Correlation between Stress and Pro-Inflammatory Cytokines in Erosive Oral Lichen Planus Patients

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Abstract

Background: The etiology of oral lichen planus (OLP) remains uncertain. However, most evidence suggests that it is a T-cell mediated autoimmune reaction precipitated by endogenous or exogenous factors in persons with a genetic predisposition to the development of LP. Certain precipitating factors have been identified including stress. IL-8 and TNF-α are found to be in a higher serum level in patients with OLP than in normal subjects and to be involved in the pathogenesis of OLP. Objective: The aim of the present study was to find a relation between stress and pro-inflammatory cytokines in erosive oral lichen planus patients (EOLP), Subjects and Methods: 30 patients with EOLP were participated in the present study. Patients were divided according to their stress scores using Hospital Anxiety and Depression scale (HADS) into three groups, group A, with high stress score, group B with moderate stress scores, and group C with normal stress score. Lesion size, visual analogue score (VAS), and serum levels of IL-8 &TNF- α were assessed for all groups. Results: A statistically significant increase in group A compared to group B and group C respectively in relation to lesion size , VAS scores and serum level of IL-8 &TNF- α. Conclusion: The study highly suggested a positive correlation between psychological stress, immune system over-activity with increased production of pro-inflammatory mediators and OLP clinical manifestations.


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INTRODUCTION

Lichen planus (LP) is a chronic autoimmune disease caused by type IV hypersensitivity reaction to antigenic alteration observed in the mucosal lining and skin (Lavanya N 2011). While the cutaneous lesions of LP usually self-regress, the lesions found in the oral cavity are chronic, rarely undergo spontaneous remission and are potentially premalignant (Eisen D 2005). Fifty percent of patients with skin lesions also manifest oral mucosal lesions, and 25% of patients with oral lichen planus (OLP) present only oral lesions (Ghislaine 2010).

Oral lichen planus tends to persist for many years with periods of exacerbation and quiescence. During periods of exacerbation, the area of erythema or erosions increases, with concomitant increase in pain and sensitivity. On the contrary, during periods of quiescence, the area of erythema or erosion decreases with decrease in pain and sensitivity (Sandhu 2014). Exacerbation of OLP has been linked to periods of psychological stress and anxiety, a predictable correlation with any condition that is related to an immune system imbalance ( Soto 2004, Rojo-Moreno 1998).

The etiology of OLP remains uncertain. However, most evidence suggests that it is a T-cell mediated autoimmune reaction precipitated by endogenous or exogenous factors in persons with a genetic predisposition to the development of LP (Shirasuna 2014). This results in altered response to self-antigens with subsequent damage to basal keratinocytes. Certain precipitating factors have been identified and these include genetic background, dental materials, drugs, stress, trauma, infections, chronic liver disease, diabetes and hypertension (Robertson 1992, Klanrit 2003, Luis-Montoya 2005).

IL-8 and TNF-α are found to be in a higher serum level in patients with OLP than in normal subjects and to be involved in the pathogenesis of OLP (Rhodos 2005). Authors proved that serum IL-8 level is a sensitive marker in monitoring the disease activity of OLP. Others. (Powell 1986, Mahmood 1983, Wang 2011) found that the elevated TNF- α serum levels may be associated with the induction and/or perpetuation of the apoptotic events in the OLP epithelium and that TNF-α concentrations varied in different clinical types of OLP, being particularly elevated in the case of the...
erosive/atrophic form of the disease. Moreover, other investigators (Fujita2009, Bai 2007, 2009, Liu 2014) identified the positive influence of inflammatory cytokine, including, TNF-α, and IL-8 in the prognosis of OLP. the psychosomatic stressors being discussed as one of the causative factors. Since the description of lichen planus (Wilson 1869). Later on, authors found a significant difference in the mental disturbance between OLP and non-OLP patients (Hampt 1987). More recently, it was found that OLP patients exhibited greater anxiety than controls, but could not establish a causal relationship between them (Rojo-Moreno 1998).

A psycho-neuro-immunological researches demonstrated clinically relevant interrelations between psychological stressors & the onset and progression of chronic diseases. Disturbances in the interaction between the nervous, immune and endocrine systems have been hypothesized to be implicated in several autoimmune diseases (Koraya 2003).


Thus the current study was performed to evaluate serum pro-inflammatory cytokines levels correlating them with stress profile in patients with erosive oral lichen planus (EOLP).

Subjects and methods:

Thirty participants were selected from the outpatient clinic of oral medicine & periodontology department, faculty of dentistry, Alexandria University.

