The role of Matrix Metalloproteinase-2 and -9 with their Tissue Inhibitors in Oral Lichen Planus

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Abstract

Background: Oral Lichen Planus (OLP) is a chronic inflammatory mucosal disease, presenting various clinical forms. Both antigen- specific and non- specific mechanisms involved in the pathogenesis of OLP.Matrix Metallo Proteinases (MMPs) and their inhibitors are one of the mechanisms that would be activated in this disease.
Objective: to explore the expression of Matrix MetalloProteinases (2&9) and their inhibitors and correlation between them in oral lichen planus.
Method: Twenty five paraffin embedded blocks of OLP and six of sequamous cell carcinoma were collected from the records of Oral Diagnosis Department. Six negative control cases were freshly obtained from Maxillofacial Center in the Specialized Surgeries Hospital in Baghdad. All samples were investigated for the expression of MMPs & Tissue Inhibitors of Matrix MetalloProteinases (TIMP) by Immunohistochemistry (IHC).
Results: Expression of MMP-2,-9, TIMP-1 and TIMP-2 were detected at higher significant level in keratinocytes and lymphocytes of OLP cases as compared to control healthy group ($p<0.0001$). There was no significant difference between the expression of MMP-2, TIMP-1 and TIMP-2 in lymphocytes and keratinocytes. However MMP-9 expression showed significant difference with MMP-2& TIMP-1&-2 in lymphocytes. There was non significant correlation between the expression of MMP-2, MMP-9, TIMP-1 and TIMP-2; and the morphological types of OLP.
Conclusion: High expressions of MMPs and TIMPs in OLP may indicate their role in pathogenesis of the disease as there is no expression of these enzymes in the control healthy; so they could be a diagnostic of aid in OLP.
Key: Oral Lichen Planus, MMP-2, MMP-9, TIMP-1, TIMP-2

الخلاصة

المقدمة: الحزاز المنبسط الفموي هو مرض مخاطي التهابي مزمن ، يظهر بأشكال سريرية متعددة ان ميكانيكية المستضد النوعي وغير النوعي هي مشتركة في تولد مرض لحزاز المنبسط الفموي.
(Matrix metalloproteinase's) ومثبطاتها هي واحدة من تلك الميكانيكات التي تنشط في هذا المرض.
الهدف من الدراسة: هو التحري عن تعبير ال(Matrix metalloproteinase-2,-9) (MMP-2,-9) ومثبطاتها (Tissue inhibitors of metalloproteinase -1,-2) في مرض الحزاز المنبسط الفموي ودراسة العلاقة بينها مقابل الأشخاص غير المصابين لتقييم متابعة الحالات وتشخيص المرض.
طريقة البحث: تم جمع 25 عينة من خلال أنسجة مطمورة بشمع البارافين للحزاز المنبسط الفموي من ارشيف قسم التشخيص الفمي في كلية طب الاسنان جامعة بغداد مقارنة مع 12 عينة (6 لحالات غير مصابة و 6 لحالات مصابة).
النتائج: أظهرت نتائج مقارنة مستوى تعبير (Matrix Metalloproteinase -2,-9) ومثبطاتها(TIMP-1,-2) في الخلايا القرنية واللمفاويه ارتفاعا ملحوظا في المرضى المصابين بالحزاز المنبسط الفموي مقارنة مع الاشخاص غير المصابين وبمستوى ذو اهمية عالي ($P<0.0001$) .كذلك أظهرت الدراسه عدم وجود إختلاف معنوي بين مستوى

تعبيرMetalloproteinase-2 والمثبطين (TIMP-1,-2) بالنسبة لتواجدهما في الخلايا القرنية والخلايا اللمفاوية أما (MMP-9) فقد ظهر له اختلاف معنوي وذلك مرجوع الى تعبيرة العالي في الخلايا اللمفاوية (النوعية وغير النوعية) مقارنة بتعبيرة في الخلايا القرنية. كذلك أظهرت الدراسة عدم وجود إختلافات إحصائية بين تعبير (MMP-2, MMP-9, TIMP-1 TIMP-2) في الخلايا القرنية و الخلايا اللمفاوية وبين الانواع الشكلية للحزاز المنبسط الفموي الشكلية.

