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Salivary Osteopontin Was Associated with Lesion Size in Patients with Oral Lichen Planus

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ABSTRACT

Introduction: Osteopontin (OPN) is recognized as a potent biomarker of Oral lichen planus (OLP) because of its vital role in inflammation and the repair process. The present study aims to assess OPN in OLP in comparison with healthy controls (HC).

Materials and Methods: To explore salivary levels of OPN, a group of 20 subjects with OLP was compared with 20 healthy controls. Salivary OPN levels were measured by enzyme-linked immunosorbent (ELISA) assay.

Results: Results indicated elevated OPN levels in lesion size<1cm compared with 1-3cm lesion size of OLP (p=0.02). In contrast, we did not find a significant difference in OPN expression level in saliva from OLP patients and healthy controls (P=0.96).

Conclusion: Osteopontin plays a role in the process of repair and healing in oral lichen planus, providing tissue protection and enhancing the capacity for tissue wound healing in these lesions.

Keywords: Oral lichen planus; Osteopontin; Saliva.

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Introduction

Oral lichen planus (OLP) is a chronic inflammatory oral disease that affects buccal membranes, tongue, and gingiva [1]. Clinical presentation of OLP is as white striations (Wickham's striae), white papules, white plaques, erythema, erosions and blisters [2]. The most common types of OLP are reticular, atrophic, plaque-like and ulcerative/erosive forms [3]. The prevalence rates of OLP vary from 0.5–2.2% of the population and the usual age of presentation is between 30 and 60 years, and it is more common in women [4]. A systematic review detected that the prevalence of malignant transformation was 1.09% and a slight predominance of females was seen among those who experienced transformation [5]. Oral squamous cell carcinoma (OSCC) is more about to develop in atrophic and erosive OLP lesions, which are usually treated with immunosuppressants [6]. At this time, histopathological investigation represents the "gold standard" for oral disease diagnosis combined with clinical oral examination [7].

Surgical biopsies are inherently invasive, expose patients to procedural risks and could delay diagnosis and treatment [8]. Salivary biomarkers could be used to develop screening techniques and early detection of OLP, which can improve treatment and patients' quality of life [3]. Some inflammatory biomarkers such as C-reactive protein (CRP), interleukin (IL) 6, IL 18, fibrinogen and adhesion molecules have been recognized to monitor inflammatory diseases. They are known as molecular biomarkers which can be objectively measured to determine the nature and development of pathological processes, such as inflammation [9]. The mechanisms underlying the immunopathogenesis of OLP have not yet been elucidated [2].

Both innate and adaptive immune responses may play vital roles in the pathogenesis of OLP [6]. OLP is characterized by a T-cell-mediated immune response against epithelial cells, with persistent T cells and few B-cells accumulation and epithelial cell damage [3,10]. Unusual productions of inflammatory mediators reflect the immune dysregulation in OLP, which may be involved in the regulation of interactions between many cell types, such as keratinocytes and T cells. Cytokines are suggested as the most major types, among these inflammatory mediators [11]. Several studies have described an abnormal expression patterns of various inflammatory cytokines, such as IL-1, IL-6, IL-8, TNFa [12], IL-2, IL-10 [13], IL-4, IL-17 [14], TGFb, IFN-γ [15] and in lesions, saliva, serum and peripheral blood mononuclear cells from OLP patients, which may appear as central players in the immunopathogenesis of OLP [11]. Osteopontin (OPN) is a secreted glycosylated multifunctional phosphoprotein which has an important role in physiological and pathological processes such as cell survival, adhesion, migration, apoptosis, inflammation and wound healing and is elevated in many autoimmune diseases [16,10]. During inflammation, OPN is expressed by both antigen-specific and non-specific immunity cells such as activated T cells and macrophages [10]. OPN plays T helper 1 (Th1) cytokine roles including stimulation of macrophage and T-cell migration [17,10]. In addition, OPN plays a role in tumor development, progression and metastasis [18]. Its initial description was a malignant transformation-associated protein [19]. Previous studies suggested that OPN is involved in inflammatory process of OLP and may serve as a candidate biomarker for OLP [10]. As OPN has important roles during the pathogenesis of OLP, the purpose of the present study is measurement of OPN levels in saliva from OLP patients and healthy controls to investigate possible alterations associated with OLP occurrence and gain more insights into the pathogenesis of this disease.

