SALIVARY CORTISOL AND DEHYDROEPIANDROSTERONE (DHEA) LEVELS, PSYCHOLOGICAL FACTORS IN PATIENTS WITH ORAL LICHEN PLANUS

Oral lichen planus: salivary cortisol and DHEA

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ABSTRACT

Objective: The aim of this study was to determine the salivary levels of dehydroepiandrosterone (DHEA) and cortisol and scores of depression, anxiety and stress in patients with oral lichen planus (OLP).

Study Design: Thirty-one patients with a diagnosis of OLP were selected; they were matched by sex and age with 31 control patients. Symptoms of depression, anxiety and stress were investigated by the instruments Beck Depression Inventory, Beck Anxiety Inventory and Lipp’s Inventory of Stress Symptoms for Adults, respectively. Saliva was collected in the morning and at night for the determination of DHEA and cortisol levels by radioimmunoassay.

Results: There was no significant difference between the groups with respect to depression ($P=0.832$), anxiety ($P=0.061$) or stress ($P=0.611$), or with respect to morning and night salivary levels of DHEA ($P=0.888$, $P=0.297$) and cortisol ($P=0.443$, $P=0.983$).

Conclusions: The results suggest an association of OLP with anxiety. However, DHEA and cortisol levels did not differ between groups, which does not support any neuroendocrine aetiology for OLP.

INTRODUCTION

Oral lichen planus (OLP) is an immunopathologic disease whose prevalence in the population varies from 0.5 to 3%.\(^1\) There is evidence suggesting that the immunologic process begins with an internal or external antigen which alters the basal epithelial cells, making them susceptible to a cell immune response.\(^2,3\) Psychological disturbances, such as depression, anxiety and stress, have been investigated in the etiopathogenesis of OLP, since patients with the disease report a more frequent development or exacerbation of lesions during periods of greater emotional tension.\(^4-8\) Stress, as well as other psychological alterations, modifies and promotes dysregulation of immune functions with alteration of the balance of Th1/Th2 cytokines and increased Th2 response, which is associated with the development of autoimmune diseases.\(^9-12\) Psychoneuroimmunological research demonstrates clinically relevant interrelations between psychological stressors and 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.\(^13,14\) Neuroendocrine hormones triggered during stress may lead to immune dysregulation or altered or amplified cytokine production, resulting in autoimmune diseases.\(^15\)

In stressful situations, there is an activation of the HPA (hypothalamus-pituitary-adrenal) axis, with release of cortisol, a hormone that shows a complex action on the metabolism of carbohydrates, proteins and lipids, besides acting on inflammatory and immunologic responses.\(^16\) High concentrations of this hormone have been found in individuals with depression,\(^17\) periodontal disease\(^18\), burning mouth syndrome\(^19\) and recurrent aphthous ulceration.\(^20\)
DHEA is a corticosteroid secreted by the adrenal gland (in response to the activation of the HPA axis) and also in the gonads and central nervous system\textsuperscript{21,22} Although its mechanisms of action in humans is not completely understood, low concentrations of this corticosteroid are found in patients with particular immunologic diseases such as rheumatoid arthritis and systemic lupus erythematosus (SLE),\textsuperscript{23,24} and in individuals with depression,\textsuperscript{25,26} panic syndrome, phobias\textsuperscript{27} and burning mouth syndrome.\textsuperscript{28} DHEA regulates the production of cytokines, as it increases the production of IL-2 by Th1 cells and decreases the secretion of IL-6 and IL-10 by Th2 cells. A fall in DHEA levels contributes to the dysregulation of the balance of cytokines, which can be involved in the pathogenesis of various autoimmune diseases.\textsuperscript{29,30}

As OLP is an autoimmune disease and since there are reports of its association with psychological variables, we examined the salivary levels of cortisol and DHEA and psychological factors (stress, anxiety and depression) with the aim of identifying risk factors for this disease.

METHODS

This study was conducted after its evaluation and approval by the Institutional Committee of Ethics in Research (protocol 07/03931). The sample consisted of 62 patients over 30 years old, who were seen at Oral Medicine Service of São Lucas Hospital, Pontifical Catholic University of Rio Grande do Sul. After the explanation of the objectives and procedures of the study, the patients signed an informed consent form. They were matched by sex and age and divided as follows:

- OLP group: 31 patients with OLP with histopathologic confirmation.
• Control group: 31 patients without lesions in the oral mucosa.

Excluded from the study were patients who had lesions in the oral mucosa besides LP, such as burning mouth syndrome, or a history of malignant neoplasias, or who had undergone chemotherapy or radiotherapy, or who had autoimmune diseases, such as lupus erythematosus, rheumatoid arthritis or Sjögren syndrome. Blood testing was requested of all the subjects, and those individuals with alterations in their hemogram or in serum concentrations of glucose, iron, folic acid and vitamin B12 were excluded. Also excluded were patients who used antidepressives, anxiolytics, anabolic steroids, oral contraceptives, corticosteroids or immunosuppressant drugs in the 30 days prior to the examination, and patients who were on hormone replacement therapy for menopause. Patients with potential lichenoid lesions due to allergic reactions were excluded from the study on the basis of the interview.

The diagnosis of OLP was established by a Clinic and an Oral Pathologist on the basis of typical clinical features and histopathological examination of the mucous membrane, based on criteria established by Pindborg et al. and Van der Meij and Van der Wall.

The group with oral lichen planus included those not receiving any form of therapy for at least thirty days; therefore, patients with painful symptoms were recruited at their first appointment.

Investigation of symptoms of depression, anxiety and stress

The presence and intensity of depression and anxiety were determined using the Beck Depression Inventory (BDI) and the Beck Anxiety Inventory (BAI), respectively.
Lipp’s Inventory of Stress Symptoms for Adults (LISS)\textsuperscript{35} was employed to evaluate the presence of stress, the predominant type and phase of existing symptom.

**Salivary DHEA and cortisol analysis**

The saliva samples were collected between 8:00 am and 10:00 am (before breakfast) and between 7:00 pm and 9:00 pm (before dinner). The patients were instructed not to brush their teeth or ingest or place any substance in their mouth for one hour before saliva collection, not to apply medication or cosmetics on their lips, to remain seated with eyes open and their head lowered, and to deposit all the saliva that had accumulated in their mouth into a flask, for 5 min. The samples were stored at -80°C until the time of analysis of the hormones which ranged from two to five months.

After thawing, the samples were transferred to polyethylene tubes and centrifuged for 5 min at 1500 rpm in order to remove precipitated salivary proteins and obtain clear saliva in the supernatant. DHEA and cortisol levels were determined in duplicate using a radioimmunoassay kit specific for each hormone. Salivary levels of DHEA were measured utilizing the DSL\textsuperscript{-8900 DHEA RIA kit} (Diagnostic Systems Laboratories Inc., Webster, Texas, USA) with a specific monoclonal antibody for this hormone following the manufacturer’s instructions. The Coat-A-Count\textsuperscript{®} Cortisol kit (Siemens Medical Solutions Diagnostics, Los Angeles, California, USA) was utilized for determination of the salivary levels of cortisol.

The radioactivity in the tubes was measured in a Gamma C12\textsuperscript{®} counter (EURO-DPC). Both measurements were expressed in counts per minute (cpm). The specific software of the equipment calculated the DHEA and cortisol levels in each saliva sample.
Data analysis

The data were analyzed using descriptive statistics. The Mann-Whitney test was applied for comparison of the groups in relation to salivary levels of DHEA and cortisol, and with respect to symptoms of depression, anxiety and stress. Spearman’s correlation coefficient was utilized to determine the correlation between DHEA and cortisol levels and scores for depression and anxiety. The Mann-Whitney test was utilized to evaluate the association of DHEA and cortisol levels with the presence of stress. The level of significance used was 5%, where \( P \leq 0.05 \) was considered significant. Analyses were carried out using the software *Statistical Package for the Social Sciences* (SPSS), version 10.0.

RESULTS

Demographic characteristics

Mean age of OLP group was 53.8 (±10.46) years and control group was 55.5 (±11.77). In both groups, 87% of patients were females and 13% were males. Fifty-five percent (n=17) of the patients showed a combination of the reticular and atrophic-erosive forms of the disease; 25.8% (n=8) exhibited reticular lesions; 9.6% (n=3) presented with atrophic-erosive lesions; and 9.6% (n=3) displayed a combination of the papular, reticular and plaque forms. Lesions at various sites were observed in 87% (n=27) of the patients, and the most affected sites were the buccal mucosa and the sides of the tongue.

Seventeen patients with OLP (55%) complained of painful symptoms. The time of development of the lesions ranged from five months to 16 years, with a median of 24
months (13.0 - 78.0 months). This information was provided by patients during the interview.

**Symptoms of depression, anxiety and stress**

The BDI scores ranged from 0 to 39 points, with a median of 10.0 (5.00 - 14.00) in the OLP group, and from 2 to 21 points, with a median of 9.0 (6.00 - 13.00) in the control individuals. The BAI scores ranged from 0 to 41 points, with a median of 9.0 (5.00 - 14.00) in the OLP group and from 0 to 27 points, with a median of 6.0 (4.00 - 9.00) in the control patients. No significant difference was observed between the groups with respect to symptoms of depression (Mann-Whitney test, \( P=0.832 \)). A borderline \( P \) value (Mann-Whitney test, \( P=0.061 \)) was found for anxiety symptoms when the LPO group was compared with the control group (Table 1).

The patients with OLP and controls were categorized with respect to the presence or absence of stress according to ISSL scores. Eighteen patients with OLP and 15 control individuals showed stress symptoms, without any statistically significant difference between the groups (\( \chi^2, P=0.611 \)). Besides, the patients were categorized according to the phases of stress (alarm, resistance, quasi-exhaustion and exhaustion) and the predominant type of symptoms (physical, psychological or both). There was no significant difference between the groups with respect to these variables (data not shown).

**Salivary levels of DHEA and cortisol**

There was no significant difference between the groups with respect to morning levels (Mann-Whitney test, \( P=0.888 \)) or night levels (Mann-Whitney test, \( P=0.297 \)) of
salivary DHEA. The concentrations of cortisol also did not differ between the OLP and control groups with regard to the morning (Mann-Whitney test, $P=0.443$) or night (Mann-Whitney test, $P=0.983$) salivary levels (Table 2).

There was no significant correlation between DHEA and cortisol levels and scores for depression and anxiety (data not shown). The DHEA and cortisol levels also did not exhibit a significant association with stress in the OLP or control groups (data not shown).

DISCUSSION

Stress causes an elevation in the concentrations of cortisol and a reduction in those of DHEA, and these alterations are associated with an imbalance of Th1/Th2 cytokines, which can predispose to the development of autoimmune diseases.\textsuperscript{9,15,29,30} Therefore, the present study examined the salivary levels of both corticosteroids and their association with stress, anxiety and depression in patients with OLP.

There was no significant difference with respect to cortisol levels between patients with OLP and controls. This corticosteroid is considered a biological marker of stress and anxiety. Its salivary concentrations and association with psychological alterations have been investigated in patients with OLP, but with conflicting results. Corroborating the finding of the present study, Rödström et al.\textsuperscript{7} did not find higher levels of cortisol in patients with OLP. Shah et al.\textsuperscript{36} observed an association of OLP with elevated levels of salivary cortisol, anxiety, depression and stress; however, they did not show a comparison of these variables between patients with the disease and controls. Salivary concentrations of cortisol and higher anxiety scores in patients with OLP were found by Koray et al.\textsuperscript{37}, but the analyses were carried out before the patients were biopsied, which may have contributed to an elevation of the levels of the variables studied.
Ivanovski et al. found higher levels of cortisol in patients with erosive OLP, but there was no difference in the values for the patients with reticular OLP compared to controls. In the present study, the patients with OLP were not distributed into distinct groups according to clinical form of the lesions. This difference between the two studies may have contributed to the contrasting results, suggesting that the psychological profile of the patient can play a more important role in the erosive-atrophic forms of the disease.

DHEA appears to play an important role in the physiopathology of depression, regulation of humor and sensation of well-being, besides being an important modulator of the immune response. The fall in DHEA levels contributes to the dysregulation of cytokine balance, which can be involved in the pathogenesis of autoimmune diseases. Since lichen planus is an immunologic disease in which there is a Th1/Th2 imbalance, with a predominance of the Th2 response, the present study examined the hypothesis that the salivary concentrations of DHEA could be lower in patients with this oral disease. However, the results did not provide evidence of a significant difference between the patients with OLP and the control individuals.

