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ORIGINAL RESEARCH

Serum and Saliva MMP-3 in Patients with OLP and Oral SCC

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ABSTRACT

Background: Matrix metalloproteinase-3 (MMP-3) plays a key role in development of cancer. The purpose of this study was to assess MMP-3 in the serum and saliva of patients with oral lichen planus (OLP) and oral squamous cell carcinoma (OSCC).

Materials and methods: Thirty patients with OLP (8 reticular and 22 erosive forms), and 20 patients with OSCC (6 in low stage and 14 in advanced stage), were enrolled in this study, conducted at the Cancer Department, Clinic of Oral Medicine, Tehran University of Medical Sciences. The serum and saliva MMP-3 was assayed by ELISA method. Statistical analysis of the Student's t-test, ANOVA and Pearson correlation coefficient was performed. The mean saliva and serum levels of MMP-3 were significantly higher in patients with OSCC compared with OLP.

Results: The serum and saliva MMP-3 concentrations increased from reticular form of OLP to erosive form of OLP, and increased further to low stage of OSCC and advanced stage of OSCC. Serum MMP-3 correlated significantly with unstimulated ($r = 0.310$, $p = 0.038$) and stimulated ($r = 0.365$, $p < 0.026$) saliva MMP-3.

Conclusion: Serum and saliva MMP-3 levels appear associated with OLP and OSCC.

Keywords: MMP-3, Oral lichen planus, Squamous cell carcinoma, Saliva.

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INTRODUCTION

Oral lichen planus (OLP) is a common, chronic, immunological mucocutaneous disease that commonly involves the oral mucosa.[1] The prevalence rate of OLP varies from 0.1 to 4% in the general population,[2] and it was diagnosed approximately in 55 years.[3] In addition, there is an obvious female predominance of 2:1.1.[4] Unlike oral lesions, skin lesions are usually self limited in majority of cases lasting only 1 year or less.[4,5] Oral lichen planus is ideal for human T-cell-mediated autoimmunity and inflammation studies.[6] Many autoimmune features of OLP supported the role of autoimmunity in pathogenesis of disease including disease chronicity, adult beginning, and female tendency; involvement with other autoimmune diseases, occasional tissue-type association, depressed immune suppressor activity in OLP patients, and the presence of auto-cytotoxic T-cell clones on lichen planus lesions.[7]

Since, the first reported case of a squamous cell carcinoma (SCC) developing from a mucosal lichen planus, transformation of this lesion to SCC is considered an important issue for the patient, although the real odds of such transformation are a matter of discussion.[2,8] In the past decades, many papers have suggested that patients with OLP are at an increased risk of developing cancer, which led the World Health Organization to classify this disease as a premalignant condition.[9] Frequencies of progression from OLP to oral SCC ranging from 0.4 to 6.5% have been reported.[10]

Squamous cell carcinoma represents more than 90% of all head and neck cancers.[9] Of all diagnosed cancers, oral cancers account for 2 to 4%. They significantly affect several aspects of a patient's quality of life, and are one of the preventable causes of disability and death. About 90% of oral cancers occur in subjects over 40 years, the average age at the time of diagnosis being in the 60s. Oral cancers are malignancies which can be diagnosed early.[11] OSCC typically presents as a persistent mass, nodule, or indurate ulcer. It can develop from precancerous lesions, or apparently normal epithelium.[9]

It has become accepted that host-related factors may be the keys to the fundamental understanding of the disease processes in many oral diseases. One of these host factors is a family of enzymes called Matrix metalloproteinases (MMPs).[12] Matrix metalloproteinases are zinc

dependent endopeptidases that are capable of degrading extra cellular matrix proteins. This group of 28 human enzymes is classified into collagenases, gelatinases, stromelysins, membrane-type MMPs and other MMPs, mainly based on the substrate specificity and molecular structure.[12,13] The coordinated and controlled synthesis, breakdown and remodeling of the extracellular matrixes and basement membrane components are critical events in normal physiological conditions.[14,15] The activity of MMPs is seen not only during normal organogenesis and wound healing, cell proliferation, differentiation, migration, apoptosis, and angiogenesis, but also in pathological conditions like inflammatory diseases and invasion.