Materials and Methods

Study Population

The Ethical Committee of the College of Dentistry, Kashan University of Medical Science, approved the protocol by IR.KAUMS.MEDNT.REC.1399.191 code and informed written consent has been signed by patients and control volunteers. This was a case-control study and samples were obtained from 20 patients who presented to Ketabchi Dental Clinic by non-random sampling technique. The characteristics of the subjects are seen in Table 1. Diagnosis was based on clinical and histological criteria. A periodontal probe was used to measure lesion size in the mouth. All subjects neither had detectable contact sensitivity in the oral mucosa, nor any visible oral lesions under careful examination. Also, they were not taking medicine inducing hyposalivation, or other drugs such as anticholinergics, antihistamines, antihypertensives and β-adrenergic blockers. Further, none of the study individuals received OLP treatment within 30 days before specimen collection, had history of radiotherapy particularly in the head and neck region or had history, symptoms or signs of infections, allergies, smoking and other autoimmune disease such as sjogren syndrome, Type 1 diabetes, rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), progressive systemic sclerosis (PSS), uncon-

trolled chronic disease such as fatty liver, AIDS, Hepatitis B and C. Control saliva samples were obtained from 20 healthy individuals who presented to Ketabchi Dental Clinic.

Enzyme-linked immunosorbent assay (ELISA)

The enzyme-linked immunosorbent assay (ELISA) was applied to determine differences in salivary OPN between OLP patients and healthy controls. All saliva samples were aliquoted and stored frozen at≤-20°C to prevent cytokine degradation. Then centrifuged for 15 min at 1500 rpm. The levels of soluble OPN were determined by means of the commercially available Kit (Zelbio's Osteopontin Human ELISA). Briefly, 100µl of diluted standard (ranging from 0.15 to 10 ng⁄ml)/ blank/samples were added to each well of Costar 96-well plate coated with anti-OPN antibody. The plate was sealed and then incubated for 2 h. Biotinylated antibody was incubated for 1 h and HRP-Conjugated Streptavidin was incubated for 1 h. Then TMB substrate-chromogen was added and developed for 15 min in the dark and then reaction was stopped by adding 100µl of stop solution (resulting in a yellow solution). All incubations were at 37°C. TBS washing buffer was used to wash all wells between each step. The absorbance was measured at 450nm, Page 5/11, by microplate reader (Epoch, BioTek). Standard curves were plotted on a log–log scale. The sample concentrations were determined by linear regression of log average column absorbance against log standard concentration. The total protein content was measured in saliva using the BCA Protein Assay Reagent with bovine serum albumin (BSA) as a standard. The concentration of the target protein was normalized to total protein of sample assayed.

Statistics

We used Statistical Analysis System (GraphPad Prism) software to analyze the data and then the Kolmogorov-Smirnov test to check normality of data distribution and the non-parametric Mann-Whitney test for comparison of OLP and healthy control groups. For comparison of OPN levels among three subgroups of patients (according to lesion size, OLP type and lesion location in the mouth), the Kruskal–Wallis test was calculated. A value of $p<0.05$ was considered to be statistically significant.

Results

The aim of this non-randomized case-control study was to identify the OPN present in saliva from patients with OLP in comparison with healthy controls. To this end, we collected saliva specimens from OLP and healthy cohorts and conducted ELISA to assess OPN levels. The demographic and clinical data of individuals included in the present study are shown in Table 1. Results from ELISA indicated that the OPN was detected in all saliva samples obtained from patients and healthy subjects. The salivary OPN levels were 0.224±0.043 (mean±SEM) and 0.231±0.054 in OLP patients and healthy controls, respectively. The salivary OPN levels in patients with OLP did not differ significantly from the levels in healthy controls ($p=0.96$) (Figure. 1).

Table 2 summarizes the patients who analyzed for OPN protein expression, including levels of OPN in the saliva of different subgroups of patients with OLP. In addition, we intended to investigate possible differences in salivary OPN expression according to the location of the lesions in OLP patients. Based on OLP types, patients were subdivided into plaque-like, erosive and reticular cohorts. We did not observe any significant statistical difference in salivary OPN expression between the three subgroups ($p=0.12$) (Figure. 2.a). Results revealed that there is no significant difference in OPN expression between buccal, buccal-tongue and buccal-gingiva groups ($p=0.24$) (Figure. 2 b). Moreover, based on lesion size, patients were subdivided into<1cm, 1–3cm and 3<cm groups. The salivary level of OPN was 0.374±0.115 (mean±SEM) in lesion size<1cm, 0.166±0.035 in lesion size 1–3cm and 0.137±0.022 in lesion size 3<cm.

