inflammatory cytokine that has been associated with several immunomodulating processes. The relationship between visfatin and periodontitis has been the subject of a few studies that have described visfatin as an inflammatory marker for periodontitis. However, studies on visfatin as a potential therapeutic target in periodontal diseases are scarce. In the present study, we evaluated the alterations in salivary visfatin levels in response to non-surgical periodontal treatment.

Materials and Methods: Twenty individuals with moderate to severe chronic periodontitis and twenty periodontally healthy individuals were selected for this study according to clinical parameters. Patients with chronic periodontitis were treated by non-surgical periodontal therapy. Clinical parameters were recorded and saliva samples were obtained from the control group and test group before (T1 group) and one month after

periodontal treatment (T2 group). Salivary visfatin concentrations were measured by standard enzyme-linked immunosorbent assay (ELISA). Statistical analysis was performed with the statistical software SPSS, version 18.

Results: Visfatin was detectable in all samples. T1 and control groups were significantly different in terms of clinical parameters and visfatin levels. Visfatin concentrations were reduced significantly after non-surgical periodontal therapy. Periodontal treatment also resulted in significant reductions of all clinical parameters with the exception of clinical attachment level.

Conclusion: The results demonstrated that salivary levels of visfatin are reduced after non-surgical periodontal therapy to the levels comparable with those found in healthy individuals. Therefore, the salivary visfatin level may have the potential to be a target marker for assessment of responses to non-surgical periodontal therapy. However, more studies with larger sample sizes are necessary to validate these findings.

Keywords: Adipokines, Chronic periodontitis, Nicotinamide phosphoribosyl transferase, Saliva, Therapy

INTRODUCTION

Periodontitis is a chronic inflammatory disease characterised by overproduction of inflammatory mediators and tissue-destructive molecules against microbial pathogens [1]. These agents move from inflamed tissues to gingival crevicular fluid (GCF), eventually enter and accumulate in the saliva [2], and have the potential to be used as biomarkers of periodontal diseases [3].

Current diagnostic procedures for periodontitis, including clinical examinations and radiological findings, are insufficient for the objective and accurate diagnosis of periodontal diseases [3-5]. Oral fluid biomarkers have recently emerged as an adjunct to traditional methods for the early detection of periodontitis and risk assessment of its progression, as well as monitoring the responses to periodontal treatments [5,6].

GCF has been considered as a source of periodontal related biomarkers in many studies but, it has inherent problems. The GCF sampling techniques are difficult and time consuming as well as invasive for gingival tissue. In addition GCF is a site specific fluid and presents in small quantity [7,8]. In contrast saliva is abundant and its' sampling is simple, fast and non-invasive. Moreover, there is no need for specialised or advanced equipment for sampling saliva [7-10]. Unlike GCF, saliva samples are pooled from all sites of the oral periodontal tissues and thus are more useful for the overall assessment of diseases and identification of susceptible patients [8].

Saliva as a target material for diagnostic tests has been extensively studied [11]. Elevated levels of a large variety of enzymes, proteins and mediators have been observed in the saliva of patients with periodontal disease compared with those in healthy control individuals [4,7,8,10-12]. In addition, reductions in the salivary levels of periodontitis biomarkers after periodontal treatments have been demonstrated in several studies [10,11,13-15]. Currently,

new candidate biomarkers for the evaluation of periodontal disease are under investigation. Several studies have demonstrated that adipose tissue secretes mediators called adipocytokines, which have important roles in immunity and inflammation [16].

Visfatin, also known as pre-B-cell colony-enhancing factor (PBEF) and nicotinamide phosphoribosyl transferase, is an inflammatory adipocytokine introduced primarily by Fukuhara et al., [17]. This mediator is generally produced by adipose tissue and can also be expressed in a variety of cell types, including macrophages, lymphocytes, peripheral blood monocytes and dendritic cells [17].

Visfatin has several immunity functions. It can stimulate the expression of interleukin 6 (IL-6), IL-1 β and tumour necrosis factor alpha (TNF α) in human monocytes. Conversely, an increase in the levels of the above mentioned proinflammatory cytokines in the periodontal tissues can induce visfatin production. Moreover, it has been demonstrated that the activation of T-cells, chemotaxis of CD4+ monocytes and CD19+ B-cells and inhibition of neutrophil apoptosis can be affected by visfatin. Several studies have indicated the elevation of visfatin levels in inflammatory diseases such as type 2 diabetes mellitus, inflammatory bowel disease and rheumatoid arthritis [16,17].

The diverse inflammatory functions of visfatin, described above, make this cytokine a candidate biomarker for investigation in periodontitis. A few studies have demonstrated the relationships between serum and GCF concentrations of visfatin and the severity of periodontitis [18,19].

