

EVALUATION OF SALIVARY AND SERUM VISFATIN IN ORAL BULLOUS EROSIIVE LICHEN PLANUS AND ORAL SQUAMOUS CELL CARCINOMA

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ABSTRACT

Introduction: Bullous Erosive Oral Lichen Planus (BEOLP) is a potentially malignant condition with high rate of transformation into Oral Squamous Cell Carcinoma (OSCC), and aiming to reduce the morbidity and mortality rate arising from OLP; the early diagnosis of oral cancer was recommended. Many biomarkers were found to be released into the serum and the saliva during the course of both diseases, BEOLP and OSCC. One of these proteins is visfatin.

Aim of the study: The aim of this study was to evaluate salivary level of visfatin and to find out if it can be used instead of its serum level as an early diagnostic biomarker for patients with either BEOLP or OSCC.

Subjects and Methods: The study was conducted on 3 groups; group (A) which included fifteen medically free subjects, group (B) which included fifteen patients suffering from BEOLP, and finally group (C) which included fifteen patients suffering from OSCC. Serum and salivary samples were collected and visfatin levels were measured using an ELISA kit (Enzyme Linked Immuno Sorbent Assay). Data were analyzed by SPSS.

Results: The present study detected no statistically significant difference between mean visfatin levels in BEOLP and OSCC groups; both showed statistically significantly higher mean levels than control group which showed the lowest mean visfatin level. It Also revealed a statistically significant positive (direct) correlation between serum and salivary visfatin levels ($r = 0.835$, P -value <0.001) i.e. an increase in serum level of visfatin is associated with an increase in salivary level of Visfatin.

Conclusion: Salivary level of visfatin can be used instead of its serum level as a biomarker for BEOLP and OSCC.

KEYWORDS: Visfatin, BEOLP, OSCC..

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INTRODUCTION

Visfatin, which is also known as pre-B-cell colony enhancing factor and nicotinamide phosphoribosyl-transferase, was originally cloned by *Samal et al. in 1994*.⁽¹⁾ In addition to adipose tissue, this protein is synthesized by various cell types and tissues, comprising dendritic cells and peripheral blood monocytes.⁽²⁾ Furthermore, visfatin is usually expressed by lymphocytes, bone marrow, liver cells, skeletal muscles, trophoblasts, and fetal membranes.⁽²⁾

This pleiotropic mediator acts as a growth factor, cytokine, and enzyme.⁽³⁾ In addition; it has several pro-inflammatory roles. It can encourage the expression of interleukin 6 (IL-6), IL-1 β and tumor necrosis factor alpha (TNF- α) in human monocytes. Contrariwise, an increase in the levels of the above mentioned pro-inflammatory cytokines in the periodontal tissues can induce visfatin production. Likewise, it has been validated 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 as for example rheumatoid arthritis, type 2 diabetes mellitus and inflammatory bowel disease.^(2,4) Visfatin was found to be one of the adipokines that may play a role in the pathogenesis and severity of psoriasis in addition to its role in diseases of the cardiovascular system.⁽⁵⁾

Moreover, visfatin has been proposed as being expressed in normal, inflamed, and tumor tissues.^(6,7) It possesses nicotinamide adenine dinucleotide (NAD) biosynthetic activity and regulates growth, angiogenesis, and apoptosis in mammalian cells.^(8,9) Apoptotic cell death was thought to be a contributory cause of basal cell destruction in oral lichen planus (OLP).⁽¹⁰⁾

Additionally, bullous erosive oral lichen planus (BEOLP) is considered a potentially malignant condition, and aiming to reduce the morbidity and mortality rate arising from oral lichen planus; the early

diagnosis of oral cancer was recommended.⁽¹¹⁾ It was found that visfatin is identifiable and measurable in saliva.⁽¹²⁾

Determining the potential for malignant transformation of oral lichen planus (OLP) is complicated by difficulties in diagnosis.⁽¹³⁾

Numerous studies have reported that the inflammatory infiltrate can be a strong risk factor for cancer development in chronic, inflammatory conditions. Adipose tissue produces several proteins (adipocytokines), such as TNF- α , IL-6, type-1 plasminogen activator inhibitor (PAI-1), adipo-nectin, leptin, resistin, visfatin and apelin are associated with the risk of cancer at various sites (e.g., breast, prostate gland, endometrium and colo-rectum).^(14,15)

The altered secretion of metabolically active, pro-inflammatory adipo-cytokines from adipose tissue is believed to play a key role in the mechanisms related to cancer.⁽¹⁶⁾

To the authors' knowledge, there have been no studies that investigated salivary concentrations of visfatin in BEOLP and OSCC. The aim of this study was to evaluate salivary level of visfatin and to find out if it can be used instead of its serum level as an early diagnostic biomarker for patients with either BEOLP or OSCC.

