A Comparison between Serum and Salivary Tumor Necrosis Factor-Alpha in Oral Lichen Planus

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ABSTRACT

Oral lichen planus is an autoimmune inflammatory disease. Tumor necrosis factor α (TNF-α) is an important cytokine with a large number of biological effects which is implicated in the pathogenesis of the disease. The present study was conducted to measure and compare the level of TNF-α in sera and saliva of patients with OLP. The study included 30 patients suffering from OLP. They were divided into 2 groups: erosive OLP (15 patients) and reticular OLP (15 patients). The study also included 10 age and sex matched healthy subjects as control group. TNF-α was measured in sera and saliva by enzyme linked immunosorbent assay method.

Results: TNF-α in sera of OLP patients was statistically higher than in control group (6.39 ± 1.82 pg/ml compared to 2.99 ± 0.52 pg/ml, P < 0.0001). Also TNF-α in sera of erosive OLP was statistically higher than in sera of reticular OLP (7.87 ± 1.16 pg/ml compared to 4.90 ± 0.90 pg/ml, P <0.0001) and TNF-α in erosive or reticular OLP was significantly higher than in the control group (P < 0.0001). Regarding salivary TNF-α the study showed that salivary TNF-α in OLP patients was statistically higher than in the control group (28.20 ± 5.00 pg/ml compared to 6.91 ± 0.98 pg/ml, P < 0.0001). The study also showed that salivary TNF-α was statistically higher in erosive type than in reticular type and each of them was higher than the control group (31.75 ± 3.75 pg/ml in erosive type, 24.56 ± 3.15 pg/ml in reticular type and 6.91 ± 0.98 pg/ml in the control group, P = < 0.0001 in all). The study also showed that a positive correlation was present between salivary and serum TNF-α, and salivary TNF-α was always higher than serum TNF-α. Conclusion: Salivary TNF-α is a non invasive more sensitive technique that can be used to detect disease activity and monitor the therapeutic response in OLP.

INTRODUCTION

Oral lichen planus (OLP) is a chronic inflammatory disease that affects the oral mucous membranes, sometimes in combination with lesions of the skin. It causes bilateral wide striations, papules or plaques on the buccal mucosa, tongue and gingivae. Erythema, erosions and blisters may or may not be present. The reported prevalence rates of OLP varies from 0.5% to 2.2% of the population. The typical age presentation is between 30 and 60 years, and it is more frequently seen in women. The cause of OLP is still not known, however T-cell mediated auto-immune damage against epithelial cells is implicated in the complex pathogenesis of the disease. The immunologic process leads to vacuolar degeneration, lysis and liquefaction of the basal cells. T cell dominate the infiltration in the subepithelial region which charac-terize OLP, and induce further release of chemokines and cyto-kines.

The large amounts of cytokines released by affected keratinocytes and the associated inflammatory elements play a key role in the selective recruitment of the T-lymphocytes and in exacerbation and perpetuation of OLP. Tumor necrosis factor-α (TNF-α) is a multifunctional cytokine that plays a prominent role in immune and host defense responses to infection. It enhances several innate immune functions and regulates the specific immune response aimed at limiting the spread of infection. TNF stimulates angiogenesis, influences tissue remodeling and takes part in the regulation of cell proliferation and differentiation, it has been identified as an important mediator of cancer development.

Previous data pointed to TNF-α as a key stone in OLP, and high serum levels of TNF-α were detected in all patients with OLP in comparison with its hardly detect-able levels in control subjects. Oral fluids analysis has obvious advantages compared with blood based analysis, such as easy access and non invasive collect-ion. Also oral fluids analysis have been widely used in drug and disease monitoring of various oral and systemic maladies. Whole saliva has been successfully applied for detection of s-IgA and proinflammatory cytokines in patients with
sjogren’s syndrome\(^{(13)}\). Other investigators use saliva for detection of nuclear factor-Kappa B (NF kappa B) dependent cytokines in patients with OLP\(^{(14)}\), oral preneoplastic lesions and oral squamous cell carcinoma\(^{(15)}\).

The aim of this study was to compare both the salivary and serum levels of TNF-\(\alpha\) in patients with OLP and compare it with the type of the disease (disease activity).