Furthermore, Raghavendra et al., indicated, for the first time, that GCF and serum visfatin levels were reduced following non-surgical periodontal therapy in patients with periodontitis. They suggested visfatin as an inflammatory marker that could be a target for the evaluation of responses to periodontal treatments [20].

In this study, along with our previous study [21], which for the first time evaluated salivary visfatin levels in periodontitis, we aimed to investigate alterations in the salivary concentrations of visfatin following non-surgical periodontal treatment.

MATERIALS AND METHODS

This longitudinal, case-controlled, clinical study was performed from March 2012 until June 2013 in the Periodontology Department of Qazvin University of Medical Sciences, 20 patients with moderate to severe chronic periodontitis (Test group) and 20 periodontally healthy individuals (control group) who visited the Department were selected from about 200 potential participants and were enrolled in the study.

Individuals completed personal, medical and dental history questionnaires, and written consents were obtained. The study was approved by the ethical committee of Qazvin University of Medical Sciences.

The participants met these inclusion criteria: age over 18 y, good general health, a minimum of 18 teeth and BMI of 18.5–25 [22].

Exclusion criteria included: history of periodontal therapy during the preceding 2 years; history of alcoholism and smoking; liver, kidney, or salivary gland dysfunction; infectious diseases; inflammatory bowel disease; rheumatoid arthritis; granulomatous diseases; hypertension; diabetes; organ transplantation; or cancer therapy. In addition, patients using glucocorticoids, cyclooxygenase inhibitors, bisphosphonates, antibiotics, or immunosuppressant medication during the preceding 6 months, those pregnant or lactating, those needing antibiotics for infective endocarditis prophylaxis during dental procedures, those with acute illness symptoms (i.e. fever, sore throat, body aches, or diarrhoea), those with orthodontic appliances, or those with the presence of an oral mucosal inflammatory condition (e.g. aphthous, lichen planus, leukoplakia or oral cancer) were excluded from the study.

Body mass index (BMI) was measured based on the World Health Organization guidelines and was recorded in charts [22].

Clinical Evaluation

Diagnosis of moderate or severe periodontitis was performed based on the measurement and recording of the periodontal indices at 4 points per tooth by means of a UNC probe, in accordance with the criteria defined by the American Academy of Periodontology [23].

This diagnosis was established based on clinical parameters, including plaque index (PI) [24], probing pocket depth (PPD), clinical attachment level (CAL) and bleeding index (BI) [25]. Individuals with periodontal disease had to have at least 5 teeth with CAL \geq 3 mm and PPD $>$ 5 mm in at least two quadrants [26]. The control group consisted of individuals with no history of periodontitis and clinical attachment loss. In addition, they had BI $<$ 0.1 and PPD \leq 3 mm in all sites.

Saliva Collection

Saliva collection was performed in the control group and the test group before (T1) and 1 month after the completion of periodontal treatment (T2). In each stage, 5 ml of unstimulated whole expectorated saliva was collected from each participant between 10.00 am and 12.00 pm, according to a modification of the method described by Navazesh [27]. The subjects were instructed to avoid eating, drinking and brushing one hour before sampling. In addition, to avoid contamination of samples with blood, clinical parameters were measured at least one hour before saliva collection. The participants were asked to swallow saliva first, and then allowed the saliva to drain passively for 5 min over the lower lip into a sterile tube. Collected saliva was immediately placed on ice prior to being frozen at -80°C . Samples were defrosted and analysed within 6 months of collection.

Non-surgical Periodontal Therapy

Periodontal therapy was performed according to the standard protocol for the non-surgical phase of periodontal treatment, consisting of full- mouth scaling, root planning and oral hygiene instructions in two sessions with a 1-week interval. Also, no antibiotic was prescribed for the patients. The stages of treatment were performed by a periodontist (ZAT).

Two weeks after the second visit, patients were re-called. Their cooperation with hygienic instructions was assessed and reinforced. After the use of medicines listed in the exclusion criteria was checked and ruled out, supragingival prophylaxis was performed.

One month after the completion of non-surgical periodontal therapy, the patients were re-evaluated and saliva was re-collected.

Biomarker Analysis

Each saliva sample (5 μl) was pipetted into a clean microcap tube and clarified by centrifugation at 10,000 g for 5 min. The supernatant was transferred to clean microcap tubes and used immediately for enzyme-linked immunosorbent assay (ELISA). The concentration of visfatin was determined with the Human Visfatin (VISFATIN) ELISA Kit (Cat. No.E0025Hu),

according to the manufacturer's instructions. The results of the visfatin assay were expressed as ng/ml for concentrations. All laboratory tests were performed in the Immunology Department of Qazvin University of Medical Sciences.

STATISTICAL ANALYSIS

Statistical analysis was performed with SPSS statistical software, version 18. Demographic variables were compared between groups by Fisher's exact test. Comparison of indices and visfatin levels between the T1 and control groups was performed with an independent t-test.

