Original Article

Immunohistochemical Expression of Stromelysin-2 (St-2) In Patients with Oral Lichen Planus and Its Clinical Significance

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ABSTRACT

Statement of the Problem: Oral lichen planus (OLP) is a chronic inflammatory disorder with various clinical features; however, its pathogenesis is still unknown. In OLP, destruction of the basement membrane and migration of T-cell may be mediated by matrix metalloproteinases.

Purpose: The aim of this study was to examine the role of stromelysin-2 (ST-2) expression in pathogenesis of OLP.

Materials and Method: A retrospective analysis of 46 samples including 26 patients with OLP and 20 control patients with oral irritation fibroma was performed. All samples were stained employing immunohistochemistry method. After immunohistochemical staining for ST-2 marker and microscopic examination of the samples, the expression levels of ST-2 were evaluated. The data were analyzed by SPSS (V.21) and applying Mann-Whitney test.

Results: The strength of ST-2 expression was seen in most cases of OLP group, whereas control group did not show ST-2 expression. Mean expression of ST-2 in connective tissue was 1.7±1.10 and in the epithelium of the OLP samples was 1.6±1.06. Likewise, the ST-2 expression in connective tissue and epithelium of the OLP erosive lesions was significantly higher in comparison with reticular lesions ($p< 0.05$).

Conclusion: According to the results of this study, we suggest that ST-2 may be involved in the formation of OLP lesions and it may play a key role in the transformation of reticular to erosive form of OLP.

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Introduction

Oral Lichen planus (OLP) is an inflammatory autoimmune disorder that affects oral mucosa. In OLP, $CD8^+$ autocytotoxic T-cells recognize self-antigen on keratinocytes as foreign agents and cause inflammation and death of keratinocytes. [1-2]

Prevalence of OLP is about 2% in general population [3] and it is more common in the fifth and sixth decades of life; [4] it affects women more than men (1.4 :1). [2]

The most prevalent site of the lesions is buccal mucosa (90% of lesions) and after that tongue and gingiva have more chance of infection. [5]

According to a simple category, OLP is classified into three forms: reticular, atrophic, and erosive lesions. [6-7] Atrophic lesions have slightest symptoms and often do not make any problem for the patients. [8] Generally, two thirds of patients with atrophic and ero-

sive lesions of lichen planus report oral discomfort. [9-10] The exact etiology of OLP is unknown. But there are many immunological theories to explain pathogenesis of this disease including autoimmune response, humoral immunity, antigen-specific cell-mediated immune response, and non-specific immunological mechanism. Non-specific mechanism consists of degranulation of mast cells and activation of matrix metalloproteinases (MMPs). [11-12]

MMPs are a group of zinc-dependent endopeptidase that these enzymes are capable of degradation of extracellular matrix and basement membrane. Currently, at least 10 different types of these MMPs have been discovered. [13] This group of enzymes, based on their special substrate are classified to: gelatinises (MMP-2 and 9) collagenases (MMP-1 and MMP-8), stromelysins (MMP-3 and 10), membrane-type MMPs and other MMPs. MMPs play an important role in many physiological process such as wound healing, immune functions, embryonic development, morphogenesis, and angiogenesis. [13]

Although the role of MMPs in various diseases is still not fully understood but this is mentioned that MMPs are upregulated via cytokines and proinflammatory mediators in inflammatory process. Moreover, it is reported that disruption of basement membrane in OLP is usually due to destruction of collagen IV, laminin and stromelysin by various MMPs such as MMP-2, MMP-9, MMP3, and MMP-10. [14] A member of MMPs, MMP- 10 or Stromelysin-2 (ST-2) is capable of degradation of extracellular matrix proteins like collagen III and V, proteoglycans, laminin and fibronectin. [15]

The role of some MMPs was evaluated for the pathogenesis of OLP in previous studies, [4] but only few studies have been done on expression of ST-2 in OLP. The aim of this study was to evaluate ST-2 expression in OLP compared to control patients and assess its clinical significance.

Materials and Method

Patient selection

For the purpose of this 10-year retrospective study, 46 tissue samples were collected from the department of Pathology in Dental School of Zahedan, Iran. The samples included 26 OLP (17 reticular forms and 9 erosive forms) and 20 irritation fibroma cases. Inclusion criteria for the study group were the presence of clinical diagnosis and the histopathological confirmation of OLP, and exclusion criteria were any history of systemic disease or inflammatory disease, oral candida infection, oral lichenoid reactions, pregnancy and using antibiotic during one month before diagnosis.