SUBJECTS & METHOD:

I. Study population:

The present study was performed on a total of 45 subjects, 13 males and 32 females. All participants were recruited from the outpatient clinic of Oral Diagnosis, Oral Medicine and Periodontology Department, Faculty of Oral and Dental Medicine, Cairo University.

Inclusion criteria:

Participants were systemically free as evaluated by the aid of Dental modification of the Cornell medical index to standardize their systemic

condition. ⁽¹⁷⁾ (*Brightman, 1994*). They had no history, symptoms, and/or signs of infection and allergy. Furthermore, they were selected to be free of any oral lesions other than OSCC or BEOLP which had to have the following criteria: (i) painful lesions (ii) no topical treatment for 2 weeks, and no systemic treatment used for BEOLP for one month before the start of sampling.

Exclusion criteria:

Pregnant or breast-feeding women or children patients, those who use corticosteroids or other immune-suppressive drugs, and patients who had allergy to macrolide antibiotics and smokers, all were excluded from the study.

Ethical procedures:

All subjects were informed about the detailed procedure and they were given written approval consent to sign. Patients were treated after the samples had been collected. The study was performed between September 2014 and February 2016.

The forty five selected participants were divided into three groups as follows:-

Group A:

It included 15 medically free subjects, 4 males and 11 females. Their ages ranged from 35-42 years.

Group B:

It included 15 patients suffering from BEOLP, 3 males and 12 females. Their ages ranged from 36-48 years.

Group C:

It included 15 patients suffering from OSCC, 6 males and 9 females. Their ages ranged from 41-58 years.

II- Clinical monitoring

The following procedures were performed for all individuals included in the study:

1. Saliva collection

Un-stimulated whole saliva was collected from all participants. Each individual was requested to abstain from eating, drinking and brushing his/her teeth for at least 60 min prior to collection. Saliva samples were collected between 8 a.m. and 2 noons. Un-stimulated whole saliva was collected using the drooling technique. Each subject rinsed their mouth with water before saliva collection, and then the subject was asked to swallow to remove saliva from the mouth. The subject was seated upright, and leaned his/her head forward over a test tube with a funnel, allowing the saliva to drain into the tube. Whole saliva (~5 mL) was obtained from each individual. During saliva collection, the test tube was placed on ice. At the end of the collection, any remaining saliva in the patient's mouth was expelled into the test tube.

2. Serum collection

Venous samples were obtained to measure the serum visfatin level using the ELISA technique after 12 hours of fasting. Blood samples were collected in sterile tubes and allowed to clot at room temperature. Sera were isolated by centrifugation and stored frozen below -20 until assayed for the visfatin level.

3-Saliva processing

Saliva obtained was centrifuged at 15,000×g (MPW-65R, MPW, Med Instrument, Warszawa, Poland) for 15 min at 4°C to remove insoluble material. Supernatant was divided into 1-mL aliquots in pre-chilled cryo-tubes. The specimens were immediately frozen (-80°C) until analysis.

4- Visfatin level detection by ELISA:

The level of visfatin was measured in serum and saliva samples by using ELISA kit provided by *EIAab*[®], China Catalog No: E0638h. The ELISA is based on the competitive binding Enzyme Linked Immune Sorbent Assay technique. The micro-titre

plate provided in this kit has been pre-coated with an antibody specific to C4a, C4a in the sample or standard competes with a fixed amount of biotin-labeled C4a for sites on a pre-coated Monoclonal antibody specific to C4a. Then Avidin conjugated to Horseradish was added to each well and incubated. Then a TMB substrate solution was added. Then termination was done by the addition of sulphuric acid solution and the color change was measured spectro-photo-metrically at a wavelength of 450 nm \pm 2 nm. The concentration of C4a in the samples was then determined by comparing the O.D. of the samples to the standard curve.



Fig. (1) A clinical photograph showing BEOLP in right side of cheek mucosa.



Fig. (2) A clinical photograph showing OSCC in floor of mouth.