A paired t-test was used for comparison of clinical parameters and visfatin levels between T1 and T2 and between T2 and control groups. The correlations between visfatin levels and clinical parameters were assessed by Spearman rank correlation analysis. p-values $<$ 0.05 were considered statistically significant.

RESULTS

In this study, along with our previous investigation, we evaluated salivary visfatin levels before and after non-surgical periodontal treatment of 20 patients with generalized moderate to severe chronic periodontitis; these were called the T1 and T2 groups, respectively. Furthermore, we compared salivary visfatin levels in the T2 group with those in healthy control individuals.

The test and control groups were matched in terms of age ($p = 0.1$), gender ($p = 1.000$) and BMI ($p = 0.23$) [Table/Fig-1].

Visfatin was detected in all samples. As also shown in [Table/Fig-1], clinical parameters were significantly lower in the control group than

Parameters	Healthy (N = 20) Control	Periodontitis (N = 20)	
		T1	T2
Age (Years)	33.85 \pm 6.84	38.45 \pm 9.98	38.45 \pm 9.98
Sex (female/male) (%)	13/7(65/35)	14/6(70/30)	14/6(70/30)
BMI (kg/m ²)	21.88 \pm 2.56	23.66 \pm 2.99	23.66 \pm 2.99
PPD (mm, mean \pm SD)	1.59 \pm 0.27	4.19 \pm 0.61*	2.55 \pm 0.74**
PI (mean \pm SD)	0.31 \pm 0.35	2.28 \pm 0.45*	0.24 \pm 0.24**
CAL(mm, mean \pm SD)	0	3.79 \pm 1.02*	3.38 \pm 1.04
BI (mean \pm SD)	0.05 \pm 0.07	0.78 \pm 0.26*	0.22 \pm 0.22**
Visfatin (ng/ml, mean \pm SD)	23.38 \pm 7.58	33.43 \pm 15.72*	23.00 \pm 9.57**

[Table/Fig-1]: Demographic and clinical characteristics and salivary visfatin levels of the three groups

*significant compared to control group

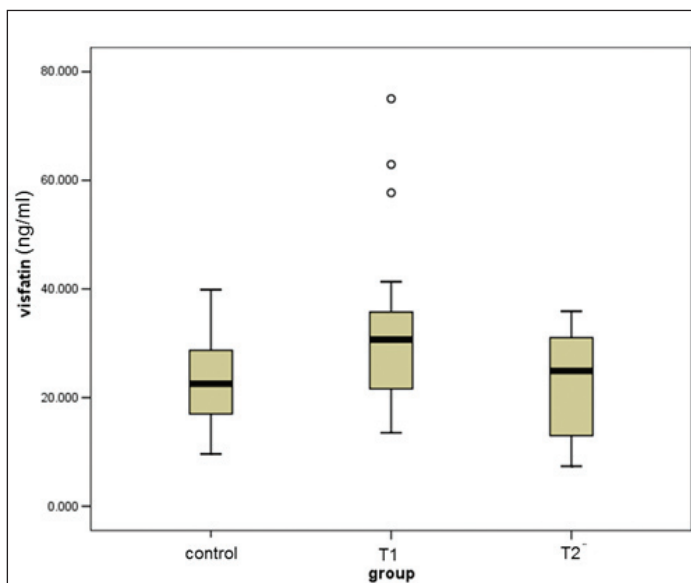
** significant compared to before therapy

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the T1 group ($p < 0.0005$). In addition, the healthy and T1 groups were significantly different in salivary levels of visfatin ($p = 0.015$).

One month after the completion of therapy, periodontal indices were recorded as shown in [Table/Fig-1]. While PD, PI and BI showed significant reductions ($p = 0.009$, $p = 0.0001$ and $p = 0.0001$, respectively), alterations in CAL amounts were not significantly different ($p = 0.18$).

Analysis of visfatin concentrations in the groups demonstrated the highest levels of visfatin in the T1 group [Table/Fig-1]. Salivary visfatin levels decreased significantly after non-surgical periodontal treatment ($p = 0.003$). Application of the independent t-test showed no significant differences in visfatin levels between the T2 group and control participants ($p = 0.89$). Distributions of visfatin concentrations among groups are shown in [Table/Fig-2].



[Table/Fig-2]: Box plot showing salivary concentrations of visfatin (ng/ml) in the groups

Clinical Parameters	Visfatin	
	r	p-value
CAL	0.514	0.157
PD	-0.083	0.821
PI	0.499	0.208
BI	0.355	0.349

[Table/Fig-3]: Correlation of visfatin concentration with periodontal clinical parameters in T2 group

[Table/Fig-3] shows the Spearman rank correlation between salivary visfatin levels and clinical parameters after non-surgical periodontal treatment. There was no significant relationship between clinical periodontal parameters and visfatin concentrations.