MMP-3 (stromelysin-1), a MMP family member, is a secretory enzyme and its expression is up regulated by several growth factors, such as epidermal growth factor and transforming growth factor-a, or can be stimulated by the wound healing process. It is also shown to promote the epithelial-to-mesenchymal transition and the carcinogenesis of mammary tumors.[16] MMP-3 is expressed by keratinocytes, fibroblast and chondrocytes.[14]

There has been increasing interest in saliva-based analyses in recent years.[9] Saliva as a diagnostic specimen can give not only the same information as serum testing, but also additional or new information that cannot be obtained from serum. From a logistical perspective, the collection of saliva is safe, patient-friendly, noninvasive, and simple, and it may be collected repeatedly without discomfort to the patient. Because of these significant characteristics, finding biomarkers in saliva for the detection of serious systemic illnesses, such as cancer, is of great interest for most salivary researchers.[17]

In the present study; we assess the serum and salivary levels of MMP-3 in OLP as compared to OSCC.

MATERIALS AND METHODS

Subjects

Twenty patients with SCC (13 males, 7 females, aged 28 to 77), proven by biopsy and pathological examination, referred to the Cancer Department of Emam Khomeini Hospital of Tehran University of Medical Sciences (TUMS) for treatment, were selected for the study. The most frequent complications in patients with OSCC were exophytic mass of their mouth interfering with eating; moderate or severe pain; bleeding; or ill-fitting dentures. Mean time of presence of the complication was about 3 to 9 months.

Thirty patients with OLP (6 men, 24 women; aged 32-77 years) were selected from those referred to the Department of Oral Medicine, Faculty of Dentistry, TUMS. Inclusion criteria consisted of being clinically diagnosed with lichen planus (presence of bilateral lesions, and presence of reticular lesions elsewhere in the oral). Exclusion criteria were histological sign of dysplasia, lichenoid drug reactions, consumption of drug in the past month, breast feeding, pregnancy, and any kind of localized or systemic disease. The inclusion and exclusion criteria in this study were the same as in our previous studies.[9]

In all groups, subjects with systemic and periodontal diseases (periodontal pocket more than 3 mm), or who were taking any medications at the time of the study, were excluded. The protocol was approved by the review Board of TUMS, and written informed consent was obtained from all patients.

Saliva and Serum Sampling

Stimulated and unstimulated whole saliva were collected under resting situations in a silence room between 10.00 AM and 12.00 PM, and at least 90 minutes subsequent to the last eating of food or drink. Unstimulated salivary samples were obtained by expectoration in the absence of chewing movements. Prestimulation was accomplished by chewing a piece of standard-size paraffin, and after 60 seconds, the individuals were requested to swallow the saliva present in the mouth. Thereafter, whole stimulated saliva was collected for about 5 minutes in a dry, deionized and sterilized plastic tube. Blood specimens were obtained by venipuncture, collected in 10 ml glass vacuum tubes without additive, and allowed to clot. The blood and saliva were then centrifuged (2500 gm, 10 minutes), and the serum and supernatants of saliva were separated and immediately stored at 80°C for later determination.

LABORATORY MEASUREMENTS

Enzyme-linked immunosorbent assay was applied to measure the serum and saliva concentrations of MMP-3 using ELISA kits from Boster biological technology, USA. Determination of MMP-3 levels was carried out according to the manufacturers' instruction.

STATISTICAL ANALYSIS

For statistical analysis, the data are given as a mean ± s.e.m. Comparison of means among groups were carried out with unpaired two-tailed Student's t-test or ANOVA followed by the Student-Newman-Keuls posthoc test. The Pearson correlation test was applied to determine association between serum and salivary concentration of MMP-3. Results were considered statistically significant if $p < 0.05$. Analyses were performed using SPSS software version 16 (SPSS Inc., Chicago, IL, USA).

RESULTS

The study involved two groups. Group I consisted of 30 patients suffering from OLP (males/females: 6/24; mean age: 51.4 years). A subgroup of eight had the reticular form, and a subgroup of 22 the erosive form. The buccal mucosa was the most widespread site (50%), followed by the gingiva (30%) and tongue (20%). Group II consisted of 20 patients suffering from OSCC (males/females: 13/7; mean age: 50 years). A subgroup of six were in the low stages (I and II), and a subgroup of 14 patients in the advanced stage (III and IV). The tongue was the most common site (45%), followed by the lip (30%), buccal (15%), the floor of the mouth (5%) and the palate (5%).

Student's t-test showed that the serum concentration of MMP-3 proved to be significantly higher in patients with OSCC compared to patients with OLP (Graph 1A, $p = 0.001$). There were significant differences in the unstimulated (Graph 1B, $p = 0.032$) and stimulated (Graph 1C, $p = 0.036$) saliva MMP-3 levels between the two study groups, with the patients with OSCC having a greater amount of salivary MMP-3 than OLP patients.