الاستنتاج: نستنتج من هذة الدراسة أن (MMP-2, MMP-9, TIMP-1 and TIMP-2) يمكن تحديدها في مناطق الاصابة بالحزاز المنبسط الفمي. كما أن زيادة هذة الانزيمات مترابط مع الحالة المرضية للحزاز المنبسط الفمي مقارنة بالحالات السليمة. يمكن اعتبار تلك الانزيمات كمرشدات للكشف عن حالات الحزاز المنبسط الفمي. هنالك علاقة معنوية بين تعبير (MMP-2, TIMP-1 and TIMP-2) في الخلايا المفاوية والخلايا القرنية على عكس MMP-9.

Introduction

Lichen planus (LP) is a chronic mucocutaneous T-cell mediated immunoinflammatory disease. The etiology of LP is unknown. An alteration in the basal keratinocytes by certain stimuli that induce humoral and cell mediated immune response has been postulated as a mechanism[1]. The histopathological features of LP include liquefaction of the basal cell layer accompanied by apoptosis of the keratinocytes, a dense band-like lymphatic infiltrate between the epithelium and connective tissue**.** The accumulation of leucocytes in the lamina propria and epithelium is also a feature of lichen planus, and most of these leukocytes are CD8 T-cells (suppressor/ cytotoxic T-lymphocytes) and CD4 T-cell (helper/inducer T-lymphocytes) [2]

Oral keratinocytes and lymphocytes to produce and release a wide range of cytokines and enzymes are known to be relevant to the pathogenesis of Lichen Planus. The cytokines released are IL-8, IL-6, and TNF and others [3] the enzymes include (MMPS) and their inhibitors (Tissue Inhibitor of MatrixmetaloProteinase) TIMPs and others. MMP-2 and MMP-9; the Gelatinases cleave collagen type IV, which is basic component of epithelial basement membrane, and this disruption is apparent in OLP [4] (Zhou *et al*., 2001).

Patients and Method

The collective number of samples enrolled in this study was 37, involved both retrospective and prospective samples.

The retrospective samples were 25 paraffin-embedded blocks retrieved from the archive of Department of Oral Diagnosis/College of Dentistry/ Baghdad University, Maxillofacial Center /Specialized Surgery Hospital/ Baghdad, and from Dermatology Clinic/ Al-Kadhimya Teaching Hospital.

Six cases of SCC which used as a positive control as diagnosed from previous studies; six healthy individuals, cheek and/or gingiva tissue around surgical extracted tooth were obtained and considered as negative control. Tissue samples were processed as recommended by Luna[20]; briefly, the sectioned slides after baking, deparaffinization and rehydration, antigen retrieval were used and a peroxidase blockwere applied. The primary monoclonal antibodies were used against MMP-2, MMP-9 USBiological,USA). Human TIMP-1, TIMP-2 (CHEMICON, USA) and IHC/Detection Kit (DakoCytomation) 10µl of primary antibody reagent were applied on each slide (except for negative control, were PBS was added instead), incubated for 90min. at 37°C. Then (10µl) of Biotinylated link was added, incubated for 60min. at 37°C. (10µl) of Streptavidin were added, incubated for 45min. at 37°C. Substrate and chromogen (DAB) were added to

each slide, incubated in a dark chamber for 10- 30min. at 37°C. Sections were dehydrated by ethyl alcohol, mounted with permanent mounting medium (DPX).

The positive slides had brown cytoplasm of the keratinocytes and lymphocytes cells with light bluish color nuclei.

Statistical analysis was performed with the statistical package for social sciences (SPSS).

Results

Twenty five patients with OLP lesions were investigated, 13 females (52%) and 12 Males (48%) Their age ranged between 19 and 68 years; the most affected age group regarding female was the third & 6th decade while that for male was 40-49 years.

Figure 1 and 2 show are showing the typical cytoplasmic immunostaining of MMP-2 in keratinocytes and lymphocytes of OLP, respectively. The results are shown in table (1) showed the MMP-2 expression in keratinocytes and lymphocytes in which there is significant increase in the MMP-2 in both keratinocytes & lymphocytes. While MMP-9 in keratinocytes & lymphocytes were as described in table-2- also shows an increase level of MMP-9 in both keratinocytes & lymphocytes.