DISCUSSION

Visfatin is an emerging adipocytokine that, in addition to have several functions in the immune system, has been linked to various inflammatory disorders [16,17]. Visfatin induces the production of pro-inflammatory cytokines such as $\text{TNF}\alpha$ and IL-8 by peripheral mononuclear cells. Furthermore, it promotes macrophages to survive and inhibit neutrophil apoptosis. Visfatin affects leukocyte recruitment by up-regulation of cell adhesion molecules, including ICAM-1, VCAM-1 and E-selectin. Furthermore, it stimulates the release of variable mediators by endothelial cells such as IL-6, IL-8 and monocyte chemoattractant protein-1 (MCP-1) [28,29].

Adya et al., showed that visfatin induces the expression and activity of matrix metalloproteinase (MMP) and, conversely, down-regulates the inhibitors of the MMPs in monocytes and endothelial cells. It has

been reported that any imbalance between MMPs and their inhibitors plays a key role in the progression of periodontitis [30,31].

When the aforementioned functions are considered, it could be hypothesised that there is a relationship between visfatin and the pathogenesis of periodontitis. The association between chronic periodontitis and visfatin levels in GCF and serum have been investigated in a few studies. It was indicated that visfatin concentration in serum and GCF would be a possible marker for inflammatory activity in periodontitis [18,19]. Furthermore, in our recent study, for the first time, we demonstrated that salivary visfatin levels increased in patients with moderate to severe chronic periodontitis [21].

Several studies have demonstrated reductions in the salivary levels of enzymes and cytokines concomitant with the treatment of periodontal disease [13-15]. The present study was designed to evaluate, for the first time, the alterations in salivary levels of visfatin after non-surgical periodontal treatment in patients with chronic periodontitis.

There is controversy regarding the relationship of visfatin with age and BMI in literatures [32]. So, we considered normal range of BMI as inclusion criteria and matched our groups in age and BMI to eliminate the confounding effects of these variables. The results of the current study indicated that salivary visfatin levels decreased significantly one month after treatment, in parallel with significant reductions in PD, BI and PI. Moreover, the levels of salivary visfatin in individuals after receiving treatment were comparable with those in healthy controls.

Our results were almost in accordance with the findings of a similar study by Raghavedra et al., [20] on the GCF and serum visfatin levels after phase 1 periodontal treatment, which indicated that visfatin concentrations in serum and GCF decreased significantly 8 weeks after non-surgical periodontal treatment in patients with moderate to severe chronic periodontitis. In that study, unlike the present investigation, CAL decreased significantly, consistent with visfatin reduction.

Nogueria et al., and Nokhbehain et al., in their recent studies showed that PDL cells can produce visfatin locally, and this production is up-regulated by key periodontopathogens such as *Fusobacterium nucleatum* and *Porphyromonas gingivalis* [31]. They demonstrated that microbial and inflammatory signals such as IL-1 β may use visfatin for their destructive effects on periodontal structures [33,34].

Considering the significant reductions in visfatin concentrations, in our study, to the same levels as for healthy individuals, and despite incomplete treatment outcomes especially in severe periodontitis patients, it may be speculated that changes in the microbial composition and the ongoing inflammatory process in the pocket environment have a close relationship with the visfatin levels in saliva. Reductions in key cells for visfatin production in the periodontium, such as macrophages and inflammatory leukocytes, following treatment may be an additional reason for diminished levels of salivary visfatin concentration after periodontal treatment.

The results of this study should be interpreted with caution due to the study's small sample size. Future studies with larger sample sizes are necessary to verify the findings of this study.

CONCLUSION

The results of the present study indicated the significant reductions of salivary visfatin concentrations in patients with chronic periodontitis to levels comparable with those in periodontally healthy individuals, as a result of non-surgical periodontal therapy. Therefore, salivary visfatin might be useful for monitoring responses to periodontal therapy. However, for visfatin to be considered as a potential therapeutic target in the treatment of chronic periodontitis, further longitudinal studies with larger sample sizes are needed.

ACKNOWLEDGEMENTS

This study has been supported by a grant from Iranian National Elite Foundation, Tehran, Iran and this article was extracted from Dr Nohekhan's thesis. Also, we would like to extend our thanks to Mr Reza Norian (Immunologist, Immunology Department, Qazvin University of Medical Sciences, Qazvin, Iran) for his assistance with the laboratory stages of this study.

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Date of Submission: **Oct 09, 2014**
Date of Peer Review: **Feb 16, 2015**
Date of Acceptance: **Feb 25, 2015**
Date of Publishing: **Apr 01, 2015**

FINANCIAL OR OTHER COMPETING INTERESTS: As declared in Acknowledgements.