Assessment of Salivary MMP-1 in Iraqi patients with Oral Lichen Planus

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ABSTRACT:

Background: Oral lichen planus (OLP) is chronic inflammatory mucocutaneous diseases. The causes of OLP are unknown and there are six different clinical shape of OLP. Aims: The salivary levels of MMP-1 in OLP in Iraqi patients and compared them to healthy individuals. Methods: saliva sample were collected from 65 patients with OLP and healthy controls. The technique used to reveal saliva level of MMP-1 Enzyme linked immune sorbent assay (ELISA). Results: The current results revealed that salivary MMP-1 concentration in OLP patient (46 6ng/l) whereas (64ng/l) in healthy group. Moreover there were highly significant differences (p>0.01) in percentages of MMP-1 between OLP patients and control group. Conclusion: This concluded that the saliva levels of MMP1 might play a crucial in oral lichen planus.

Key words: Oral lichen planus, Saliva, MMP-1, ELISA.

Introduction

Oral lichen planus (OLP) is common chronic inflammatory disease of the oral mucosa. Years of clinical, histological, immunological, immune histochemical and genetic studies have not identified the pathogenic pathway and initial trigger leading to the formation of lesions. Some general diseases, such as genetic predisposition, hypertension, chronic liver disease and diabetes, are also associated with the underlying causes of the disease(Carrozzo M etal.,2014; Roopashree MR etal.,2010). The prevalence of OLP is about 1% to 2% in the general population (Mozaffari HR etal., 2016). OLP is usually between middle-aged and older individuals (Ingafou M etal.,2006) with a female to male ratio around 2:1 (Shen ZY etal.,2012 ). Reticular, plaque-like and papular forms are white keratotic lesions that form non-symptomatic lesions, accidentally discovered by the patient or during clinical examination within the mouth. However, some patients may feel edema or grossness of the oral mucosa. The reticular form can transform into more advanced erosive, atrophic or bullous form. These occur in the form of red lesions and are associated with a pain triggered and burning sensation or intensified by spicy, hot or salty food products, chemical irritating factors (Eisen D,2002; Van der Meij EH etal.,2003 ). The diagnostic criteria for oral lichen planus first identified and then revised by the World Health Organization are accurate, well known and describe both clinical and histopathological features(Rad M etal.,2009 ). Cytokines play an important role in the causing of OLP and a bulk of evidence proposing that OLP is a T-cell mediated disease (Lavanya N etal.,2011). The large amount of cytokines released by infected keratinocytes and their associated inflammatory agents plays a key role in the selective recruitment of the T lymphocytes. T-cell predominated infiltrate in the subepithelial region, which characterizes OLP, stimulates further release of chemokines and cytokines that belong to either Th1 or Th2 groups (Thornhill MH,2001). Adding to the increased interest in recent years in the role of different cytokines in the immune pathogenesis of lichen planus, which include interleukin-2 (IL-2), interleukin-10 (IL-10) and interleukin-6 (IL-6) (Prime SS etal., 2004). Matrix metalloproteinase (MMPs) are a large family of calcium-dependent zinc-containing endopeptidases (EC 3.4.24.7), which are responsible for the tissue reconstruction and demolition of the extracellular matrix (ECM), including collagens, gelatin, matrix glycoproteins, elastin, and proteoglycan throughout organogenesis, growth, normal tissue turnover, wound healing, teeth morphogenesis, and teeth eruption(Beertsen etal.,2002; Kapoor etal.,2016 ). MMPs act in following manner: cell migration, angiogenesis, proteolysis activation of

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growth factors and factors of normal tissue remodeling, repair and metastasis (Pickering V et al.,2007; Roopashree et al.,2010). The Analysis of saliva components was used for the evaluation of biomarkers (SugermannPB et al., 1996).because Saliva has advantages compared to blood and is increasingly being used in the diagnosis of diseases. It offers discriminatory advantages over serum because it eases of access and evaluation and preventing stress and pain in patients. Salivary analysis offers a reliable correlation of various parameters that are routinely evaluated in blood (Abbas AK et al.,2009; Pardali K and Moustakas A,2007).The purpose of this study was to evaluate salivary MMP-1 in Iraqi patients suffering from OLP.

MATERIALS AND METHODS

- **The patient and control:** Thirty Five Iraqi patients (20 female and15 male) attending the dermatological outpatient clinic at medical city in Baghdad during the period from July 2016 till October 2016. The control group consisted of thirty healthy volunteers (17 female and 13 male). They were all diagnosed by specialist physician based on the clinical pictures. Excluded criteria patients without chronic periodontitis, gingivitis and without systemic diseases, no history or clinic evidence of malignant disease. Data were collected through direct interview with patient and by seeking his/her previous medical reports included name, age, gender, personal history of smoking, alcohol drink, blood transfusion, previous surgery, cupping and hepatitis infection.

- **Saliva collection and processing:**

  Three ml of saliva was collected from each patients and control in sterile cup after 5 minutes from rinse of the mouth by water. Saliva samples were processed immediately after collection they were centrifuged at 2,600g for 15 minutes at 4°C. Separate salivary fluid was stored in the disposable storage vials at -80 °C until analysis time. Care was taken not to collect sputum (Heba F. Hassan et al.,2017).

- **Determination of MMP-1 levels in saliva:**

  The level of MMP-1 concentrations have been measured by using commercially available ELISA and performed as recommended in leaflet with kit (Mybiosource-MBS355314, USA).