All selected patients were diagnosed with erosive oral lichen planus lesions (EOLP) clinically and confirmed by histopathological examination according to modified WHO criteria (van der Meij and Van der Wall 2003). patients were otherwise systemically healthy. Any psychiatric illness, and those who are taking anti-depressives as well as any systemic medications for the previous 3 months of sample collection were excluded patients with other skin, mucosal or systemic diseases and Pregnant women or women using oral or injectable contraceptives were excluded.

Materials:

- AviBion Human TNF-α ELISA Kit
- AviBion Human IL-8 ELISA Kit

Methods:

1- Full history was obtained including name, age, onset, symptoms, medical history, drug history, any extra-or lesions and any previous treatments for LP.

2- Patients were informed about the nature and objectives of the study, read, approved and signed a written informed-consent form.

3- Patients with EOLP were evaluated according to Hospital Anxiety and Depression Questionnaire (HADS) (Zigmond 1983). accordingly, the selected thirty patients will be divided into three groups as follow:

- **Group A:** 10 patients with EOLP with (HADS) scores of ≥11 considered abnormal or high stress value.
- **Group B:** 10 patients with EOLP with (HADS) scores of 8-10, considered as borderline or moderate stress value.
- **Group C:** 10 patients with EOLP with (HADS) scores of 0-7 considered as normal or low stress value.

4- Clinical examination:

**Lesion scores:**

Lesions were examined clinically according to the criteria of Thongprasom et al. 2003, where:

- Score 0: No lesion/normal mucosa
- Score 1: Mild white striae only
- Score 2: White striae with erythematous area < 1cm²
- Score 3: White striae with erythematous area ≥1cm²
- Score 4: White striae with erosive area < 1cm²
- Score 5: White striae with erosive area ≥1cm²

A ruler and a sterile graduated (figures 1&2) periodontal probe will be used to measure the size of the most sever erythematous area in mm

Visual Analogue Scale (Huskisson 1976).

The severity of pain and pain sensation will be evaluated according to following scales

- Scale 0: no pain: VAS=0
- Scale 1: mild pain: 0<VAS≤3.5
- Scale 2: moderate pain: 3.5<VAS≤7
- Scale 3: severe pain: 7<VAS≤10 (Thongprasom 1992)

4- Sample collection:

Venous blood samples (5ml) were collected from the antecubital fossa from all subjects. The whole blood samples collected were transferred to tubes, without using anticoagulants, and sent to the laboratory of the Department of Clinical Pathology, Faculty of Medicine, Alexandria University.

Blood samples were left for 30 minutes to clot, and then the blood was centrifuged at 2,000 rpm for 15 minutes. The yellow serum layer was pipetted off without disturbing the white buffy layer. Serum was then stored in -80°C till analyzed.

**Determination of IL-8 and TNF-α assay:**

Concentrations of serum TNF-α and IL-8 will be determined using the Human TNF-α ELISA Kit and
Human IL-8 ELISA Kit respectively (Hotamisligil 1998). The assay will be performed according to the manufacturer’s instructions.

Results:
The results of the present study showed a statistically significant increase in the mean value of stress, anxiety and depression between all the studied groups (P<0.001) table I. According to the VAS scores, results showed a statistically significant increase in VAS scores in relation to group A(high stress values group) when compared to group B(moderate stress values group) and group C (as a normal stress values group) respectively and statistically significant increase in relation to group B when compared to group C (P<0.004, P<0.001) table II.

According to the lesion size, there was a statistically significant increase in the lesion size in group A when compared to both group B and C, and in group B when compared to group C.( P1<0.018, P<0.001) table III.

According to the serum level of pro-inflammatory cytokines IL-8 and TNF-α, there was a statistically significant increase in both group A and group B when compared to group C and in group B when compared to group C. (P<0.001) Table IV and V.

Discussion:
Lichen planus is a common multifactorial disease. Immunological mechanisms are fundamental in the initiation and perpetuation of LP (Scully 2008, Zhou 2001). Although the etiology and pathogenesis of OLP are not fully understood, oral lichen planus has been associated with multiple disease processes and agents, such as viral, bacterial infections, autoimmune diseases, medications and vaccinations (Rubaci 2012, Ertugrul 2013, Tavassol 2008). Lately, it is generally agreed that OLP is associated with various psychogenic factors, the most frequent psychogenic conditions which may lead to it are depression, anxiety and stress (Sreedhar 2004).

The present study results showed statistically significant increase in the clinical features represented by lesion size and VAS scores in relation to the high stress values group A when compared to group B and group C respectively.

![Fig. 1: lichen planus lesion size measurement.](image1)

![Fig. 2: sterile graduated periodontal probe and ruler used for lesion measurement.](image2)

Table I: The mean values of anxiety and depression scale of the three groups.

<table>
<thead>
<tr>
<th>HDA Scale</th>
<th>Group A (n=10)</th>
<th>Group B (n=10)</th>
<th>Group C (n=10)</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anxiety</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Min – Max</td>
<td>14.0-18.0</td>
<td>9.0-10.0</td>
<td>6.0-7.0</td>
<td>321.030*</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>16.20±1.32</td>
<td>9.50±0.53</td>
<td>6.80±0.42</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>16.0</td>
<td>9.50</td>
<td>7.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sig. bet. Groups</td>
<td>P1&lt;0.001*</td>
<td>p2&lt;0.001*</td>
<td>p3&lt;0.001*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Depression</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Min.- Max. Meas±SD</td>
<td>11.0-16.0</td>
<td>8.0-10.0</td>
<td>4.0-7.0</td>
<td>124.025*</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Median</td>
<td>13.0</td>
<td>8.0</td>
<td>5.50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sig. bet. Groups</td>
<td>P11&lt;0.001*</td>
<td>p2&lt;0.001*</td>
<td>p3&lt;0.001*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

F: F test (ANOVA)
P1: P value for Post Hoc Test (LSD) for comparing between Group A and Group B.
P2: P value for Post Hoc Test (LSD) for comparing between Group A and Group C.
P3: P value for Post Hoc Test (LSD) for comparing between Group B and Group C.
*: Statistically significant at p ≤ 0.05
**: Sig. bet. Grps: significant between groups.
The is in line with several investigators who found a positive correlation between stress scores, and clinical features and manifestations of OLP giving an evidence that stress could aggravate the signs and symptoms of EOLP as visual analogue scale (VAS) and lesions size scoring (Shetty 2010, Girardi 2011). Additionally, Evidences showed that stressful life events and psychological agents could

**Table II:** The mean pain scores in the three groups.

<table>
<thead>
<tr>
<th>VAS</th>
<th>Group A (n=10)</th>
<th>Group B (n=10)</th>
<th>Group C (n=10)</th>
<th>Test of significance</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>VAS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. pain(0)</td>
<td>0</td>
<td>0.0</td>
<td>0</td>
<td>0.0</td>
<td>0</td>
</tr>
<tr>
<td>Mild(1)</td>
<td>0</td>
<td>0.0</td>
<td>0</td>
<td>0.0</td>
<td>6</td>
</tr>
<tr>
<td>Moderate (2)</td>
<td>0</td>
<td>0.0</td>
<td>6</td>
<td>0.0</td>
<td>4</td>
</tr>
<tr>
<td>Severe (3)</td>
<td>10</td>
<td>100.0</td>
<td>4</td>
<td>40.0</td>
<td>0</td>
</tr>
</tbody>
</table>

Sig. between Groups

**Chi square Monte carlo test sig. between groups.
KW $\chi^2$: Chi square for Kruskal Wallis test, sig. between groups using Mann Whitney test.
P1: P value for comparing between Group A and Group B.
P2: P value for comparing between Group A and Group C.
P3: P value for comparing between Group B and Group C.
*: Statistically significant $p \leq 0.05$
**: Sig. bet. Grps: significant between groups.

**Table III:** The mean lesion size score of the three groups.

<table>
<thead>
<tr>
<th>Lesion Size (cm²)</th>
<th>Group A (n=10)</th>
<th>Group B (n=10)</th>
<th>Group C (n=10)</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Min.- Max.</td>
<td>4.0-5.0</td>
<td>3.0-4.0</td>
<td>2.0-4.0</td>
<td>29.400*</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Mean±SD</td>
<td>4.70±0.48</td>
<td>4.0±0.67</td>
<td>2.60±0.70</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>5.0</td>
<td>4.0</td>
<td>2.50</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Sig. between Groups

F: T test (ANOVA)
p1: P value for post Hoc Test (LSD) for comparing between Group A and Group B.
p2: P value for post Hoc Test (LSD) for comparing between Group A and Group C.
p3: P value for post Hoc Test (LSD) for comparing between Group B and Group C.
*: Statistically significant $p \leq 0.05$
**: Sig. bet. Grps: significant between groups.