**PATIENTS, MATERIALS & METHODS**

This study was done in the Department of Microbiology and Immunology, Benha Faculty of Medicine, Benha University, during the period from February 2008 to November 2008. The study included 30 patients with oral lichen planus (OLP), 15 with reticular form and 15 with erosive form. The patients were selected from the Dermatology Outpatient Clinic, Faculty of Medicine, Benha University Hospitals. OLP was diagnosed according to the standard clinical criteria, and confirmed by incisional biopsy. Their age ranged from 18 to 58 years (mean \(\pm SD = 39.63 \pm 9.80\) years). The study also included 10 age and sex matched healthy individuals as control group.

All subjects neither had gingival and/or periodontal inflammation, nor any visible oral lesion as examined by a dentist.

**Exclusion criteria:**
- Patients with hepatitis, diabetes mellitus, autoimmune disease or systemic infection.
- Patients who are receiving drugs that induce hyposalivation or anticholinergic, antihistaminic, antihypertensive and beta adrenergic blockers (in the previous 3 months).
- Patients who are smoking.
- Also patients who received treatment for OLP within 30 days of specimen collection.

All individuals were subjected to the following:
- Full history taking including site of the lesion and its progression, history of dental procedure, history of drug intake, past or family history of lichen planus also symptoms suggestive of systemic diseases.
- Thorough clinical examination.
- Investigation for the exclusion criteria:
  - Complete blood count (CBC).
  - Erythrocyte sedimentation rate (ESR).
  - Serum urea and creatinine.
  - SGOT – SGPT (liver enzymes).
  - HCV antibodies (ELISA).
  - Fasting blood sugar (FBS) and postprandial blood sugar (PPBS) if needed.

**TNF-\(\alpha\):**
- 5ml of venous blood was taken from each individual under complete aseptic conditions and kept in the refrigerator. After clotting, the specimen was centrifuged at 5000 rpm to separate serum. Sera were stored at freeze (-20\(^\circ\)C) till use.

The whole unstimulated saliva (WUS) was collected in the morning using standard techniques described by Navazesh 1999\(^{(16)}\). Briefly subjects were refrained from eating, drinking, using chewing gum, mints, etc.. for at least 1 hour prior to specimen collection. The subjects were requested to swallow first, hit their head forward and then expectorate all saliva into sterile centrifuge tube for 5 minutes without swallowing. The samples were centrifuged at 5000 rpm for 20 minutes, the clarified supernatants were drawn off and immediately frozen at -80\(^\circ\)C till use in the ELISA assay.

Quantitative determination of human tumor necrosis factor alpha (TNF-\(\alpha\)) in sera and saliva was determined by use of commercially available kit (Quantikine\textsuperscript{\textregistered} human TNF-\(\alpha\) immunoassay) R & D system–catalog number DTAOOC, and according to the manufacturer's instructions. Briefly 50 ul of assay diluent was added to each well, then 200 ul of standard, sample or control were added, and incubated at room temperature for 2 hours. The wells were aspirated and washed 4 times, then 200 ul of TNF-\(\alpha\) conjugate were added to each well and incubated for 2 hours at room temperature (1 hour only in saliva). The wells were again aspirated and washed 4 times. 200 ul substrate solution were added to each well, and incubated at room temperature for 20 minutes, protected from light. 50 ul stop solution were added to each well. The wells were read at 450 nm within 30 minutes. A standard curve was prepared by plotting the absorbance values of the standards versus corresponding concentrate-ions. The concentration of TNF-\(\alpha\) in the samples was determined from the standard curve.
RESULTS

Our results are shown in the following 4 tables and 3 figures:

Table (1): Sex distribution of patients and control groups

<table>
<thead>
<tr>
<th></th>
<th>Females</th>
<th>Males</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>OLP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Erosive OLP</td>
<td>9</td>
<td>63.33</td>
<td>11</td>
</tr>
<tr>
<td>- Reticular OLP</td>
<td>10</td>
<td>66.67</td>
<td>5</td>
</tr>
<tr>
<td>Control group</td>
<td>6</td>
<td>60</td>
<td>4</td>
</tr>
</tbody>
</table>