- **Statistical Analysis**

  By using Statistical Package for Social Science (SPSS 24 IBM, Armonk, USA), the results were expressed as mean ± standard deviation (SD). In this study the differences in means of the variables between control and patient groups and between smoker and non-smokers in patient group were analyzed by independent sample t-test.

Results:

- Reticular OLP their age ranged from 30 to 60 years with mean (46.26±8.56) The control group consisted of thirty healthy volunteers (17 female and 13 male) matched according to age and gender of patients the mean age of control (47.23±9.02) (table 1), and female was more predominance (57%) than male patients.

<table>
<thead>
<tr>
<th>Age(Year)</th>
<th>Healthy group</th>
<th>Patient group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Mean ±SD</td>
<td>47.23 ±9.02</td>
<td>46.26 ±8.56</td>
</tr>
</tbody>
</table>

- **Assessment of salivary MMP-1 in OLP patients and healthy control:** The current results revealed that salivary MMP-1 concentration in OLP patient (466ng/l) whereas (64ng/l) in healthy group, Figure (1). Moreover there...
were highly significant differences (p>0.01) in percentages of MMP-1 between OLP patients and control group, Table (2).

Table 2: Assessment of MMP-1 in OLP patients and healthy control

<table>
<thead>
<tr>
<th></th>
<th>Healthy Group</th>
<th>OLP Patient Group</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Year)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>30</td>
<td>30</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td>Mean ±SD</td>
<td>47.23±9.02</td>
<td>46.26±8.56</td>
<td></td>
</tr>
<tr>
<td>MMP-1 (ng/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>64</td>
<td>466</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Mean ±SD</td>
<td>190.93±20.23</td>
<td>400.03±148</td>
<td></td>
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</tbody>
</table>

Fig 1: concentration of MMP-1 in OLP patients and healthy control

Assessment of salivary MMP-1 in smoker and non smoker OLP patients: The results of assessment of MMP-1 in OLP patients according the smoking habit there was significant difference (p<0.05) between the studied groups, the MMP-1 in smokers OLP patients were (352 ng/l) while (466 ng/l) were non-smokers. (Table 3).

Table 3: Assessment of MMP-1 in OLP patients (smoker and non smoker)

<table>
<thead>
<tr>
<th></th>
<th>OLP Patient</th>
<th>OLP Patient (Non-Smoker)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Year)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>22</td>
<td>30</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>Mean ±SD</td>
<td>50.8±6.83</td>
<td>42.85±8.27</td>
<td></td>
</tr>
<tr>
<td>MMP-1 (ng/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>352</td>
<td>466</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td>Mean ±SD</td>
<td>368.67±101.76</td>
<td>423.55±173.72</td>
<td></td>
</tr>
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</table>
DISCUSSION

OLP is a chronic inflammatory disorder. Although the precise causes of OLP are still unclear, immunological aberration plays a difficult role between different causative factors (Pickering V et al., 2007). It affects 1.27% of the population and is more common in women (1.57%) than men (0.96%) and The predominance of females ranging from 54% to 65%. Overall OLP is a middle-aged and elderly disease (Miller CS et al., 2010; Malamud D, 1992; Nishanian R et al., 1998). The cause of OLP is very complex and involves possible antigen presentation by oral keratinocytes that can be either exogenous or endogenous produced (Carrozzo M, 2008). This antigen trigger is accompanied by a mixed inflammatory response which mainly involves T cells, mast cells, macrophages, as well the association of cytokines and cytotoxic molecules. (Sugarman PB and Savage NW, 2002; Scully C and El-Kom M, 1985). The current results showed that there is no significant difference in mean of age between OLP patients and healthy control which is comparable with other Iraqi study conducted by Heba et al., (2017). And also the present study is corresponding with Heba et al., indicated that the female predominance among patients, about (62.5%) of OLP patients females, while only (37.5%) of patients were males (Farhi D. and Dupin N, 2010). In addition, the results obtained in our study consistent with the study conducted by Bermejo-Fenoll et al., (2010) stating The percentage of infection of oral lichen planus (OLP) in south-eastern Spain in females were (76.7%) and in males (23.3%). Also another study showed OLP in 674 China patients 65.9% were women and 34.1% were men (Gonzalez-Moles MA and Scully C, 2008). Add to concordance with study down by Vice Budimir et al., (2014) on 563 Croatian patients with OLP Where the proportion of olp female about (73.5%) and male (26.5%). Our patients who had OLP were mostly non-smokers (57.14%), Moreover, results reported by Vice Budimir et al., (2014) showed that non-smoker OLP patient around (78.4%) while (21.6%) they were smoker. Also Neumann- Jensen B et al., (1977) stated that OLP was less common in smokers than non-smoking. OLP is chronic inflammatory mucocutaneous diseases. 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 (Tahereh Nosratzehi et al., 2017). 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 V et al., 2007; Roopashree MR 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. 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 to Kim et al., (2006) study, high levels of MMP-1 and 3 can be promoted as the development of erosion in various forms of LP. Ertughrul et al., (2013) examined the level of MMP-1 in OLP patients with gingivitis and periodontitis and both groups had chronic periodontitis. Mutlak et al. (2017) that findings of the increased levels of the MMP-1n association with immunohistological changes in the tissue expression of the MMP-1 in different dental diseases. 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. This study affirmed the influencing of immune cytokine in pathogenesis of OLP and the significant changes were evident in the saliva levels of MMP1 might play a crucial in oral lichen planus. Add to the measurement of this marker in saliva may be more useful than serum measurements for determining diagnostic and therapeutic aims.

References:


