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Research Paper: Salivary evaluation of p53 and MMP-3 in patient with oral lichen planus

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ABSTRACT

Introduction: Lichen planus is an inflammatory chronic illness with unknown cause that can irritate the oral mucosa. P53 is associated with malignant changes in oral epithelial cells. MMPs can destroy intercellular junctions and cause acantholysis. It seems that MMP-3 plays a significant role in this regard. The purpose of this study was to evaluate the salivary levels of P53 and MMP-3 in the patients with Oral Lichen planus (OLP) compared with the control group.

Materials and Methods: 30 salivary samples were collected from patients with OLP (15 with erosive Oral Lichen planus (EOLP) and 15 with reticular Oral Lichen planus (ROLP)) and 30 salivary samples from healthy people as a control group. The salivary P53 and MMP-3 level was assayed by ELISA method. Statistical analysis of the Student's t-test, ANOVA and Pearson correlation coefficient was performed.

Results: The salivary concentrations of P53 and MMP-3 in patients with EOLP were significantly higher than patients with ROLP and control group, but no significant difference was found between control group and patients with ROLP.

Conclusion: The salivary concentrations of P53 and MMP-3 were significantly different between different clinical types of OLP.

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Introduction

The lichen planus is an autoimmune and chronic inflammatory disease that can irritate the skin and mucosa (1-3). Oral and genital mucosa are most commonly involved(2). The oral lichen planus (OLP) occurrence varies from 0.5% to 3.0% of population (2, 4). Females predominate in most series of cases and the disease occurs between the age of and 60(2, 4). The cause of this disease is unknown, but genetic and environmental factors such as drugs and some infectious agents are likely to contribute to the disease(2, 5-7). Cellular immunity plays a major role in clinical manifestations of this disease(5). OLP has two distinct clinically forms: 1 – Atrophic or erosive lesion (with or without reticular lesions) 2 - Reticular or plaque-like lesions(4, 5). Unlike lichen planus, oral lichen planus tends to be less self-limiting and has more resistance to topical treatments(8). The risk of malignancy is reported to be 0 to 1.2% (1, 4, 9). Erythematous and erosive form of OLP has the highest potential for malignant transformation(4). The disease causes hydropic degeneration in the basal membrane(2, 5). The basal membrane degradation in OLP is accomplished by MMPs and mast cell kinases(5). MMPs are a large family of zinc-dependent proteases, and MMP-3 is an important member of this family(5, 10) . In fact, MMPs interfere in many stages of tumor progression, including cancer cell growth, differentiation, apoptosis, migration, invasion and metastasis(10, 11). MMP-3 is a stromalysine that can reduce many non-collagenic matrix compounds, such as proteoglycans, fibronectin, laminin and gelatin in addition to collages type 3, 4 and 5(5).

MMP-3 is capable of lysing collagen in the basal membrane and stimulating the synthesis of other MMPs, such as MMP-1 and MMP-9(10). MMP-3 is also associated with tumor growth and metastasis in breast, colon, neck and six cancers in humans(10, 11). Increased expression of MMPs has been reported in OLP(12). MMP-3 has potential ability for tumor progression in mammary glands and lung and is also reported to be associated with tumor growth and metastasis in breast, colon, cervical, and lung cancers(11). A few studies showed that MMP-3 expression increased in OLP epithelium(5). P53 is a tumor suppressor gene discovered about two decades ago(1, 7, 13). P53 is a key factor that focuses and coordinates stress-related signals and converts them into a sequence of responses such as cell cycle control, cell death, apoptosis, or DNA repair(7, 14). The importance of P53 in cell death and apoptosis has been discussed in many studies, and many studies have suggested that OLP has a malignant potential and P53 protein plays a major role (1, 4, 7). Activation of P53 after DNA destruction is an important protective mechanism that facilitates DNA repair and stimulates malignant apoptosis(14). In addition, there has not been a study on the simultaneous evaluation of these two markers in these patients yet. Therefore, in this study, we decided to use a completely quantitative method to study the salivary level of P53 and MMP-3 in OLP lesions, in order to find a more real role in local tissues of OLP. Among the available techniques, studies are done on the blood, tissue of lesion, and various liquids in the body. Study on the saliva is a dynamic field and its diagnostic value is proven(8). Saliva analysis has more benefits than tissue or blood analysis, such as easier access, non-invasiveness, and being more practical to analysis and evaluation. According to the mentioned points, this study was conducted based on the evaluation of P53 and MMP-3 in saliva (whole unstimulated saliva) of patients with OLP by ELISA method.