Table (2): Age distribution of the studied groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Range</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erosive OLP</td>
<td>20 – 58 y</td>
<td>40.67 y ± 9.85 y</td>
</tr>
<tr>
<td>Reticular OLP</td>
<td>18 – 57 y</td>
<td>38.60 y ± 9.98 y</td>
</tr>
<tr>
<td>OLP (total)</td>
<td>18 – 58 y</td>
<td>39.63 y ± 9.80 y</td>
</tr>
<tr>
<td>Control group</td>
<td>21 – 56 y</td>
<td>37.40 y ± 10.15 y</td>
</tr>
</tbody>
</table>

Table (3): TNF-α in sera of patients and control groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Level of TNF-α (pg/ml)</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A) Erosive g. (n=15)</td>
<td>7.87 ± 1.16</td>
<td>A vs C</td>
<td>12.4</td>
</tr>
<tr>
<td>(B) Reticular g. (n=15)</td>
<td>4.90 ± 0.90</td>
<td>B vs C</td>
<td>6.05</td>
</tr>
<tr>
<td>(C) Control g. (n=10)</td>
<td>2.99 ± 0.52</td>
<td>D vs C</td>
<td>5.77</td>
</tr>
<tr>
<td>(D) OLP g. (n=30)</td>
<td>6.39 ± 1.82</td>
<td>A vs B</td>
<td>7.84</td>
</tr>
</tbody>
</table>

$P = < 0.0001$ = Highly significant

Table (4): TNF-α in saliva of patients and control groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Level of TNF-α (pg/ml)</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A) Erosive g. (n=15)</td>
<td>31.75 ± 3.75</td>
<td>A vs C</td>
<td>20.3</td>
</tr>
<tr>
<td>(B) Reticular g. (n=15)</td>
<td>24.56 ± 3.15</td>
<td>B vs C</td>
<td>17.11</td>
</tr>
<tr>
<td>(C) Control g. (n=10)</td>
<td>6.91 ± 0.98</td>
<td>D vs C</td>
<td>13.2</td>
</tr>
<tr>
<td>(D) OLP g. (n=30)</td>
<td>28.20 ± 5.00</td>
<td>A vs B</td>
<td>5.68</td>
</tr>
</tbody>
</table>

$P = < 0.0001$ = Highly significant
Fig. (1-a): Level of TNF-alpha in sera of the different groups

Fig. (1-b): Level of TNF-alpha in saliva of different groups

Fig. (2): Correlation between TNF-α in serum and saliva in OLP patients.

(A) Erosive OLP
r = 0.5467
p = <0.01 (significant)

(B) Reticular OLP
r = 0.8191
p = <0.001 (significant)
Fig. (3): Histopathology of OLP showing: degeneration of basal cells, lichenoid infiltrate, and infiltration of inflammatory cells into the subepithelial layer of CT some colloid hyaline (Civatte) bodies and elongation of rete ridges that resemble a saw tooth pattern.

DISCUSSION

OLP is a persisting inflammatory disorder of oral mucous membranes. The disease occurs in a number of different clinical forms. However reticular and erosive forms are the most common ones, and the erosive form has been taken as the acute (more severe) phase of the disease, when the superficial epithelium desquamate and leaves areas of ulcerated or erosive mucosa surface (17).

The mechanisms involved in the pathogenesis of OLP are unknown. However T cell mediated autoimmune damage against epithelial cells is implicated in the complex pathogenesis of the disease (3).

Recently TNF-α which is one of the proinflammatory cytokines has been reported to play a role in the pathogenesis and inflammatory process of OLP (18). TNF-α is involved primarily in T cell mediated cytotoxic reactions, and through its interactions with positive and negative regulators of apoptosis, seems to be an important cytokine involved in basal cell death seen in OLP epithelium (19).

Our results showed that OLP is a disease of middle age and affect females more than males. The age of OLP patients in our study ranged from 18 – 58 years, the mean ± SD of the age was 39.63 ± 9.80 years. The ratio between females and males in our study was 19 : 11 i.e. 63.33% females compared to 36.67% males. These results are in agreement with that of Osman et al. 2004 (20), who found that the mean age of the patients was 38.3 ± 15.8 years, and the disease was common in females compared to 26.67% for males.