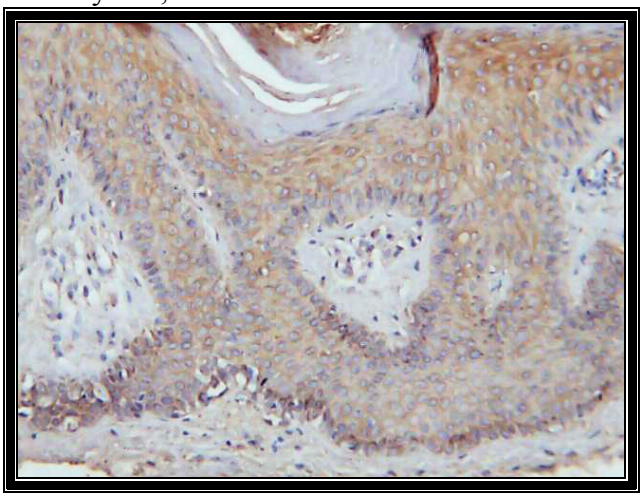

Figure 1. Expression of MMP-2 in keratinocytes of OLP (patients). Brown staining of cytoplasm of keratinocytes. (x10)

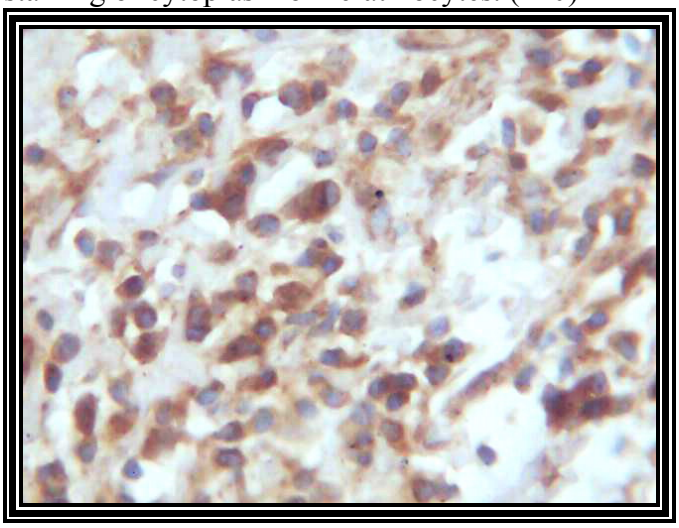

Figure 2. Expression of MMP-2 in lymphocytes of OLP patients. (x10)

Table 1. Immunohistochemical expression of MMP-2 in keratinocytes & lymphocytes were as described

		N	Mean	Std. D.	Std. E.	P
MMP2 Keratinocyte expression	Patient	19	7.58	14.226	3.264	.000
	Negative control	6	.67	1.033	.422	
	Positive control	6	36.50	17.615	7.191	
MMP2 Lymphocyte expression	Patient	19	7.84	7.726	1.773	.000
	Negative control	6	1.00	.632	.258	
	Positive control	6	45.00	.000	.000	

Table 2.While MMP-9 in keratinocytes & lymphocytes were as described in

		N	Mean	Std. D.	Std. E.	
MMP9 Lymphocyte expression	Patient	21	7.14	5.121	1.118	.000
	Negative control	6	1.00	0.632	0.258	
	Positive control	6	35.33	11.553	4.716	
MMP9 Keratinocyte expression	Patient	20	2.50	3.069	0.686	.000
	Negative control	6	1.50	0.548	0.224	
	Positive control	6	23.17	6.676	2.725	

The results of TIMP1 and TIMP2 were described in table (3) and table (4) respectively. Both showing the significant increase of TIMP1 and TIMP2 in keratinocytes & lymphocytes in OLP patients over the control group.

TIMP1 in keratinocytes & lymphocytes result in table-3-

		N	Mean	Std. D.	Std. E.	
TIMP1 Lymphocyte expression	Patient	18	31.28	18.339	4.323	0.001
	negative control	6	0.83	0.983	0.401	
	Positive control	6	25.00	0.000	0.000	
	Total	30	23.93	18.478	3.374	
TIMP1 Keratinocyte expression	Patient	18	27.78	14.490	3.415	0.000
	Negative control	6	0.33	0.516	0.211	
	Positive control	6	25.00	11.402	4.655	
	Total	30	21.73	16.284	2.973	

TIMP-2 in keratinocytes & lymphocytes in table -4-

		N	**Mean**	**Std. D.**	**Std. E.**	
TIMP2 Keratinocyte expression	Patient	22	40.68	22.404	4.777	0.000
	Negative control	6	1.00	0.632	0.258	
	Positive control	6	20.83	9.174	3.745	
TIMP2 Lymphocyte expression	Patient	22	31.55	18.918	4.033	0.001
	Negative control	6	0.83	0.983	0.401	
	Positive control	6	32.00	8.246	3.367	

