Estimation of Salivary Cortisol Level in Oral Lichen Planus and Oral Leukoplakia Patients

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ABSTRACT

Introduction: - Cortisol, also known as stress hormone has been used as an indicator for the evaluation of stress.
Aims: - The aims of the present study was to determine salivary cortisol levels in patients with oral lichen planus, oral leukoplakia and normal oral mucosa and its correlation with DAS (Depression, anxiety and stress) scale.
Materials and method: - A cross-sectional study was carried out on 10 patients each of oral lichen planus, oral leukoplakia and normal oral mucosa. DAS scale was applied to each patients and morning unstimulated saliva was collected. From the sample, salivary cortisol levels were analysed using enzyme-linked immunosorbent assay method.
Results: - In the present study, depression, anxiety and stress along with salivary cortisol levels were higher in oral lichen planus as compared to oral leukoplakia and normal oral mucosa patients which were in the normal range. A positive correlation was found between salivary cortisol levels in oral lichen planus and psychological factors.
Conclusion: - DAS scale plays an important role in estimating the psychological state of the patient which can be related with the salivary cortisol level. Thus, depression, anxiety and stress play an important role in the pathogenesis of oral lichen planus.

Key words: Stress, Saliva, Lichen planus, Leukoplakia, Depression, Anxiety

INTRODUCTION

Cortisol, also known as stress hormone was first discovered by Edward Kendall, Tadeus Reichstein and Philip Hench in the year 1930s. [¹,²] In the morning, cortisol level in the human body varies from 0.2 to 1.41 μg/dl but in the afternoon, it ranges from 0.04 to 0.41 μg/dl. [³] It is the main glucocorticoid secreted in humans by adrenal gland and has been used as a predictor in stress related studies. The hormone cortisol has a major role on physiological condition like cell metabolism, vascular responsiveness, immunoregulation, cognition and behaviour but also has a strong influence on pathological conditions like inflammatory autoimmune disorders. [⁴,⁵]

The term stress was defined by a Canadian physician named Hans Selye in 1930 as “non-specific response of the body to any demand for change.” Stress is considered as an etiological or predisposing factors in various diseases like lichen planus, oral cancer, aphthous stomatitis etc. [⁶]

The two parameters of stress measurements are physiological and
psychological. Physiological measures are biological response to situation along with biochemical measures comprising of blood pressure, heart rate and increased secretion of stress hormones like catecholamines, cortisol and epinephrine. Psychological measures evaluate the levels of stress through self-report and scales based on life events or daily hassles for stress evaluation. [6]

Saliva can be used as a tool for the evaluation of free cortisol levels along with plasma. Salivary cortisol has various advantages in addition to plasma cortisol as salivary cortisol is non-invasive, constant at room temperature for a week, easy collection of sample and bulk of the cortisol which occurs in free form determines the free cortisol level or biologically active cortisol in human serum. However in plasma, the majority of the cortisol is bound to protein thus the analysis of free cortisol is expensive, difficult and laborious along with sampling of blood might interfere with the experimental stress procedures. [7,8] During the period of stress, an activation of the HPA (hypothalamus-pituitary-adrenal) axis occur which result in the release of a hormone i.e. cortisol that indicates a complex effect on the metabolism of carbohydrates, proteins and lipids, as well as acting on inflammatory and immunological responses. [5]

Literally; the word leukoplakia means a ‘white patch’ which was derived from Greek word leukos- white, plakia- patch. In 1978, World Health Organization (WHO) group defined oral leukoplakia as “A white patch or plaque that cannot be characterized, clinically or pathologically as any other disease”. [9] Warnakulasuriya et al in 2007 defined leukoplakia as “A plaque of questionable risk having excluded (other) known diseases or disorders that carry no increased risk for cancer”. [10] The various etiological factors that predispose to leukoplakia are tobacco smoking and chewing, alcohol, candida albicans infections, HPV infections, persistent trauma etc. [11] It occurs mostly on the buccal mucosa in the vicinity of retromolar area followed by lips, floor of the mouth, ventral or lateral borders of the tongue etc. [12]

Lichen Planus was derived from the Greek leikhen, meaning "a tree moss” and the Latin planus, meaning “flat”. Oral lichen planus is a common mucocutaneous disease. It was first described by British physician Erasmus Wilson in 1869 and it is thought to affect 0.5-1 per cent of the world’s population. The condition can affect either the skin or mucosa or both. It is usually present as a symmetrical, bilateral white striations, papules or plaques on the buccal mucosa, tongue and gingiva. Erythema, erosions and blisters may or may not be present. [13,14] The exact etiology of the lichen planus is not clearly known but various predisposing factors like genetic background, dental materials, drugs, infectious agents, autoimmunity, immunodeficiency, food allergies, stress, habits, trauma etc. [15]

The aim of the present study was to estimate the salivary cortisol levels in patients with oral lichen planus, oral leukoplakia and normal oral mucosa (healthy patients) along with the determination of depression, anxiety and stress in these patients using DAS scale.

MATERIALS AND METHODS
A cross-sectional study was conducted on 30 subjects who were divided into three groups each of 10 subjects.
Group A- 10 subjects of oral lichen planus
Group B- 10 subjects of oral leukoplakia
Group C- 10 subjects of normal oral mucosa

Patients with history of systemic diseases (diabetes mellitus, cardiovascular diseases, renal dysfunction etc.), autoimmune diseases, patients on medication which alter the salivary cortisol level i.e. corticosteroid were excluded from the study. The histological confirmation was done on all the cases. The study was approved by ethical committee and an informed consent was taken from study subjects.
Saliva samples were collected in the morning between 9 am and 10 am before any meal from the subjects so as to prevent diurnal variation. In each subject, 5ml of saliva was collected in a plastic container and stored at -20°C. After collection, the saliva was subjected to centrifugation at 3000 rpm for 15 minutes and 2ml of supernatant clear fluid was used for the detection of cortisol using ELISA with a commercial kit (DetectX), following the manufacturer’s instructions.

