RESEARCH ARTICLE

Salivary MMP-1, MMP-2, MMP-3 and MMP-13 Levels in Patients with Oral Lichen Planus and Squamous Cell Carcinoma

Tahereh Nosratzehi[1]*, Ebrahim Alijani[2], Marziyeh Moodi[3]

Abstract

The aim of present study was to evaluate salivary matrix metalloproteinase-1 (MMP-1), MMP-2, MMP-3 and MMP-13 levels in patients with oral lichen planus (OLP) and squamous cell carcinomas (SCC) as well as in healthy controls. Thirty cases of OLP (bilateral lesions, papular and reticular lesions, and Wickham lines) clinically and histopathologically (group A), 30 with oral SCCs (group B), and 30 with no history of oral cancer, other lesions or lichen planus (group C) were enrolled at the Department of Oral Medicine School of Dentistry, Zahedan, Iran. Unstimulated whole saliva was collected and laboratory measurement of salivary concentration of MMP-1, MMP-2, MMP-3 and MMP-13 was conducted by immuno-sorbent enzyme-linked methods. Data analysis was performed with Kruskal-Wallis and Mann-Whitney tests and Pearson's correlation coefficients. In the present study, MMP-2 and MMP-13 levels were higher in oral SCC patients than in OLP and healthy individuals. More research is required to assess MMP links with tumor invasion.

Keywords: Salivary- MMP- Oral Lichen Planus- Squamous Cell Carcinoma

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Introduction

Lichen planus is a common chronic inflammatory mucocutaneous disease and the involvement of oral mucosa is often seen in 50% of cases. So far, the etiopathogenesis of oral lichen planus (OLP) has still been poorly understood, but T cell-mediated immunity and inflammatory pathways play a role in its pathogenesis (Nylander et al., 2012). Many studies proposed autoimmune properties of OLP such as interaction with other autoimmune diseases, reduced immune-suppressing activity in patients with OLP, and the presence of auto-toxicity cells cause them to be selected for research about inflammation and autoimmunity (Agha-Hosseini et al., 2010; Roopashree et al., 2010). From the first reported squamous cell carcinomas (SCC) developed from LP so far, several studies have focused on malignant transformation of OLP lesions to oral SCC (OSCC), as it has become a concerning topic in the world's health. Nowadays, the term "oral lichen planus" is known as a precancerous condition, using the World Health Organization (WHO) classification. The molecular mechanisms underlying the development of oral cancer are not clearly known in patients with OLP, but OLP lesions can evolve from normal epithelium or precancerous lesions and the basement membrane disruption may trigger the apoptotic keratinocytes. OSCC is the most common neoplasia of the oral cavity and is a serious worldwide health problem; so, understanding the SCC biomarkers is essential for early diagnosis as well as better prognosis and prevention of disease recurrence and is a good way to decrease the mortality of patients (Roopashree et al., 2010). Malignant transformation of oral mucosa due to gene mutation in cell growth and its regulation cause increased proliferation of cells, abnormal keratinization, epithelial dysplasia, increased cell motility and angiogenesis. Cancer occurs by genetic changes that cause deregulation of protein, poor cell division and tissue differentiation, invasion and metastasis (Pickering et al., 2007).

Suppressed immune system is an established phenomenon in patients with cancer, especially in ones with squamous cell cancer and can include changes in cytokines and the balance of immune cells. Th cells play a key role in the regulation of immune response. Th1 cells are involved in cell-mediated immunity and are generally considered as the host of anticancer mechanisms, while Th2 cells act as mediated antibody and for a humoral reactions against extracellular pathogens (Pickering et al., 2007).

They are responsible for a broad range of activities, including angiogenesis, the proteolytic activity of growth factors, soft tissue reconstruction, wound healing and

*[1]Oral and Dental Disease Research Center, Department of Oral Medicine, [2]Clinical Immunolgy Research Center, Department of Immunolgy, [3]Dentist Zahedan University of Medical Science, Zahedan, Iran. *For Correspondence: taherehnosratzahi@yahoo.com*

tumor invasion.