Material and Methods

This case-control study was performed on 30 patients (17 women and 13 men, age: 47.5±12.2) with oral lichen planus referring to the dental school of Babol University of Medical Sciences and the city clinics (15 patients with Erosive OLP and 15 patients with Reticular OLP). Patients who were diagnosed with oral lichen planus based on WHO modified lichen planus diagnostic criteria were participated in our study. H & E slides of all specimens were

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also re-prepared for confirmation of diagnosis. Also, 30 healthy individuals (16 women and 14 men, age: 45±10.6) without a history of known blood diseases were included as control group. It should be noted that the age and sex were matched between patients and control groups. Exclusion criteria included systemic diseases, smoking, pregnancy for women, chronic periodontitis, use of nonsteroidal anti-inflammatory drugs, corticosteroids and antihistamines, and a history of chronic lung disease. All samples were collected between 9 and12 a.m. and participants were banned from eating, drinking, chewing gum and smoking for at least two hours before sampling. People first swallowed their saliva, then rotated their heads forward and poured all their saliva for centrifugation in two dry and sterile tubes for 5 minutes without dipping their saliva (1-5 ml for each factor). All samples were immediately centrifuged at 4 ° C for 20 minutes at a speed of 6000 rpm to separate the cell debris and then stored at -80 ° C.

MMP-3 and P53 salivary concentration were measured by ELISA according to the manufacturer's instructions (E1711Hu and E0907Hu, SHANGHAICRYSTAL DAY BIOTECH CO, China). We used one-way ANOVA and Kruskal-Wallis tests for Statistical analysis.

Result

17 women (7 with Erosive OLP and 10 with Reticular OLP) and 13 men (8 with Erosive OLP and 5 with Reticular OLP) participated in our study with the diagnosis of OLP. Buccal mucosa was the most common location for lichen planus (80%). One-way Anova test showed a significant difference in saliva p53 concentration between control group, patients with Erosive OLP, and patients with Reticular OLP [p<0.05](table 1).

Table1. Unstimulated whole saliva concentration of P53 and MMP-3 in patients with Erosive OLP, Reticular OLP and control individuals. Data are expressed as mean ± SD.P<0.05 is significant

MMP-3	P53	Specimen
74.85±81.52(30.26)	1434.80±348.50	Erosive OLP
16.82±9.30(14.35)	731.38±380.32	Reticular OLP
45.41±67.35(18.73)	1011.14 ±655.32	Control
0.004	0.002	p-value

Kruskal-wallis test showed a significant difference in saliva MMP-3 concentration between control group, patients with Erosive OLP, and patients with Reticular OLP. (p<0.05). A post hoc analysis showed that P53 saliva concentration in patients with erosive OLP (mean± SD: 1434.80±348.50) was significantly higher than patients with reticular OLP (mean± SD: 731.38±380.32; p=.000) and control group (mean± SD: 1011.14 ±655.32; p= 0.019). However, no significant difference was observed between the reticular OLP and control groups (p= 0.18). A mann-whitney analysis showed that MMP-3 saliva concentration in the patients with Erosive OLP (mean± SD: 74.85±81.52; median:30.26) was significantly higher than patients with Reticular OLP (mean± SD: 16.82±9.30; median: 14.35; p= 0.002) and control group (mean± SD: 45.41±67.35; median: 18.73; p= 0.008). However, no significant difference was observed between the Reticular OLP and control groups (p= 0.324). The independent t-test showed no significant difference in P53 saliva concentration between men (mean± SD: 1168.64±494.48) and women (mean± SD: 1017.67±520.47) with OLP (p= 0.754) (Figure 1). The mann-whitney test showed no significant difference in MMP-3 saliva concentration between men (mean± SD: 64.87±81.87; median: 24.84) and women (mean± SD: 31.27±43.83; median: 24.37) with OLP (p= 0.517)

Discussion

Lichen planus is a chronic inflammatory disease(2, 3). This autoimmune and muscocutaneous disease involves some organs including oral mucosa, skin, genital mucosa, scalp and nails(15). Although there is no clear evidence of malignancy in oral lichen planus, the pre-malignant potential of OLP has been shown in previous studies(1, 3, 16, 17). The OLP pathogenesis consists of a series of interactions between inflammatory cells, chemokines and cytokines that cause apoptosis of keratinocytes found in the basal membrane. In our study, accurate clinical and histological evaluation have been used to detect OLP. Many studies have suggested that genetic changes in the early stages