**Table IV:** Comparison between three studied groups according to Interleukin-8.

<table>
<thead>
<tr>
<th>Interleukin-8</th>
<th>High stress (n = 10)</th>
<th>Moderate stress (n = 10)</th>
<th>Mild stress (n = 10)</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Min. – Max.</td>
<td>43.20 – 75.50</td>
<td>20.40 – 42.40</td>
<td>2.90 – 13.10</td>
<td>95.185*</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>56.17 ± 10.92</td>
<td>31.60 ± 7.18</td>
<td>7.61 ± 3.88</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>52.10</td>
<td>31.10</td>
<td>6.65</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Sig. bet. Groups

F: T test (ANOVA)
p1: P value for Post Hoc Test (LSD) for comparing between High stress and Moderate stress
p2: P value for Post Hoc Test (LSD) for comparing between High stress and Mild stress
p3: P value for Post Hoc Test (LSD) for comparing between Moderate stress and Mild stress
*: Statistically significant at $p \leq 0.05$

**Table V:** Comparison between three studied groups according to TNF- $\alpha$.

<table>
<thead>
<tr>
<th>TNF- $\alpha$</th>
<th>High stress (n = 10)</th>
<th>Moderate stress (n = 10)</th>
<th>Mild stress (n = 10)</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Min. – Max.</td>
<td>19.0 – 30.20</td>
<td>9.70 – 17.0</td>
<td>1.90 – 9.0</td>
<td>133.625*</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>25.95 ± 3.74</td>
<td>12.54 ± 2.34</td>
<td>4.61 ± 2.58</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>26.54</td>
<td>12.65</td>
<td>3.95</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Sig. bet. groups

F: T test (ANOVA)
p1: P value for Post Hoc Test (LSD) for comparing between High stress and Moderate stress
p2: P value for Post Hoc Test (LSD) for comparing between High stress and Mild stress
p3: P value for Post Hoc Test (LSD) for comparing between Moderate stress and Mild stress
*: Statistically significant at $p \leq 0.05$
play a role in development and exacerbation of skin disease (1995)demonstrated that LP develops in relation to stress, emotional events and stressful life events that could exacerbate both skin and oral lesions with more severe symptoms and eruption of new lesion (Girardi 2011, Lami 2005).

The results of the present study showed also a statistically significant increase in the serum level of pro-inflammatory mediators IL-8 and TNF-α, in relation to high stress values group A and moderate stress values group B when compared to normal stress values group C.

These results are in accordance with Chiappelli et al.,1992,1994 in their study where they found a significant positive correlation between the psychological status and the ratios of lymphocytes in patients with LP. This finding is parallel with the associations between stress and immune response observed in OLP and provides support that OLP also involves psycho-physiological and immune changes that can be of significance for its onset and course.

The results of present study could be explained by previous studies that found a close correlation between central nervous system (CNS) and immune system. The immune system cells are found to express some receptors for many molecules such as neurotransmitters, neuropeptides and steroid hormones, that are modulated by nervous system, thus they could play an important role in health maintenance or disease development. In OLP the stressful situation may cause the HPA-axis to release corticosteroids; simultaneously there are psychoneuro-immunologic interactions that ultimately stimulate lymphocytes to release various cytokines important in OLP development ( Lundqvist 2006 , Valter 2013).

Neuro-endocrine hormones triggered during stress may lead to immune dysregulation or altered or amplified cytokine production, resulting in autoimmune diseases (Miller 1995).

Corticosteroids used in management of immune mediated diseases is mainly due to the effects of cortisol on immune system inhibiting the inflammatory response through suppressing synthesis and decreases the release of a number of inflammatory mediators and they decrease leukocyte emigration into inflamed sites ,also decreases differentiation and proliferation of local mast cells, while suppress immune response by decreasing the number of circulating T4 lymphocytes and by decreasing the production of interleukins (IL) and & - interferon that are critical mediators of immune response. Although cortisol also decreases recruitment and activation of B lymphocytes, its main effects are exhibited on cell-mediated immunity (Herold MJ 2006, Holsboer 1997, Miller 1995).

Moreover , a randomized clinical trial studied the effect of using psychological drug therapy for 6 months in OLP patients comparing them with control group and at end of study ,they found significant decrease in the size of the lesions. Hence the authors recommended the combination of psychiatric drug therapy and routine treatment of OLP (Delavaian et al. 2010) . Based on these results several investigators recommended the use of psychotherapy in adjunct to OLP treatment for reducing pain and the lesion size (Schiavone 2012, Mollaoglu 2000, Seoane 2004).

The effect of stress on immune system is mediated by a complex network of signals that function in a bidirectional manner in the nervous, endocrine, and immune systems. The mediators of these interactions are mainly neurotransmitters, neuropeptides, hormones, and cytokines. Neuroendocrine hormones triggered during stress may lead to immune dysregulation or altered or amplified cytokine production, resulting in autoimmune diseases (Felten 1994).

Conclusions:

Increased stress may be directly related to increased immune system activity causing increase in serum levels of pro-inflammatory cytokines as TNF-α and IL-8 levels which intern could be related to increased severity and exacerbation of OLP lesions.

Recommendations:

1-Further researches should be directed at assessing the psychoimmune interactions in the pathogenesis of OLP.

2-The benefit of combined therapy of psychotherapy along with corticosteroid for management of OLP should be investigated.

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