Matrix metalloproteinase 1 (MMP-1), also known as interstitial collagenase or fibroblast collagenase, breaks down ECM by the cleavage of type I , II and III collagens. Matrix metalloproteinase-2 (MMP-2) or collagenase type IV destroys Collagen type IV, the main glycoprotein component of the basement membrane, and is involved in the regulation of vascular and inflammatory processes. Matrix metalloproteinase-3 (MMP-3) is a stromelysin and breaks down many components of the extracellular matrix such as proteoglycans, fibronectin, laminin and collagen type III, IV, V. Matrix metalloproteinase-13 (MMP-13) or collagenase 3, preferably breaks down type II collagen (Al-Azri et al., 2013). Saliva-based analysis has been proposed in recent years and the potentially abnormal markers of oral cavity appear in saliva directly or indirectly. Therefore, its application as a diagnostic fluid can be of special significance. Saliva is a diagnostic tool to assess markers due to its several advantages: low-cost tool for monitoring, safe collection, non-invasive, convenient, simple and reproducible without causing discomfort for the patient.

With regard to the different results of studies and the importance of cytokines in chronic inflammatory diseases such as OLP and OSCC as well as the high incidence of OSCC in the region of Sistan and Baluchestan, the evaluation of these markers is essential for early diagnosis and appropriate treatment. Therefore, the purpose of this study was to assess salivary MMP-1, MMP-2, MMP-3 and MMP-13 in patients with OLP and OSCC.

Materials and Methods

Ninety patients were referred to the department of Oral Medicine at School of Dentistry in Zahedan University of Medical Sciences, including 30 patients with clinical lesions of OLP (bilateral lesions, papular and reticular lesions, and Wickham lines) and, if necessary, they had histological confirmation (characterized by band-like inflammatory infiltrate cells, limited to the surface area of the connective tissue; they are predominantly mature lymphocytes, accompanied by vascular degeneration of the basal layer of the epithelium); they had no other oral lesions (group a), 30 patients with new SCC and reports of pathologist proving SCC and except for SCC, they had no oral lesions (group B), and 30 individuals with no history of SCC or LP (group C). Unstimulated whole saliva were collected in a quiet room between 10 to 12am under resting conditions and they were banned from eating, drinking and smoking at least 120 minutes before sampling; 0.5 mL unstimulated saliva samples were collected by spitting without chewing movements. One of the best ways is spitting method to collect whole saliva (2500 g, 10 minutes). After centrifuge, the superficial layers of saliva immediately separated and it was stored at -70°C for further assessment. Laboratory measurements of salivary concentration for MMP-1, MMP-2, MMP-3 and MMP-13 were performed by immuno-sorbent enzyme-linked method and ELISA kits and BOSTER biological made in China with a sensitivity of 95%. The determination of the levels of MMP-1, MMP-2, MMP-3 and MMP-13 was performed according to the manufacturer's instructions. OSCC and OLP patients received treatment and follow-up. The data entered into SPSS version 21 software, were analyzed using descriptive, Kruskal-Wallis and Mann-Whitney and Pearson's correlation coefficient.

Table 1. The Mean and Standard Deviation (SD) of MMP-1 in Participants of Each Group (Healthy Controls, SCC, OLP)

Group	Mean ± Deviation MMP-1	Kruskal-Wallis Test
Normal	29.80 ± 23.81	P = 0.203
SCC	33.31 ± 19.81	
OLP	37.34 ± 27.23	

MMP, Matrix metalloproteinase; SCC, Squamous Cell Carcinomas; OLP, Oral Lichen Planus

Table 2. The Mean and Standard Deviation (SD) of MMP-2 in Participants of Each Group (Healthy Controls, SCC, OLP)

Group	Mean ± Deviation MMP-2	Kruskal-Wallis Test
Normal	1,073.57 ± 1,016.71	P= 0.014
SCC	1,813.00 ± 1,085.73	
OLP	1,485.90 ± 1,057.03	

MMP, Matrix metalloproteinase; SCC, Squamous Cell Carcinomas; OLP, Oral Lichen Planus

Results

In this study, the data collected from 90 participants in three groups of 30 patients (healthy control, patients with OSCC and patients with OLP) were analyzed. The results of this analysis are listed in subsequent paragraphs.

According to the data in Table 1, Kruskal-Wallis test showed that there was no significant difference in mean values of salivary MMP-1 among the three groups (P = 0.203).

According to the data in Table 2, Kruskal-Wallis test showed that the mean values of salivary MMP-2 among the three groups was statistically significant (P = 0.014). The Mann-Whitney test, used to compare the groups, showed significant differences between the healthy group and SCC (P = 0.003), but the healthy group with OLP (P = 0.105) and SCC with OLP (P = 0.237) had no significant differences.

According to the data in Table 3, Kruskal-Wallis test showed that there was no significant difference in mean values of salivary MMP-3 among the three groups (P = 0.288).