III. Statistical Analysis

Quantitative data were presented as mean, median, standard deviation (SD), range (Minimum – Maximum) and 95% Confidence interval (95% CI) for the mean values. Data were explored for normality by checking the data distribution and using Kolmogorov-Smirnov and Shapiro-Wilk tests. Visfatin levels in serum showed parametric distribution while visfatin levels in saliva showed non-parametric distribution.

For parametric data; one-way ANOVA followed by Tukey's test were used to compare between the three groups. For parametric data; one-way ANOVA followed by Tukey's test were used to compare between the three groups. For non-parametric data; Kruskal-Wallis test was used to compare between the three groups. Mann-Whitney U test with Bonferroni's adjustment was used for pair-wise comparisons when Kruskal-Wallis test is significant. Spearman's correlation coefficient was used to determine the correlation between visfatin levels in serum and saliva.

ROC (Receiver Operating Characteristic) curve was constructed to determine the cut-off values of VISFATIN for detection of BEOLP. Areas under the ROC curve (AUCs), sensitivity, specificity, predictive values and diagnostic accuracy was calculated.

The significance level was set at $P \leq 0.05$. Statistical analysis was performed with IBM® SPSS® Statistics Version 20 for Windows.

RESULTS

Demographic data

There was no statistically significant difference between mean age values in the three groups. There was also no statistically significant difference between gender distributions in the three groups.

TABLE (1): Descriptive statistics and results of one-way ANOVA and Chi-square tests for comparison between demographic data in the three groups

	OSCC	BEOLP	Control	P-value
Age (mean, SD)	47.7 (14.1)	42.3 (11.0)	38.5 (7.8)	0.095
Gender (n, %)				0.469
Male	6 (40.0)	3 (20.0)	4 (26.7)	
Female	9 (60.0)	12 (80.0)	11 (73.3)	

*: Significant at $P \leq 0.05$

TABLE (2): Descriptive statistics and results of one-way ANOVA, Tukey’s tests, Kruskal-Wallis and Mann-Whitney U tests for comparison between visfatin levels in the three groups

	Group	Mean	SD	Median	Minimum	Maximum	95% CI		P-value
							Lower bound	Upper bound	
Serum	OSCC	34.5 ^a	7.0	32.6	22.8	51.3	30.6	38.4	<0.001*
	BEOLP	36.4 ^a	5.7	35.5	29.7	50.1	33.2	39.5	
	Control	13.8 ^b	2.3	13.0	10.9	18.3	12.5	15.1	
Saliva	OSCC	2184.7 ^a	898.0	2018.0	1068.0	3681.0	1687.5	2682.0	<0.001*
	BEOLP	2133.5 ^a	570.0	2047.0	1236.0	3247.0	1817.9	2449.2	
	Control	160.1 ^b	24.9	159.0	117.0	191.0	146.3	173.9	

*: Significant at $P \leq 0.05$, Different superscripts in the same column are statistically significantly different

Visfatin levels

Whether in serum or saliva, there was no statistically significant difference between mean visfatin levels in OSCC and BEOLP groups; both showed statistically significantly higher mean levels than control group which showed the lowest mean visfatin level.

Correlation between serum and salivary visfatin levels

There was a statistically significant positive (direct) correlation between serum and salivary visfatin levels ($r = 0.835$, P -value <0.001) i.e. an increase in serum level of visfatin is associated with an increase in salivary level of visfatin.

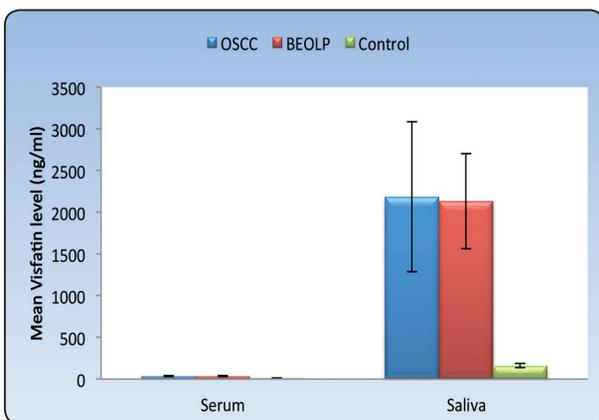


Fig. (3): Bar chart representing mean and standard deviation values of visfatin levels in the three groups