Psychological assessment was done with the help of DAS (Depression, Anxiety, Stress) scale. The questionnaire comprised of 21 questions with each score ranging from 0 to 3, 0 as “completely being not applicable to me” to 3 being “most applicable to me”. The final score of the test was given as normal, mild, moderate, severe and extremely severe as given below:

- Normal (0-78)
- Mild (78-87)
- Moderate (87-95)
- Severe (95-98)
- Extremely severe (98-100)

The data was analysed by using statistical software (SPSS version 19.0). Mean and standard deviation were calculated for each individual group. They were compared by using chi-square test and Post-hoc test. A probability value (p) of ≤0.05 was considered to be statistically significant.

RESULTS

In the present study, majority of the patients were males as shown in Table 1. In Group A, B and C, the mean age of the patients were 39.51±10.73, 44.52±9.34 and 23.75±4.09 respectively (Table 2).

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of cases</th>
<th>Gender</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>10 (100%)</td>
<td></td>
<td>7 (70%)</td>
<td>3 (30%)</td>
</tr>
<tr>
<td>Group B</td>
<td>10 (100%)</td>
<td></td>
<td>8 (80%)</td>
<td>2 (20%)</td>
</tr>
<tr>
<td>Group C</td>
<td>10 (100%)</td>
<td></td>
<td>6 (60%)</td>
<td>4 (40%)</td>
</tr>
</tbody>
</table>

Table 1: Gender wise distribution in oral lichen planus, oral leukoplakia, and normal oral mucosa

Results from DAS scale revealed that the mean depression score in patients with oral lichen planus, oral leukoplakia and normal oral mucosa were 12.44%, 6.98% and 5.45% respectively. The p value (<0.001) was found to be statistically significant. The mean depression score was higher in oral lichen planus patients than oral leukoplakia and normal oral mucosa (Table 3).

Using DAS scale, the mean anxiety score in oral lichen planus patients was 11.42%, in oral leukoplakia patients was 6.45% and in normal oral mucosa was 6.17%. The mean anxiety score was higher in patients with oral lichen planus as compared to oral leukoplakia and normal oral mucosa. A statistically significant (p<0.001) correlation was found in all the groups (Table 3).

The mean stress score by Das scale in patients with oral lichen planus, oral leukoplakia and normal oral mucosa were 20.08%, 8.04% and 7.92% respectively. The p value (<0.001) was found to be statistically significant. The mean stress score was higher in oral lichen planus patients than oral leukoplakia and normal oral mucosa (Table 3).

The mean cortisol level in oral lichen planus patients was 12.34±8.03ng/dl, in oral leukoplakia patients was 6.89±3.39ng/dl and in normal oral mucosa patients was 5.87±2.90ng/dl. The mean cortisol level in saliva was found to be higher in oral lichen planus as compared to oral leukoplakia and normal oral mucosa. The result was found to be statistically significant with the p value of <0.001 (Table 4).
DISCUSSION

Stress endangers the constant state of homeostasis with the help of internal and external stressors and measured by physiological and psychological parameters. Physiological parameters measure the increased secretion of stress hormones like cortisol, catecholamines and epinephrine. Cortisol also known as stress hormone which is responsible for fight or flight behaviour and acts as an indicator in stress evaluation studies.\[16\]

Level of cortisol can be measured in plasma, serum and urine. The serum cortisol method is an invasive procedure as it involves collection of blood and provides false positive results as compared to salivary cortisol level which is a reliable, non-invasive and easy procedures.\[5\]

In the present study, oral lichen planus patients revealed higher mean depression, anxiety and stress when evaluated by DAS scale as compared to oral leukoplakia and normal oral mucosa patients which was in accordance with the study done by Shah B et al,\[7\] Kalkur C et al,\[17\] Sonule S\[18\] determined higher anxiety, stress and depression levels in oral lichen planus patients evaluated by DAS scale.

Salivary cortisol levels was found to higher in oral lichen planus patients than oral leukoplakia and normal oral mucosa which was in agreement with the study done by Girardi C et al\[19\] and Rabiei M et al\[20\] who found higher depression, anxiety and stress along with higher salivary cortisol values than normal range.

In our study, a statistically significant correlation was found in the depression, anxiety and stress scores in oral lichen planus, oral leukoplakia and normal oral mucosa which were in contrast with the study done by Hirota SK et al\[21\] who observed negative correlation of oral lichen planus with depression, anxiety and stress scores.

A study was done by Nosratzehi T et al\[22\] showed higher anxiety score which was in agreement in our study but revealed cortisol level (3.2±1.9ng/ml) in the normal range in lichen planus which was in contrast with the present study.

In the present study, a hypothesis can be suggested that psychosomatic factors may play a major role in the predisposition of oral lichen planus.

The limitation of the present study was that the determination of salivary cortisol was observed only in the morning sample collection and for the complete observation of HPA axis, cortisol rhythms should be evaluated during 24 hours as it is essential to recognize the variabilities of cortisol levels over a day (i.e. analysis of circadian rhythms).

CONCLUSION

From this study, we can conclude that the DAS scale play an important role in evaluating the psychological state of the patient which can be associated with the
salivary cortisol level. Depression, anxiety and stress level along with salivary cortisol levels were higher in oral lichen planus as compared to oral leukoplakia and normal oral mucosa patients which were in the normal range. A positive correlation was found between salivary cortisol levels in oral lichen planus and psychological factors. In this study, a negative correlation was observed between psychological factors and salivary cortisol levels in oral leukoplakia and normal oral mucosa patients.

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