Regarding TNF-α levels in sera of OLP patients our study showed that, TNF-α level in higher in sera of OLP patients than in the control group (6.39 ± 1.82 pg/ml compared to 2.99 ± 0.52 pg/ml) a difference which is statistically significant (P = < 0.0001). Also the study showed that TNF-α levels in sera of erosive OLP patients is significantly higher than that in sera of reticular OLP patients (7.87 ± 1.16 pg/ml compared to 4.90 ± 0.90 pg/ml, and P = < 0.0001), and the level in each group is significantly higher than in the control group P = < 0.0001 in both). These results are compatible with other results. Zhang et al. 2008 (21), in a study on 30 OLP patients and 30 as healthy subjects control group, proved that TNF-α levels in sera of OLP patients were much higher than in sera of control group (4.56 ± 2.36 pg/ml compared to 2.40 ± 2.57 pg/ml) a difference which is statistically significant. They also proved that TNF-α levels in sera of patient with erosive OLP (5.68 ± 2.45 pg/ml) was significantly higher than in sera of patients with reticular OLP (3.29 ± 1.51 pg/ml). The study also showed that TNF-α levels in sera of patient with erosive or reticular OLP is significantly higher than in the control group (2.40 ± 2.57 pg/ml). These results indicate that the serum level of TNF-α may be used as a marker of disease activity. Our results also correlate with that of sun et al. 2007 (22), who detected higher levels of TNF-α in sera of patients with erosive OLP (12.0 ± 1.7 pg/ml) compared to normal control subjects (3.8 ± 0.2 pg/ml), (P = < 0.005) and also TNF-α in sera of patients with non erosive OLP was higher than in control group (6.1 ± 1.7 pg/ml compared to 3.8 ± 0.2 pg/ml, P < 0.05). Similar results were also obtained by Yammamoto et al. (23), Zahran et al. (24) and Porter et al. (25).
Regarding TNF-α levels in saliva of OLP patients, our study showed that TNF-α in saliva of OLP patients is higher than in the control group (28.15 ± 5.00 Pg/ml compared to 6.91 ± 0.98 pg/ml), a difference which is statistically significant (P=0.0001). Also, the study showed that TNF-α in saliva of erosive OLP is significantly higher than in saliva of reticular OLP (31.75 ± 3.75 pg/ml compared to 24.56 ± 3.15 pg/ml, P =<0.0001), and the level in each of the 2 groups is significantly higher than in the control group (P=0.0001 in both). These results are in agreement with other results. Pezelj-Ribaric et al. in 2004(4) examined 40 patients with OLP (20 were erosive OLP and 20 were reticular OLP) and 20 healthy subjects as control group. They found that salivary level of TNF was significantly higher in patients than in the control group (P = <0.0001). Also the levels was significantly higher in erosive and reticular group when compared with the control group (P = < 0.0001 in both). The study also showed that the level in erosive OLP is significantly higher than in reticular OLP (P=<0.0001). Rhodes et al.(2005)(14) found that TNF-α in saliva of 13 patients with OLP is significantly higher than that of 13 healthy subjects (control group) (35.63 ±9.67 pg/ml compared to 2.24 ± 0.78 pg/ml, P=<0.0001). Also Zhang et al. 2008(21) found that salivary levels of TNF-α in 30 patients with OLP is significantly higher than that of 30 normal control subjects (29.92 ± 9.99 pg/ml compared to 6.16 ± 1.93 pg/ml).

In our study there is a positive correlation between concentration of TNF-α in saliva and serum in patients with erosive OLP (r =0.5467, P=<0.01) and also in patients with reticular OLP (r=0.8191, P < 0.001).

The study also showed that the salivary concentration of TNF-α in patients with OLP is much higher than their concentration in serum. These results are in agreement with that of Zhang et al.(2008)(21).