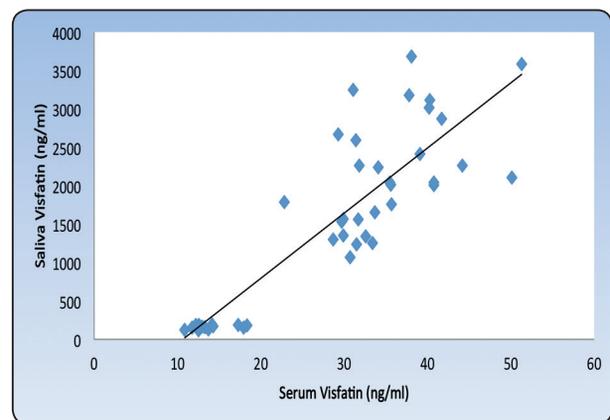


Fig. (4): Scatter diagram representing positive correlation between serum and salivary levels of visfatin

ROC curve analysis (Cut-off values)

Results of ROC curve analysis are presented in Table (3) and Figures (5-6).

ROC curve analysis of serum and salivary levels

of visfatin in the present study showed cut-off values of 18.3 and 191.0 ng/ml, respectively. At these cut-off values, the diagnostic accuracy of visfatin as a marker for detecting OSCC and BEOLP is 100.0%.

TABLE (3): Sensitivity, specificity, predictive values, diagnostic accuracy, Area under the ROC curve (AUC), 95% confidence interval (95% CI) of serum and salivary visfatin levels

Visfatin (ng/ml)	Cut-off	Sensitivity %	Specificity %	+PV %	-PV %	Diagnostic accuracy %	AUC	95% CI
Serum	18.3	100.0	100.0	100.0	100.0	100.0	1.000	0.884 – 1.000
Saliva	191.0	100.0	100.0	100.0	100.0	100.0	1.000	0.884 – 1.000

+PV: Positive Predictive Value, -PV: Negative Predictive Value

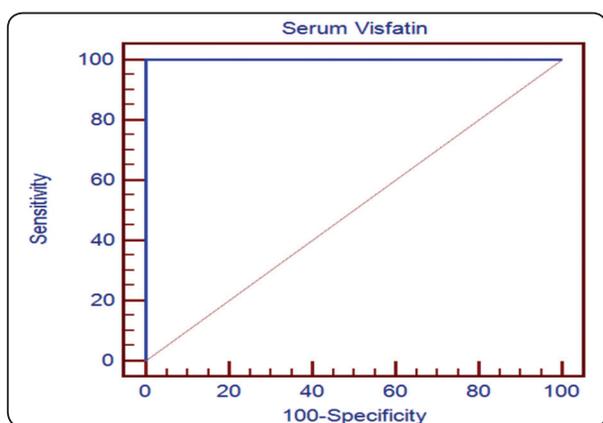


Fig. (5): ROC curve for Visfatin level in serum.

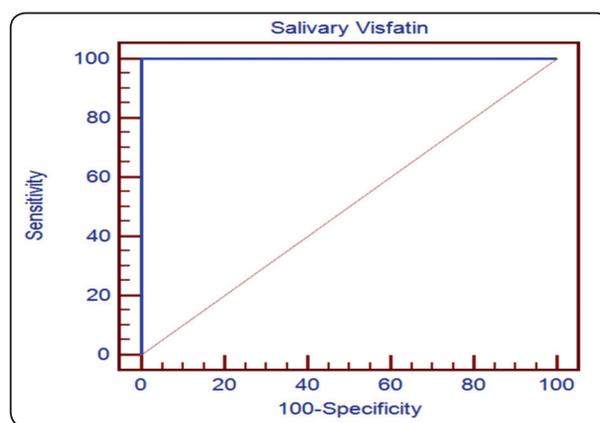


Fig. (6): ROC curve for Visfatin level in saliva.

DISCUSSION:

The aim of this study was to evaluate salivary level of visfatin and to find out if it can be used instead of its serum level as an early diagnostic biomarker for patients with either BEOLP or OSCC.