The control group consisted of mucosal fibroma cases (because of its non-inflammatory origin) without any systemic disease. Clinicopathological data including age, gender, location and type of the lesion were obtained from the patients' records. Besides, samples without clinicopathological data and sufficient paraffin-embedded tissues were excluded.

Immunohistochemistry (IHC)

For IHC, the paraffin-embedded tissues were cut into 4-micron sections. The sections were deparaffinized in Xylene and rehydrated with graded ethanol. Then to stop the endogenous peroxidase activity, the slides were immersed in 3% hydrogen peroxidase /methanol for 30 min. and were washed with phosphate‑buffered saline (PBS) for 20 min.

For antigen retrieval, the sections were placed in citrate solution (PH=6) and were maintained in a microwave oven for 30 min. The sections were incubated for 1 hour at room temperature with primary antibody. Then the sections were irrigated with PBS at room temperature for three times, and subsequently the secondary antibody was used. The immune complexes were incubated with streptavidin peroxidase (Novo Link Polymer Detection system). The antibody-antigen reactions was visualized with Diaminobenzidine (DAB) and counterstained with Mayer hematoxylin, dehydrated in gradient ethanol and cleared in xylene, and slides were then mounted. [16] For primary antibody, mouse monoclonal anti-human antibody MMP-10 (ST-2) [Code NCL-MMP-10-6016706, Novocastra, United Kingdom Dilute 1:50] were used according to the manufacture's instruction (Novocastra). Sections of ulcerative colitis were used as positive control and as a negative control, primary antibody was eliminated.

Evaluation of immunohistochemically-stained sections

For assessment of ST-2 positivity, using light microscopy (Nikon; Type2, Tokyo, Japan), the number of positive-stained cells (the brown staining of cell cytoplasm was considered as positive) was counted in 1000 cells of each sample at 400 magnifications. Cell staining was

Table 1: Distribution of lesions according to site of occurrence

Study group	Lesion location					
	Buccal N (%)	Tongue N (%)	Lip N (%)	Gingiva N (%)	Palate N (%)	Total N (%)
Case	16 (76.2%)	4 (19%)	1 (4.8%)	0 (0.0%)	0 (0.0%)	21 (100%)
Control	10 (55.6%)	1 (5.6%)	2 (11.1%)	4 (22.2%)	1 (5.6%)	18 (100%)
Total	26 (66.7%)	5 (12.8%)	3 (7.7%)	4 (10.3%)	1 (2.6%)	39 (100%)

*The location of 5 OLP and 2 control sample was uncertain.

Table 2: The level of MMP10 expression in connective tissue and epithelium of Lichen planus erosive and reticular lesions

Tissue	Lesion type	The level of MMP10 expression				
		Neg. N (%)	+1 N (%)	+2 N (%)	+3 N (%)	Total N (%)
Connective tissue	Reticular	4 (23.5%)	7 (41.2%)	4 (23.5%)	2 (11.8%)	17 (100%)
	Erosive	0 (0%)	0 (0%)	2 (22.2%)	7 (77.8%)	9 (100%)
	Total	4 (15.4%)	7 (26.9%)	6 (23.1%)	9 (34.6%)	26 (100%)
Epithelium	Reticular	5 (29.4%)	5 (29.4%)	5 (29.4%)	2 (11.8%)	17(100%)
	Erosive	0 (0%)	1 (11.1%)	4 (44.4%)	4 (44.4%)	9 (100%)
	Total	5 (19.2%)	6 (23.1%)	9 (34.6%)	6 (23.1%)	26 (100%)

scored according to other studies; [17] including Negative: no stain, score 1: less than 10%, score 2: more than 10% and less than 50% score 3: more than 50% positive staining, then the average of these scores was taken and reported.

Statistical analysis

Data were analyzed using SPSS 21(SPSS Inc.; Chicago, IL), and applying Mann-Whitney test. P-value less than 0.05 were considered statistically significant.

Results

Forty six tissue samples obtained from 26 patients with OLP and 20 control subjects were included in our study. The OLP group consisted of 17 females, 6 males and 3 with unknown gender (23.1% males, 65.4% females, 11.5% unknown) and the control group consisted of 12 females,7 males and 1 with unknown gender (35% males and 60% females, 5% unknown). The age range was from 12 to 80 years with mean age of 41.8 (42.86 in OLP group and 40.56 in control group). The most prevalent location of the lesions was buccal mucosa in both OLP (76.2%) and control (55.6%) group (Table 1).