The increase in salivary TNF-α concentration in patients with OLP may be due to two aspects: (1) Local production from the cells of the inflammatory infiltration and/or by keratinocytes themselves(26). (2) Loss of structural barriers in oral mucosa (particularly in the active inflammatory phase of erosive OLP)(26) Zhang et al. (2008)(21) proved that local production is the major cause for the high levels of salivary TNF-α in OLP. The proposal was supported by the study of Yamamamoto et al.(29) in which they had found that the epithelia from OLP lesions produce TNF-α and IL-6, 10 to 20 folds greater than those in keratinocytes from normal gingivae, hence interacting with infiltrated lymphocytes and subsequently contribute to the pathogenesis of OLP(29).

Furthermore cytokine and anticytokine therapies have shown some potential in treatment of autoimmune diseases, for example, agents directed against TNF-alpha are effective in the treatment of chronic disorders such as rheumatoid arthritis and Sjogren's syndrome(27). Since OLP is a chronic disease with some characteristics of autoimmune disorders, and TNF-α may play an important role in OLP pathogenesis as a triggering and fostering factor, these agents may be used in treatment of OLP in the near future(14).

**Conclusion:** Measurement of TNF-α in saliva may be a non invasive, more sensitive technique to evaluate the severity of OLP and to follow up the therapeutic response of the disease.

**REFERENCES**


مقارنة بين مستوى عامل النخر الورمي ألفا في المصل واللعاب في مرض الحزاز المسطح الفمياً
جمال عبدالرحمن عامر – أسامة محمد الرفاعي* – عبد اللطيف محمد البلشى**
قسم الميكروبيولوجيا والمناعة – قسم الأمراض الجلدية وأمراض الذكورة – قسم البيولوجيا**

كليّة طب بها

يعتبر مرض الحزاز المسطح الفمياً أحد الأمراض التناسلية المناعية وينتج غالبًا عن اختلاس في المناعة الخلويّة.
ويعتبر عامل نخر الورم ألفا منظماً هاماً للكثير من الوظائف البيولوجية في جسم الإنسان. وقد أظهرت دراسات عديدة تدوّن
هذا السيتوكين (عامل نخر الورم ألفا) في حدوث مرض الحزاز المسطح الفمياً. وقد أجريت هذه الدراسة تقييم ومقارنة
مستوى عامل نخر الورم ألفا في مصل ولعاب مرضى الحزاز المسطح الفمياً. واستمتعت الدراسة على 30 مريض قسموا إلى
مجموعتين الأولى 15 مريض بنم مرض الحزاز المسطح الفمياً التاكلكي والثانية 15 مريض بنم مرض الحزاز المسطح الفمياً
الشبيكي (غير تاكلكي). واستمتعت الدراسة أيضاً على 10 أفراد أصحاء كمجموعة ضابطة. وأجري لهم جميعاً قياس مستوى
عامل نخر الورم ألفا باستخدام طريقة الالتيز ودأب النتائج كالآتي:

١ - لوحظ ارتفاع في مستوى عامل نخر الورم ألفا في مصل المرضى بالمقارنة بالأصحاء وكان الارتفاع ذو دلالة
٢ - إحصائياً
٣ - لوحظ ارتفاع في مستوى عامل نخر الورم ألفا في مصل المرضى بنم مرض الحزاز المسطح الفمياً التاكلكي
٤ - بالمقارنة مع المجموعة الضابطة.
٥ - وجد ارتفاع ذو دلالة إحصائية في مستوى عامل نخر الورم ألفا في مصل مرضى الحزاز المسطح الفمياً
٦ - التاكلكي بالمقارنة مع مرضي الحزاز المسطح الفمياً الشبيكي
٧ - أو الشبيكي بالمقارنة مع المجموعة الضابطة.
٨ - كان مستوى عامل نخر الورم ألفا في اللعاب أعلى منه في المصل مع وجود علاقة طردية (إيجابية) بين المستوى
٩ - في اللعاب والمستوى في الدم.

الخلاصة:
قياس مستوي عامل نخر الورم ألفا في لعاب مرضى الحزاز المستعرض يعتبر طريقة أمنة وأكثر حساسية لمعرفة
شدة المرض – ومنطقة تطور مع العلاج.