Discussion

Oral Lichen Planus (OLP) is a chronic inflammatory mucosal disease. The present study demonstrated that the expression of MMP-2, -9, TIMP-1 and TIMP-2 were over expressed in keratinocytes and lymphocytes as compared to healthy control & this is in accordance with [5] (Sugerman, *et al*., 2002), who found over expression of these enzymes in OLP& in other malignant disease like OSCC [6, 7, 8] (Kusukawa**,** *et al*., 1993; Sutinen, *et al.*, 1998; Yorioka, *et al*., 2002). Both enzymes are increased in malignant tissues compared to their benign counterparts [9] (Iurlaro *et al.* 1999)

It has been found that one of the most characteristic features of OLP is the presence of dense subepithelial lympho-histiocytic infiltrate which is almost exclusively of T-cells [5, 10] (Jungell *et al*., 1991; Sugerman *et al*., 2002). Lymphocytes that are aggregated or accumulated in the submucosa or subepidermis showed high expression of MMP-2 and-9 & its inhibitors, this could be explained on the bases that interleukin-1 and other cytokines released by epidermal and migrating cells under inflammatory process which stimulate fibroblasts to produce both collagnases and its inhibitors[11, 12] (Postlethwaite, *et al*., 1983; Murphy, *et al*., 1983); at the same time OLP have an elevated level of interleukine-1α (IL-1α) as has been found by Al-Mosawi, 2006 [3].

It has been established that most mesenchymal cells are able to produce TIMPs, and some of them are produced by white blood cells like TIMP-1 and -2 [13] (Bjerkeli *et al*., 2004).

Results of this study showed that the high infiltrations of lymphocytes in the subepithelial layer of OLP cases are T-cells which contained mRNA of TIMP-1 so this explains its high expression in subepithelial inflammatory cells infiltrates [4] (Zhou, *et al*., 2001). Furthermore TIMP-2 expression is restricted to T-cells as suggested by Oelmann[14], *et al*., in 2002; however Hayakawa[15], *et al*., in 1994 conducted that TIMP-2 is to be secreted by leukocytes, fibroblasts as well as epithelial cells and interestingly TIMP-2 thought to stimulate their proliferation, a reciprocal regulation of their expression, which may depend on endogenously expressed (growth) factors and cytokines [16] (Gomez, *et al.,* 1997). Interleukin-1 and other cytokines released by epidermal and migrating cells under inflammatory process, stimulate fibroblasts to produce both collagenases and its inhibitors[11, 12] (Postlethwaite, *et al*., 1983; Murphy, *et al*., 1983); TNF- α stimulates the expression of several MMPs, and thus contributes to tissue degradation in inflammatory conditions[17] (Shimizu et al., 2005). Thus, in general, changes in cytokine expression may affect the MMP/TIMP balance; and at the same time OLP have an elevated level of interleukine-1α (IL-1α) as has been found by Al-Mosawi[3], 2006. Rhodus[18], *et al* in 2005 demonstrated an elevation in all inflammatory cytokines in saliva of patients with oral sequamous cell carcinoma (OSCC) and oral premalignant lesions (OPML) as compared to controls, TIMP-1 can be co-expressed with MMPs. Ogura[19], *et al*., 2005; found that chronic inflammatory diseases appear to be initiated and/ or maintained partly by cytokines activity, that's why MMPs and TIMPs are over expressed in the present study, It has been found that the over expression of MMPs is not necessarily means that they are active, because the quantitative expression of the enzymes is in no way reflecting their functional integrity, hence the defunct MMPs will develop further keratosis caused by TIMPs which is more expressed as has been also shown in this study . Results of this study showed that there was a significant correlation between MMP-2, TIMP-1 and TIMP-2 expression in keratinocytes and lymphocytes which could be attributed to the following:

1-The defunct tissue proteinases (MMPs) could be envisaged to be refractory to the regulatory influence of TIMPs, which further may lead to excessive keratosis at tissue sites.

2- The excessive keratosis taking place in the oral mucosa of OLP and/or OLP lesional site set into motion a regulatory reflex mechanism causing an up regulation in the matrix enzyme expression.

3- Over activity of MMPs results from reduced expression of TIMPs, and *vice*

versa, but one of the difficulties of this study is the simultaneous increase in reactivity of MMPs and TIMPs which pose difficulty in interpreting the results of this study.

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