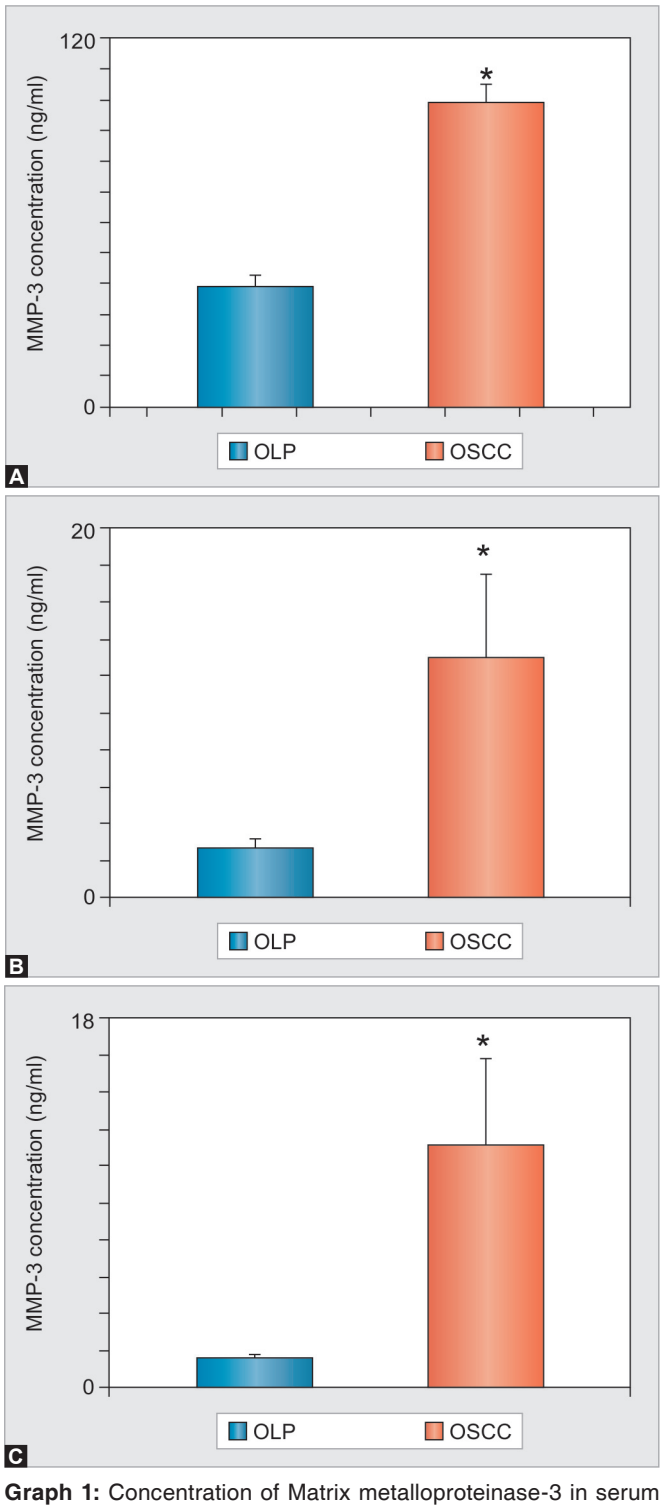
A one-way ANOVA indicated that there were significant differences in serum and saliva MMP-3 concentration between erosive and reticular forms of OLP; and OSCC patients in low and advanced stages (Table 1; $p < 0.05$). Post hoc analysis showed that the serum MMP-3 concentration was significantly higher in both OSCC subgroups than in both OLP subgroups; and also higher in advanced stage OSCC patients compared with low stage. However, stimulated and unstimulated saliva MMP-3 levels were significantly higher only in advanced stage of OSCC than in both OLP sub-groups.

Statistical evaluation of data using Pearson analysis indicated a weak correlation between salivary concentration of MMP-3 and its serum concentration. Pearson correlation coefficient, r, was 0.310 for unstimulated salivary concentration of MMP-3 ($p < 0.038$) and 0.365 for stimulated salivary of MMP-3 ($p < 0.026$).

DISCUSSION

OLP is important from several aspects. It is the most frequent non-infectious disease referred to the Oral Medicine Department with unknown etiology.[10] It has three clinical subtypes including reticular, erythematous (atrophic) and erosive (ulcerated, bullous). Lesions of reticular form are less symptomatic, although some patients with this form complain of roughness bothering them, while those presenting as atrophic and erosive types are more symptomatic, ranging from a mild burning sensation to intense pain. These latter types may also cause difficulties during speaking, eating and swallowing.[8]

The pathogenesis of OLP is unclear. Both mechanisms of antigen specific and non-specific may be implicated. Nonspecific mechanisms include mast cell de-granulation and MMP activation in OLP lesions. In the past decades; many papers have suggested that patients with OLP are at an increased risk of developing cancer, which led the World Health Organization to classify this disease as a premalignant condition.[9] Oral lesions are chronic and