Of the 26 cases of OLP, 65.4% were reticular form and 34.6% of them were erosive form. The most common location of reticular lesions were buccal mucosa with 78.6% prevalence rate, followed by tongue with 21.4%, while 71.4% of erosive lesions were found in buccal mucosa and 14.3% in lip and 14.3% in tongue. The number of positive cells was counted in the epithelium and connective tissue of the lesions and classified as scores 0, 1, 2 and 3 based on the protocol described in the methods section (Table 2). Then average ±SD of the results was calculated that is shown in Table 3.

Table 3: The comparison of MMP10 expression in connective tissue and epithelium of Lichen planus erosive and reticular lesions

Tissue	Lesion type	Number	Mean	S.D	P
Connective tissue	Reticular	17	1.23	1.03	<0.001
	Erosive	9	2.33	0.70	
Epithelium	Reticular	17	1.23	0.97	0.01
	Erosive	9	2.77	0.44	

Mann-Whitney test

In most cases of OLP, ST-2 staining showed strong expression throughout the epithelium particularly in basal layer and stratum spinosum, and also in the surface of the connective tissue particularly in lymphocytic inflammatory infiltration. (Figure 1)

The ST-2 expression was significantly higher in OLP group than control group, so that in all of the samples of the control group it was negative ($p< 0.05$). The ST-2 expression was negative in the 15.4% of connective and 19.2% of epithelium tissues of the OLP group. Mean expression of ST-2 in connective tissue was 1.7±1.10 while it was 1.6±1.06 in the epithelium of the OLP samples.

Also the ST-2 expression in connective tissue and epithelium of OLP erosive lesions was significantly higher in comparison with reticular lesions ($p<0.05$). ST-2 expression in epithelium and connective tissue of erosive OLP and reticular lesions is shown in Table 3.

Discussion

OLP is a chronic inflammatory disorder with various

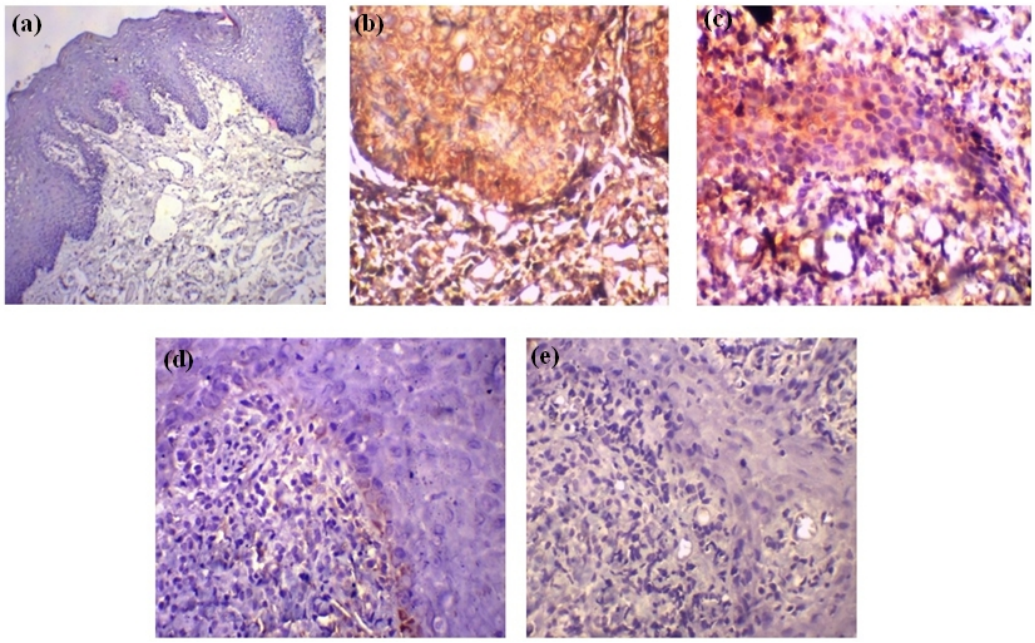

Figure 1a: Negative expression of MMP-10 in irritation fibroma (control group) (×100). **b, c:** Strong expression of MMP-10 in OLP in basal layer and sratum spinosum and in connective tissue (×400). **d:** Mild expression of MMP-10 in OLP in basal layer (×400). **e**: Negative expression of MMP-10 in OLP in epithelium and convective tissue.

clinical features but its pathogene sis is still unknown. Lichen planus is considered as a premalignant lesion, so early detection, diagnosis and treatment of these lesions are of great importance. [4]