According to the data in the Table 4, Kruskal-Wallis test showed statistically significant differences in mean values of salivary MMP-13 among the three groups (P = 0.012).

Table 3. The Mean and Standard Deviation (SD) of MMP-3 in Participants of Each Group (Healthy Controls, SCC, OLP)

Group	Mean ± Deviation MMP-2	Kruskal-Wallis Test
Normal	1,073.57 ± 1,016.71	P= 0.014
SCC	1,813.00 ± 1,085.73	
OLP	1,485.90 ± 1,057.03	

MMP, Matrix metalloproteinase; SCC, Squamous Cell Carcinomas; OLP, Oral Lichen Planus

Table 4. The Mean and Standard Deviation (SD) of MMP-13 in Participants of Each Group (Healthy Controls, SCC, OLP)

Group	Mean ± Deviation MMP-3	Kruskal-Wallis Test
Normal	9.64 ± 9.09	P= 0.012
SCC	15.26 ± 10.31	
OLP	10.62 ± 6.41	

MMP, Matrix metalloproteinase; SCC, Squamous Cell Carcinomas; OLP, Oral Lichen Planus

Mann-Whitney test showed a significant difference between the healthy group and SCC (P = 0.009), but the healthy group with OLP (P = 0.063) and SCC with OLP (P = 0.080) had no statistically significant difference.

Discussion

OLP is one of the most common chronic inflammatory mucocutaneous diseases (Culhaciet al., 2004; Marcos et al., 2009; Mazzarella et al., 2006). The etiopathogenesis of OLP is yet unknown; however, both specific and non-specific immunologic reactions may be involved. Non-specific mechanisms include cell degranulation and activation of matrix metalloproteinase (MMP) in OLP lesions. A significant increase in the risk for malignant transformation of OLP lesions has been mentioned in many articles. As a sequence, these chronic refractory oral lesions and cancer phobia are often seen in the treatments of OSCC and OLP patients. When confronted with these patients, we do not know who is really prone to change to OSCC. Furthermore, we decided to measure the levels as prognostic markers or predictors in patients with OLP and OSCC (Roopashree et al., 2010).

The complex interactions between inflammatory cells and cytokines lead apoptosis, which is induced by T lymphocytes binding to specific antigen-presenting basal cells. One of The histopathological features of OLP is the disruption of basal membrane (BM) that permits lymphocytes to migrate, and requires proteolytic activity of basal membrane–dependent enzymes. MMPs act in following manner: cell migration, angiogenesis, proteolysis activation of growth factors and factors of normal tissue remodeling, repair and metastasis (Pickering et al., 2007; Roopashree et al., 2010). For the first time, investigation about association between OLP and MMP was performed by Giannell et al., (1996). They indicated that gelatinase A expressed increasingly in acute changes of LP and suggested that change of the timp-2/MMP-2 proportion played an important role in the disruption of basal membrane. In 1998, the result of a comparative study between OLP, dysplasia and SCC showed that MMP-1, 2, 3 expressions were higher in SCC than in OLP and high expression of TIMPs and MMPs was determinately presented during oral SSC metastasis (Sutinen et al., 1998).

Subsequently, Zhou et al., (2001) reported MMP-3 and MMP-2 expressions in epithelium of OLP and the increased expression of MMP-9 in infiltrated inflammatory cells. They proposed that MMPs additionally act as the distractive of basal epithelium membrane in OLP. Kim et al., (2006) Showed raised MMP-3 levels of expression correlated with cells acantholysis and erosive changes in OLP patients.

OSCC is the most common lesion that has more likely developed from precancerous lesion such as LP (Agha-Hosseiniet al.,2012; Stott-Miller et al., 2011). The finding of Sutinen et al., (1998) investigated the involvement of MMPs in OSCC and OLP.

The evidence showed upregulation of MMP-1 mRNA in stromal fibroblast cells, neoplastic peripheral cell and metastatic lymph nodes. There were limited and low expression of MMP-1 and 2 in OLP and dysplastic lesions (Sutinen et al., 1998). The review of Jordan et al., (2004) study showed that MMP-1 and 9 upregulation can be utilized as markers for malignant transformation to oral cancer.

In contrast to our study, in two Kim study, high levels of MMP-1 and 3 can be promoted as the development of erosion in various forms of LP (Kim et al., 2006).

Ertughrul et al. examined the level of MMP-1 in OLP patients with gingivitis and periodontitis and both groups had chronic periodontitis. The participants were divided to two groups: OLP patients (27 samples) and healthy groups (30 samples). They found significantly elevated MMP-1 levels of gingival crevicular fluid (GCF) liquids in patient with OLP compared with control samples. In our study, the salivary levels of MMP-1 were not significantly different in all three groups (Ertugrulet al., 2013).