Graph 1: Concentration of Matrix metalloproteinase-3 in serum (A) unstimulated (B) and stimulated saliva (C) in patients with oral lichen planus and oral squamous cell carcinoma. Data presented as mean ± s.e.m.; *$p < 0.05$

Table 1: Matrix metalloproteinase-3 concentration in patients with reticular and erosive form of oral lichen planus and in oral squamous cell carcinoma at low and advanced stages

	Serum (ng/ml)	*Unstimulated saliva (ng/ml)*	*Stimulated saliva (ng/ml)*
Reticular form of OLP (n = 8)	29.00 ± 6.21	1.065 ± 0.27	1.28 ± 0.11
Erosive form of OLP (n = 22)	43.13 ± 4.82	3.22 ± 0.64	1.48 ± 0.24
OSCC in low stage (n = 6)	69.41 ± 9.34*#	8.89 ± 7.01	5.09 ± 2.19
OSCC in advanced stage (n = 14)	114.73 ± 2.00*#†	13.05 ± 5.89*#	14.52 ± 7.7*#

Data are expressed as mean ± s.e.m; *Different from reticular form of oral lichen planus; $p < 0.05$; # Different from erosive form of oral lichen planus; $p < 0.05$; †Different from oral squamous cell carcinoma in low stage; $p < 0.05$

often refractory to conventional therapies, as most patients have cancer phobia.[4] When we encounter these patients, we do not know who is really prone to transformation to OSCC. Therefore, we decided to measure the levels of biomarker that may be prognostic or predictive in OSCC, in patients with OLP. To the best of our awareness, this is the first study about the effects of MMPs on OSCC and OLP simultaneously, and has assessed this biomarker in stimulated and unstimulated saliva and serum in OSCC and OLP. Our results show the levels of serum MMP-3 in advanced stages III and IV are double those in stages I and II. Of 30 patients with OLP, 22 were erosive and eight were reticular. Interestingly, the mean level of MMPs in stage I and II patients is about twice those with erosive OLP; in turn, their mean level is approximately double those with reticular form.

Changes of epithelial basement membrane (BM) are common in oral lichen planus and consist of duplications, breaks and branches. Basement membrane degeneration causes weaknesses at the interface of epithelial-connective tissue.[18]

Both keratinocyte apoptosis and BM disruption may be implicated in the OLP pathogenesis. For example, BM disruption may trigger keratinocyte apoptosis, and apoptotic keratinocyte seems to be unable to repair the disrupted BM. Such a cyclical mechanism may underlie chronicity of disease.[19] Contrarily, progress in cancer research in the last decade has demonstrated that at least five cancer causing factors exist: direct DNA injuries, spontaneous replication errors in DNA, cytotoxic and/or inflammatory, ultra violet and transduction of viral oncogenes. Infection/inflammation is unquestionably among them.[19]

Although the exact role of individual MMPs in various diseases are not fully understood, it is clear that MMPs are often up-regulated in groups forming activation cascades both in the inflammatory and malignant diseases.[12] As we know, OLP is a chronic and inflammatory disease with the potential ability to undergo malignant transformation Therefore, MMPs may play multiple roles in pathogenesis of OLP and its cancerization.[15] The process from OLP to OSCC is complicated and regulated by many factors.[15] Erosive form is more susceptible than reticular to malignant transformation, Our results confirm this process well and support the earlier report by Mazzarella et al,[20] It may mean that if we screen the MMP-3 in OLP periodically, its rises may indicate a signal toward malignant transformation. This would be very important for both patients and clinician.

Salivary testing, a non-invasive alternative to serum testing, can be an effective modality for diagnosis, and prognosis predicting of diseases, and very few studies have examined tumor markers in the saliva of OSCC patients.[21] It has been shown that the concentrations of MMP-3 elevate in the saliva of patients suffering OSCC compared to controls.[22] Therefore, salivary screening of MMPs would be a performance modality in patients with OLP.

Performing studies on patients with dysplasia, e.g., leukoplakia, lichenoid lesions, OLP with dysplasia, along with healthy control groups would complete this process. Since, one of the exclusion criteria of OLP was lack of dysplasia, these results are consistent with MMP's effectiveness.

CONCLUSION

Salivary MMP-3 correlated positively with serum MMP-3 in patients suffering from OLP and OSCC. Serum and salivary MMP-3 levels are higher in OSCC, especially in advanced stage, than OLP. MMP-3 level appears associated with OLP and OSCC.

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