of oral cancer can be identified, and patients with chronic oral mucosal inflammation have a higher chance of developing malignant changes(18). Although a large number of studies have not been able to identify the risk factors affecting OLP malignancy, a number of studies have suggested that this may be due to changes in the cell cycle control mechanism(19-24). Although a large number of studies have not been able to identify the risk factors affecting OLP malignancy, a number of studies have suggested that this may be due to changes in the cell cycle control mechanism(19). Epithelial proliferation and anti-apoptotic action in OLP seems to have been caused by inflammation that may produce mutagenic irritants on the DNA. These actions can be an important activation in the P53 system, which can stop the cell cycle to repair DNA, which can justify malignant changes in the presence of high levels of P53 (2). P53 is detected on chromosome 17 and is a main tumor suppressor gene which expresses important proteins(3). P53 is associated with malignant changes in oral epithelial cells(2, 6, 20-24). The long-term presence of OLP, especially its erosive form in the oral cavity, is probably playing the main role in its malignant changes(16, 17, 25). MMPs are either secreted from cells or are present in the membrane, and can digest all the extracellular matrix and basal membrane compounds(5, 10). This is a key event in the invasion and metastasis of most malignancies(10). MMPs increase isolation and invasion by reducing cell adhesion and extracellular matrix molecules(11). It has been observed that increased expression of MMPs can be involved in the progression of tumors, including tumors in the cervical region(10). On the other hand, MMPs play a role in cell migration, angiogenesis and activation of the proteolytic action of growth factors, which are effective in tumor invasion(5). In addition, MMP-1 and MMP-3 are probably related to the processes involved in wound progression(26).

In fact, MMPs can eliminate intercellular junctions and cause acantholysis, with MMP-3 playing a significant role in this event(27). MMPs often increase in the groups causing active triggers associated with inflammatory and malignant diseases(28). OLP is a chronic inflammatory with a potential of malignancy. In addition, it is clear that chronic infection and tissue inflammation are important risk factors for the development of various types of cancer and malignancy.Therefore, it can be said that MMPs probably play an important role in OLP and its malignant changes(12). Mircea Tampa et al. in a review article in 2018 showed that biomarkers such as MMPs and P53 are elevated in OLP and can be useful markers to predict futures malignancy(3). This finding is in accordance to our study. Shaini Basheer et al. in 2016 reported a positive correlation between P53 and OLP(13). Agha Hosseini et al. Showed that the level of P53 in the saliva of patients with plaque like OLP was significantly higher than the erosive form of OLP and control group. However, despite the higher level of P53 in patients with Erosive OLP than the control group, there was no significant difference between these two groups(29). In 2014, Agha- Hosseini et al. also compared salivary P53 level in patients with OLP, Oral Squamous Cell Carcinoma (OSCC) and healthy people, and finally concluded that the expression of P53 in patients with OSCC was significantly higher than patients with OLP and control group, but there was no significant difference between OLP and control group. They also suggested that high levels of P53 expression could not indicate the pre-cancerous nature of OLP(2). In another study, Elvira Lloes et al. examined the expression of P53 in patients with OSCC, OLP, and epithelial dysplasia, and finally concluded that the expression of P53 in these three groups was approximately the same, and overexpression of P53 in OLP can provide suitable conditions for malignant changes to OSCC and epithelial dysplasia(30). Another study by Elba-Rosa Leyva-Huerta et al. showed that the immune expression of P53 in the OLP group was most commonly observed. They also suggested that P53 plays a major role in malignant changes by stopping the apoptotic process of a cell cycle(30). M Farzin et al. in their study compared the serum level of MMP-3 in patients with OLP with the control group. They observed

that the serum level of MMP-3 in the patients with OLP was higher than the control group, but this difference was not statistically significant. In addition, they found that there was a significant difference in the level of MMP-3 between patients with Erosive OLP and patients with reticular form of the disease. In fact, patients with Erosive OLP had higher serum levels of MMP-3 (5). Additionally, Agha- Hosseini et al. also revealed that serum and saliva level of MMP-3 in patients with OSCC was more than those with Erosive OLP, and in patients with Erosive OLP this level was more than patients with Reticular OLP. There was also significant correlation between serum and salivary level of MMP-3(8). This study also presented that there were no significant differences in the level of these two proteins between men and women, which was probably because gender does not affect these markers expression.

Conclusion

The result of our study showed that salivary levels of P53 and MMP-3 in patients with Erosive OLP were significantly higher than patients with reticular OLP and control group. This issue is justified by the important role of these two proteins in the process of chronic inflammation and infectious diseases.

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Conflicts of interest

There are no conflicts of interest

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