No significant differences were observed with respect to scores and levels of depression, anxiety and stress between patients with OLP and controls, although the scores of the case patients were higher. In the evaluation of anxiety, the difference between the groups was very close to being significant ($P=0.06$), demonstrating a tendency of an association of this variable with OLP. In a larger sample of patients, this association between OLP and anxiety could have been better demonstrated. However, due to the exclusion criteria, 31 patients with the disease were selected. There remains conflicting evidence for an association between psychological factors and the development and persistence of OLP. Rojo-Moreno et al., Vallejo and Zarabozo, Chaudhary, Ivanovski
et al. and Manolache et al. found a positive association between OLP and the presence of depression, anxiety and stress. However, the studies of Allen et al., Macleod, McCartan and Rödström et al. do not support such hypothesis. Psychometric inventories have been administered to patients with OLP to evaluate their psychological profile, for the purpose of determining the psychogenic causality of this disease. The utilization of different inventories, as well as their subjectivity and the lack of methodological standardization of studies can be pointed out as possible factors responsible for the existing controversy in relation to this theme. These factors make it impossible to arrive at a conclusion in relation to the role of psychological disturbances in the etiopathogenesis of OLP. In this study, the inventories BDI, BAI and LISS were utilized for the determination of the intensity of the symptoms of depression, anxiety and stress, respectively. These three instruments show high reliability and elevated internal consistency and can be applied in psychiatric patients as well as in the general population. As these are self-administered questionnaires and of rapid application, they are widely utilized in the investigation of depression, anxiety and stress. Patients on therapy with anxiolytics and/or antidepressives were excluded, because these drugs could influence the salivary levels of cortisol and DHEA. Therefore, it is possible that the exclusion of these patients, presumably with a depressive, anxious and/or stressed profile, influenced the scores of the instruments in the group with OLP.

The etiopathogenesis of OLP is complex and presumably dependent on the interaction of genetic and environmental factors, as well as lifestyle of the individuals and deficiencies in strategies for coping with stress. In the present study, the hormones analyzed did not exhibit distinct levels between patients with OLP and controls. Also, no significant association was found between OLP and the symptoms of depression and stress, but there was a tendency for an association with anxiety, suggesting that this psychological factor
may be related to OLP. This is the first study to investigate salivary DHEA levels in patients with OLP, as this hormone appears to be related to depression and to the modulation of the immune response. However, the patients with OLP did not show altered levels of this hormone or of cortisol, which does not support any neuroendocrine aetiology for this disease.
REFERENCES


43. Vallejo MJ, Zarabozo GH. Anxiety as an etiologic factor in oral lichen planus (OLP).


**LEGENDS FOR TABLES**

**Table 1** – Scores of depression and anxiety in patients with OLP and controls, median (25th-75th percentiles).

**Table 2** – Morning and night salivary levels of DHEA and cortisol (nmol/L) of patients with OLP and controls, median (25th-75th percentiles).
Table 1 – Scores of depression and anxiety in patients with OLP and controls, median (25th–75th percentiles).

<table>
<thead>
<tr>
<th>Variable</th>
<th>OLP group</th>
<th>Control group</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scores of depression</td>
<td>10.0 (5.0–14.0)</td>
<td>9.0 (6.0–13.0)</td>
<td>0.832</td>
</tr>
<tr>
<td>Scores of anxiety</td>
<td>9.0 (5.0–14.0)</td>
<td>6.0 (4.0–9.0)</td>
<td>0.061</td>
</tr>
</tbody>
</table>

Mann–Whitney test.

Table 2 – Morning and night salivary levels of DHEA and cortisol (nmol/L) of patients with OLP and controls, median (25th–75th percentiles).

<table>
<thead>
<tr>
<th>Variables</th>
<th>OLP group</th>
<th>Control group</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>DHEA M</td>
<td>0.75 (0.46–0.99)</td>
<td>0.66 (0.51–1.22)</td>
<td>0.888</td>
</tr>
<tr>
<td>DHEA N</td>
<td>0.41 (0.32–0.65)</td>
<td>0.50 (0.34–0.76)</td>
<td>0.297</td>
</tr>
<tr>
<td>Cortisol M</td>
<td>13.50 (10.50–21–30)</td>
<td>14.10 (8.60–18–30)</td>
<td>0.443</td>
</tr>
<tr>
<td>Cortisol N</td>
<td>3.80 (2.80–5.10)</td>
<td>3.90 (3.00–4.40)</td>
<td>0.983</td>
</tr>
</tbody>
</table>

Mann–Whitney test, M = morning; N = night.