Significantly elevated levels of salivary OPN were detected in lesions size<1 when compared with size 1–3 ($p=0.02$). In contrast, the difference was not statistically significant between the other groups of lesion size (Figure. 2.c). Evaluation of OPN levels based on OLP type (a), OLP lesion site in the mouth (b) and OLP lesion size (c). Table d displays salivary protein levels of OPN as mean±SEM in subgroups of type, location and size of OLP lesions. OPN protein levels were assessed by ELISA and normalized to total protein of samples. To Page 10/11 compare the OPN levels among subgroups of OLP patients, we applied the Kruskal–Wallis test. A value of $p<0.05$ was considered to be statistically significant. The colored circle shows the amount of protein in each patient

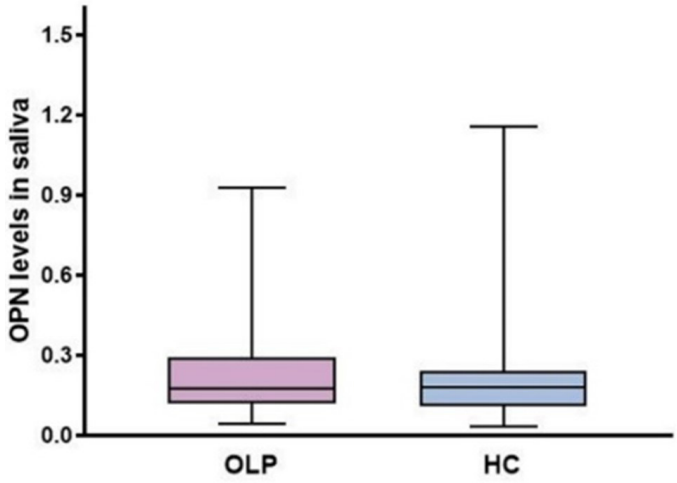

Figure 1. Salivary levels of Osteopontin (OPN) in Oral lichen planus (OLP) patients and healthy controls (HC). OPN protein levels were assessed by ELISA and normalized to total protein of samples. Box plots depict OPN levels obtained from OLP (n=20) and HC (n=20) groups. Horizontal lines indicate the median, quartiles, minimum and maximum. The Mann-Whitney test was applied to analyze data from the OLP and HC groups. A value of $p<0.05$ was considered to be statistically significant.

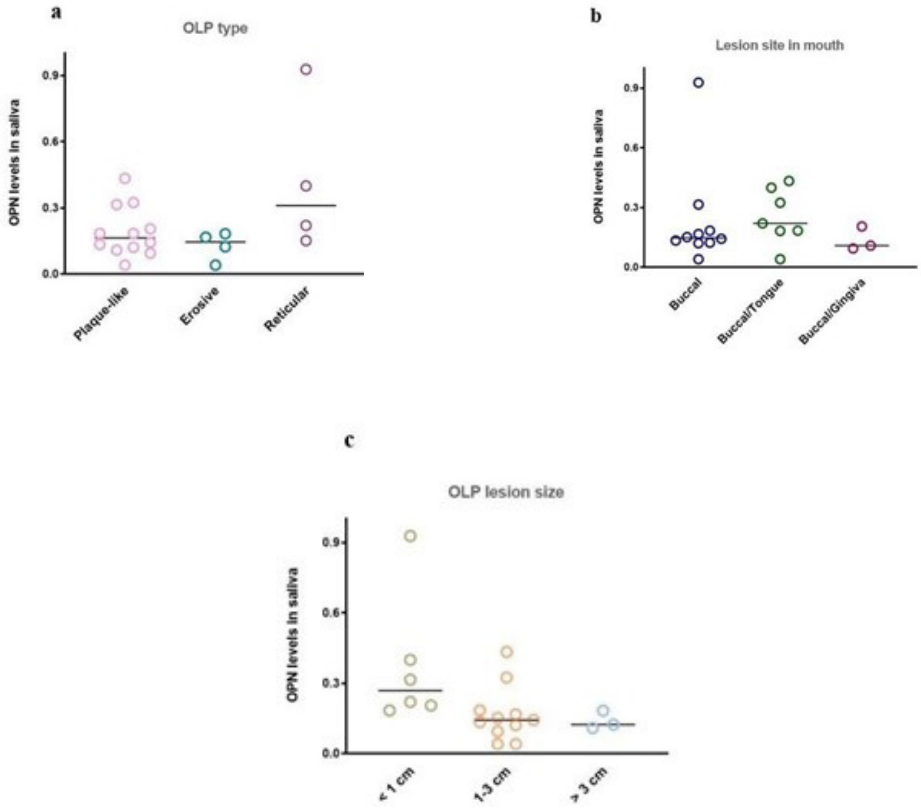

Figure 2. *********

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Table 1. Demographic and clinical description of the study groups (N=20 in each group).