Salivary samples were chosen for evaluation, as saliva is considered a diagnostic material, (18) abundant and has a simple sampling technique, fast and non-invasive; also, has no need for specialized equipment for its sampling. (19,20)

The present study showed no statistically

significant difference between both sex and mean age values in the three groups, as shown in table (1). These results are not in agreement with the study performed by Eisen in 2003. (21) His study detected that both OLP and OSCC were more common in females and, on average, being in the sixth decade of life similar to the results of Fitzpatrick et al in 2014. (13)

Regarding the mean visfatin levels, whether in serum or saliva, the present study showed no statistically significant difference between mean visfatin levels in OSCC and BEOLP groups;

however, both showed statistically significantly higher mean levels than control group which showed the lowest mean visfatin level. These results are in agreement with *Tabari et al in 2014* ⁽²²⁾ who found that salivary visfatin levels were significantly higher in the patients with CP (chronic periodontitis) than healthy controls. In addition, a positive and significant correlation was found between CAL (clinical attachment level) loss and salivary concentrations of visfatin. They explained their results by the role of visfatin in the pathogenesis of periodontal disease by local immune-inflammatory reactions in periodontal tissues that can result in degradation and resorption of the periodontal structures, respectively. Also, the present results are similar to the study performed by *Özcan et al in 2015*. ⁽²³⁾ They demonstrated higher salivary visfatin levels in patients with gingivitis and periodontitis compared to those of healthy subjects.

Moreover, these results are in accordance with the study of *Yu-Duan et al in 2013* ⁽²⁴⁾, it revealed that visfatin serum concentrations were significantly elevated in a fully adjusted model in patients with OSCC. Furthermore, they found that visfatin was gradually increased with stage progression. This could be attributed to the role of visfatin in activation of leukocytes and hence production of cytokines such as IL-1 β , IL-6 and tumor necrosis factor. Therefore, visfatin could be regarded as an adipo-cytokine with pro-inflammatory, immune-modulating, and apoptosis-inhibiting potentials. ⁽³⁾

Similarly, *Badran et al in 2014* ⁽⁵⁾ found that the serum level of visfatin was statistically significantly higher in patients suffering of psoriasis than in the healthy control group. Moreover, *Ismail & Mohamed in 2012* ⁽²⁵⁾ detected that serum visfatin level was significantly higher in patients with severe psoriasis than those with mild and moderate psoriasis with no statistically significant difference between normal weight, overweight, and obese patients in the serum level of visfatin. On the same

hand, *Gerdes et al* ⁽²⁶⁾ found that serum visfatin is increased in patients with psoriasis independent of other factors such as basal metabolic index (BMI).

The present study detected a statistically significant positive (direct) correlation between serum and salivary levels of visfatin i.e. an increase in serum level of visfatin is associated with an increase in salivary level of visfatin.

To author's knowledge, little studies were performed about the salivary level of visfatin in oral diseases. However, these results are similar to the study performed by *Tabari et al*, ⁽²²⁾ who concluded that salivary concentration of visfatin could be a beneficial and convenient alternative for serum and GCF visfatin in the evaluation of periodontal disease.

On the same hand, *Abolfazli et al in 2015* ⁽²⁷⁾ demonstrated higher level of visfatin in the saliva more than in serum and this could be explained by the leakage of saliva from salivary glands, and they considered it as a biomarker that reflects the function of periodontal tissues.

The diagnostic accuracy of visfatin measurements as a salivary marker was evaluated by the ROC plot method that proved to have very high sensitivity, specificity, and diagnostic accuracy. Actually, it has been suggested that a test's diagnostic accuracy should not be lower than 80% to be considered applicable, valid and suitable for diagnostic purposes. ⁽²⁸⁾ In ROC analysis, an AUC (area under curve) value denotes the combined effects of both specificity and sensitivity for an assay system. The present study determined the AUC values in both serum and saliva as 1.000, and 1.000, respectively. It is known that an AUC value of 0.9 or higher refers to an excellent diagnostic marker. ⁽²⁹⁾

For using saliva as a diagnostic material ⁽¹⁸⁾, different candidate biomarkers for the assessment of periodontal disease were investigated; in addition, several studies have demonstrated that

adipose tissue secretes adipo-cytokines, which are mediators that have important roles in immunity and inflammation. ⁽⁴⁾

Moreover, as quite a lot of studies have testified the elevation of visfatin level in many inflammatory illnesses such as Behcet syndrome, chronic kidney disease, chronic obstructive pulmonary disease, appendicitis, metabolic syndrome ^(3,30,31) rheumatoid arthritis, type II diabetes mellitus and inflammatory bowel disease. ^(2,4)

On the same hand, visfatin is considered as a risk factor for cardiovascular diseases. ^(32,33) Also, an increase in the expression of visfatin by macrophages in unstable atherosclerotic lesions indicates the possible role of visfatin in destabilization of the atherosclerotic plaque ⁽³⁴⁾

Accordingly, it can be concluded that salivary level of visfatin can be used over its serum level as a biomarker for OSCC and chronic inflammatory and immune modulating conditions such as BEOLP.

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