In a retrospective study, Munde *et al.* [18] reported the clinical and demographic profiles of 128 OLP patients. Mean age of patients at diagnosis was 36 years. Most of lesions were among men (61.7%) and the most prevalent site of lesions was buccal mucosa (88.2%). Reticular lesions involved 83.5% of the lesions. In current study, the mean age of patients at the time of diagnosis was 42.86 and most of the patients were women (65.4 %). Similar to the study of Munde *et al.*, most of the lesions of current study were in reticular form (65.4%) and occurred in the buccal mucosa (76.2%). [18]

Destruction of basement membrane which leads to lymphocytes migration and keratinocytes apoptosis is one of the typical views in association with OLP and in this process, MMPs as the proteolytic enzymes are involved. [19-20]

Giannelli *et al.* [21] did the first study to examine the correlation between OLP and MMP. They reported an increase in MMP-2 during acute phase of OLP and suggested that a balance between MMP-2 and TIMP-2 may contribute to basement membrane degeneration. [21]

Mozzarella *et al.* [22] reported an increase in MMPs expression in erosive OLP in comparison with reticular OLP. They also stated that MMP1 and MMP3 are associated with erosive form of OLP and that clinical features of OLP could be in relation to different levels of MMPs. [22]

Also Zhang *et al.* [23] reported increased incidence of TIMP2, MT1-MMP and MMP-2 in OSCC compared to atrophic, non-atrophic and also normal mucosa. In that study, MT1-MMP and MMP-2 expression in atrophic OLP was significantly higher in comparison to non-atrophic OLP. Therefore, they suggested that MMPs expression would be a good marker to judge about malignancy transformation in OLP lesions. [23]

Li *et al.* [24] reported that MMP-7 expression in OSCC is higher than lichen planus and normal mucosa, also MMP-7 expression in OLP compared with normal mucosa showed an increase. This indicates that MMP-7, which tends to destroy type IV collagen and is the only epithelial-specific product of MMP family, is closely connected with evaluation of tumor and poor diagnosis of treatment. In this study, it was also noted that OLP had epithelial hyperplasia and as soon as epithelial cell proliferation overtook normal rate, it could change into cells with abnormal differentiation and eventually cancer. Furthermore, application of MMP7 could give useful clinical preventive information in premalignant le-

sions to block malignant transformation of epithelial cells. [24]

Paulusova *et al.* [19] also examined the expression of MMP-9 in OLP. In their study, MMP-9 was expressed in the lamina propria and epithelium of all OLP samples. Moreover, MMP-9 was also expressed in all control samples especially in lamina propria. They mentioned that although MMP9 in OLP samples expressed as a common inflammatory enzyme, its precise role in OLP pathogenesis remained unclear; [19] whereas in Zhou *et al.* study, [25] unlike MMP-2 and 3 that were expressed in OLP epithelium, the expression of MMP-9 was reported more in inflammatory cells. They stated that MMP-9 derived from T-cells may involve in OLP pathogenesis and partial increase of MMP-9 expression could be the reason of basement membrane destruction and intra-epithelial migration facilitation in OLP. Finally, they noted that OLP samples with higher presentation of MMP2 in histopathologic feature require a regular follow-up to examine malignant transformations. [25]

To our best knowledge, only one study about ST-2 expression in OLP has been conducted, [4] that evaluated expression of TIMP-1, MMP-2, 7 and 10 and their role in pathogenesis of OLP. In that study MMP2, 7 expressions were significantly higher in connective tissue and epithelium, but MMP-10 (ST-2) expression only in connective tissue was significantly higher in comparison with normal tissue and TIMP-1 expression in both connective tissue and epithelium showed no significant difference compared to normal tissue. Also the ratio of MMP2/TIMP1 and MMP7/TIMP1 in lichen planus was significantly higher than normal tissue but the ratio of MMP-10/TIMP-1 did not show any increase in lichen planus and in control group. They reported that overexpression of MMPs and impaired balance through MMP and TIMP could be contributed in the pathogenesis of lichen planus. Similarly, in our study enhanced level of ST-2(MMP-10) expression in connective tissue (84.6%) and epithelium (80.8%) of the lichen planus samples was observed whereas ST-2 was not presented in control samples. In addition, in the present study, tissue expression of ST-2 tended to change from reticular to erosive form of OLP.

Furthermore, it is suggested that overexpression of ST-2 in OLP may be involved in OLP pathogenesis and it seems ST-2 may play a role in the transformation of reticular to erosive form of OLP. Understanding the role and biologic function of MMPs is highly important for the development and employment of MMPs inhibitors in diseases treatment, therefore, further studies with larger sample size and different techniques about MMPs in OLP are recommended.

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This study was approved by the ethics committee of Zahedan University of Medical Sciences (Project No. 6918).

Conflict of Interest

The authors of this manuscript certify no financial or other competing interest regarding this article.

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