Study Groups	Demographic and Clinical Features											
OLP[1]	Age (Mean ± SEM)	Sex		OLP type			OLP site			OLP size(cm)		
	49± 2.46	Male	Female	Reticular	Erosive	Plaque-like	Buccal	Buccal Tongue	Buccal Gingiva	<1	1-3	3<
		N=2(10%)	N=18(90%)	N=12(60%)	N=4(20%)	N=4(20%)	N=10(50%)	N=7(35%)	N=3(15%)	N=6(30%)	N=11(55%)	N=3(15%)
HC[2]	43 ±1.71	N=2(10%)	N=18(90%)									

Abbreviations:
[1] Oral Lichen Planus.
[2] Healthy Controls.

Table 2. Salivary protein levels of OPN as mean±SEM in subgroups of type, location and size of OLP lesion.

Characteristics	Type of OLP	OPN[1] expression	P value
Type	P[2]	0.190±0.032	P VS E>0.99
	E[3]	0.127±0.031	P VS R=0.3
	R[4]	0.424±0.175	E vs R=0.14
Location	B[5]	0.230±0.080	B VS BT=0.61
	BT[6]	0.254±0.052	B VS BG>0.99
	BG[7]	0.135±0.034	BT VS BG=0.37
Size	<1cm	0.374±0.115	<1 vs 1-3 =0.03
	1-3 cm	0.166±0.035	<1 vs 3<=0.09
	3<cm	0.137±0.022	1-3 vs 3<=0.99

Abbreviations:
[1] Osteopontin.
[2] Plaque-like.
[3] Erosive.
[4] Reticular.
[5] Buccal.
[6] Buccal Tongue.
[7] Buccal Gingiva.

Discussion

Saliva is a readily accessible fluid that consists of compounds and proteins produced locally and systemically which can serve as markers for many disease diagnosis. Salivary biomarkers have been Page 6/11 increasingly used in the diagnosis of disease following the improvement of highly sensitive analyses with simple and non-invasive saliva collection methods. Several proteins have been suggested as salivary biomarkers for OLP. Particularly, C-reactive protein, Immunoglobulin A (IgA), matrix metalloproteinase 8 (MMP8), CTX I, CD14 and toll-like receptor-2 exhibit upregulation in OLP patients' saliva [3]. OPN, the first extracellular matrix protein, has vital immunological roles [20]. Some studies recommended that OPN has anti-inflammatory and tissue repair effects at sites of inflammation [21]. We selected OPN that appears to have the potential to serve as repair and inflammation biomarker for OLP and validated the salivary levels of this protein by ELISA technique. Our study confirms elevated OPN levels in smaller lesion sizes of OLP. This observation emphasizes the critical role of OPN in the protection of the mucosa and the repair process. Therefore, OPN levels evaluation can be important in patient's follow-up for lesion repair. During inflammation, OPN helps the formation of granulation tissue and connective tissue remodeling around the epithelial ulcer [22]. Epithelial cells secrete OPN that is necessary for epithelial barrier integrity maintenance and helps the

transition from innate to adaptive immune response with initiation of repair [23]. In addition, we did not find a significant difference in OPN expression level in saliva from OLP patients and healthy controls. To our knowledge, until now, there is no study in which OPN expression levels were assessed in saliva from OLP patients. Zhou et al. showed that serum concentrations of OPN were significantly higher in OLP patients than control subjects [10]. Santarelli et al. detected high expression of OPN in biopsy specimens of patients with OLP [24]. This discrepancy in these studies may have been caused by the difference in the tissue type of the samples. Several previous studies have compared the expression of cytokines related to OPN in saliva and serum. Results from these investigations revealed that the expression pattern of some cytokines in saliva and serum was different [25]. One of the study's limitations is that we used a small sample size. So, a larger sample size is required to increase the power of such studies. In conclusion, this study has provided proof of concept for the biomarker role of salivary OPN to monitor the repair process in OLP.

Conclusion

In this study, the level of osteopontin in the unstimulated saliva of patients with oral lichen planus with extended lesions was found to be decreased. It seems that osteopontin plays a role in the process of repair and healing in oral lichen planus, providing tissue protection and enhancing the capacity for tissue wound healing in these lesions.

Conflict of Interest

There is no conflict of interest to declare.

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