In Y Chen's study, MMPs, TIMPs, and TGF-b1 were expressed in atrophic OLP, non-atrophic OLP, OSCC and normal oral mucosa. There were positive correlations in overexpression of MMP-2, MMP-9, MMP-14 and OSCC with cancerization of OLP. Therefore, MMPs were involved in the OLP malignant transformation to OSCC (Chenet al., 2008).

In contrast with our study, Rubaci an immunohistochemical study analyzed the tissue inhibitor of metalloproteinase-1 and matrix metalloproteinases 2-7-10 expression in the epithelium and connective tissue of OLP lesions. They propounded that MMPs can biologically act as the objectives of the treatment of disease and based on their results they suggested that MMP-2, MMP-7 and TIMP-1 were involved in OLP lesions (Rubaciet al., 2012). Delavarian et al., (2010) also found the high expression levels of MMP-2 and 9 in erosive OLP epithelium, which appear mostly in lymphocytic areas. However, the mean of MMP-2 and 9 expressions was higher in dysplastic OLP than in non-dysplastic OLP. In a similar study, overexpression of MMP-2 was seen in atrophic OLP compared to non-atrophic OLP. Statistically, they found a significant difference between the means of MMP-2 and 9 expressions in atrophic OLP and non-atrophic OLP only in the lymphocytic layer (Zhang et al., 2006). Moreover, other studies reported that the highest expression was related to cancerous lesions. It emphasizes the MMP activity and its role in malignant cell invasion to adjacent connective tissues (Lotfi et al., 2015).

In Lotfi et al., (2015) study assessed the serum matrix metalloproteinases 2 and 9 in patients with OSCC. This cross-sectional study was performed on 20

patients with OSCC and 20 healthy controls. Moreover, they evaluated the association between these markers and clinico-pathological finding such as lymph node involvements. They found positive correlation in MMP-2 with grade T, the involvement of lymph nodes and the history of smoking, as well as between MMP-9 and lymph node metastasis. They expressed that MMP-2 can be a good predictor for lymph node metastasis and grade in SCC (Farzin et al., 2012).

Our findings about MMP-2 showed significant differences between the control and the OSCC groups. Using ELISA, RT-PCR, immunohistochemistry and zymography, Zhou et al., (2001) concluded that OLP epithelial matrix changes may be associated with MMP-2,3,9-derived keratinocytes and T lymphocytes Farzin et al., (2012) investigated serum levels of MMP-3 in various clinical forms of LP using ELISA method. They reported MMP-3 expression in OLP compared to its atrophic higher. As OLP lesions have a high sensitivity to malignant transformation, in conclusion, the period screening of MMP-3 can be useful in patients with OLP. Several studies have demonstrated the expression of MMP-3 in OSCC. Contrary to our results, in Agha-Hosseini's study, salivary and serum levels of MMP-3 decreased in erosive LP compared to the atrophic form as well as in higher grades to lower grades of OSCC and also it was higher in OSCC patients compared with OLP, especially in advanced stages (Agha-Hosseini et al., 2015). However, in all three groups, there was no significant difference in the concentration of salivary MMP-3. MMP-13 was expressed on malignancy transformed epidermal keratinocytes which leads to applying this factor in the field of oral malignant and pre-malignant diseases (Luukkaa et al., 2008). Agha Hosseini et al., (2015) found no significant correlation between the mean values of serum and salivary MMP-13 in OSCC and OLP subtypes. Nevertheless, Luukkaa et al. , (2008) discovered that MMP-9 and 13 played an important role in the invasion and prognosis of salivary tumors. Similar to our study and according to Chiang et al., (2006) findings, MMP-13 can be used as a tumoral marker. In the present study, there was a significant difference between control and OSCC groups, whereas no significant difference was found between healthy patients and OLP as well as between OSCC and OLP groups.

Furthermore, we found that MMP-13 may be able to determine prognosis and oral tumor invasions, especially in high levels.

Despite that different studies mentioned that MMP levels were higher in OSCC and OLP patients than healthy controls, in this study, the levels of MMP-2 and 13 were only higher in patients with OSCC than healthy controls. However, it requires future researches in this field to conclude the helpfulness of these factors in tumor invasion.

Ethical considerations

The study is performed according to Helsinki principal. All patients were aware of the study and signed a written constant.

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