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Original Article

Dentistry Section

Association of Salivary Cortisol and Anxiety Levels in Lichen Planus Patients

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ABSTRACT

Background: Oral lichen planus (OLP) is a frequently encountered chronic inflammatory disease of oral mucosa and skin, where the patients often relate the onset and aggravation of oral symptoms to increased levels of stress. Cortisol, also called as "stress hormone" has been used as an indicator in various stress evaluation studies.

Aim: The aim of this cross-sectional study was to determine any association between anxiety and salivary cortisol levels in OLP patients.

Materials and Methods: A total of 20 OLP patients along with same number of age and sex matched healthy controls were included in the study. Saliva was collected from all the subjects between 9.00 to 9.15 am to avoid diurnal variations of cortisol levels. The saliva samples were analysed for cortisol levels by competitive enzyme-linked immunosorbent assay (ELISA) method. Anxiety levels of 40 patients were measured by using Hamilton's anxiety scale. Student's t-test was used to compare the anxiety and salivary cortisol levels between both groups.

Results: The mean salivary cortisol level of the OLP group showed highly significant difference ($p<0.001$) from the controls. The mean anxiety scores of the OLP group showed highly significant difference ($p<0.001$) from the controls. A positive correlation was found between anxiety and salivary cortisol levels in the OLP patients.

Conclusion: These findings suggest that anxiety play a vital role in the pathogenesis of OLP, thus besides traditional treatment, psychological support is also needed.

Keywords: ELISA, HPA (Hypothalamo adrenal)- axis, Stress

INTRODUCTION

Many systemic diseases manifest in the oral cavity. In some conditions oral cavity provides the first clue for the detection of underlying systemic disease. Dermatological disorders are one such class of diseases that have oral manifestations. Thus the dentist may be in a position to establish the diagnosis of a dermatologic disease before the cutaneous lesions become apparent. Lichen planus, is one of the examples of dermatologic diseases that have oral manifestations [1]. Lichen planus is a chronic inflammatory mucocutaneous disease of unknown etiology, occurs in approximately 0.02 – 4% of the adult population, affecting the skin and / or oral mucosa [2]. It is an obstinate disorder baffling not only the patients but also the practitioner. Lichen planus is not an infectious disease. The cause is unknown, but it is classified as an autoimmune disorder which may be precipitated or exacerbated by psychosocial stressors [3]. Many patients with painful OLP are frustrated and distressed as a consequence of their disease [4]. The waxing and waning nature of the lesions has been attributed to the perception of stress [5].

Lichen planus is to some extent a psychosomatic or somatopsychic disease or both. Emotional factors are important as evidenced by higher frequency of psychiatric symptoms, poor quality of life, higher level of anxiety and neuroendocrine and immune dysregulations [3]. In conditions involving pain, anxiety, fright or acute tissue damage, many metabolic and endocrine changes occur, and among these a rise in the levels of blood cortisol is one of the most important physiological effects [6]. Cortisol is the major glucocorticoid in humans and has a wide range of influences on metabolism, immune regulation, vascular responsiveness, cognition and behaviour. It also has an impact on numerous pathological conditions including inflammatory autoimmune disorders [7].

In serum, the majority of cortisol is bound to protein, and the analysis of free cortisol is difficult, laborious and expensive and sampling of blood may induce some stress in patients [8]. Salivary cortisol may actually provide a better measure than serum cortisol of the stress response as it more accurately measures the amount of unbound cortisol compared to serum measures [9]. Salivary samples have certain advantages over serum samples. Collecting saliva is a relatively stress free and non – invasive procedure, does not require trained personnel and change in environmental conditions like temperature, motion and growth of organisms does not alter concentration of cortisol in the saliva samples [10]. There are, however few disadvantages in the use of saliva. The analysis of steroids in saliva can present analytical problems since they are present at far lower levels than in circulation. Freeze/thaw cycles and centrifugation are often used to break up mucins [11].

Therefore, measurement of the salivary cortisol and anxiety which reflect response to stress seems a promising parameter in the investigation of OLP. Therefore, it can be a useful aid in not just understanding the pathogenesis of OLP but also determining the progression of these lesions. There have been few studies done in the area of stress as a predisposing factor in OLP lesions. Thus, there is a great need to understand the etiopathogenesis and progression of this lesion. The aim of the study was to determine the association between cortisol levels and anxiety in OLP.

MATERIALS AND METHODS

The study was conducted in the Department of Oral Medicine and Radiology, Kamineni Institute of Dental Sciences, India, after approval by institutional ethics committee. The study enrolled 20 OLP subjects diagnosed clinically and histological as study group and 20, age, sex and socio-economic status (SES) matched healthy subjects as control group after a written informed consent. The SES was determined using modified Kuppuswamy's SES scale [12]. None of the 40 patients had any systemic disease, including endocrine or metabolic disorders. No history of prior medications and management. All patients were non smokers.

Patients with clinical diagnosis of OLP were given an appointment at 9:00 AM. Biopsy was performed for histological confirmation after

	Study group OLP patients (n=20)	Control group (n=20)	p - value
Salivary cortisol	17.0 ± 3.82 ng/ml	5.34 ± 1.33 ng/ml	<0.001
Anxiety	27.2 ± 6.07	10.6 ± 3.18	<0.001

[Table/Fig-1]: Comparison of Salivary cortisol levels and anxiety scores (mean ± S.D.) of patients, *t- test

Variable	B	Wald	d.f.	p-value
Salivary cortisol	-1.23	6.77	1	0.00
Anxiety	-0.05	4.32	1	0.01

[Table/Fig-2]: Logistic regression analysis, in which the group membership (OLP or control) was dependent variable

collecting saliva samples and filling the anxiety questionnaire forms to avoid stress due to the invasive procedure. .

Saliva samples from both the study and control groups were collected between 9 to 9:15 am, before meal without stimulation by spitting method directly into a sterile glass tube. Patients were allowed to spit saliva until 5ml is collected. All participants were asked to wash their mouth properly before sample collection. The collected salivary samples were centrifuged for 15min at 3000 rpm and frozen at -20°c until shortly before assay. During assay the samples were thawed at 37°C.

Salivary cortisol was measured by competitive enzyme linked immunosorbent assay (ELISA) method, by using cortisol EIA (Diametra kit, Korea). The normal cortisol concentrations that were given as a guide line according to the kit are in the range of 3 – 10 ng / ml at morning time and 0.6 – 2.5 ng / ml at evening collected samples.

After saliva collection, the patients were subjected to psychological evaluation. Anxiety levels were measured by using "Hamilton's anxiety scale" (HAM-A) that provides the measures of overall anxiety, psychic anxiety (mental agitation and psychological distress) and somatic anxiety (physical complaints related to anxiety) [13]. This scale consists of 14 questions, among which seven address the psychic and the remaining seven addresses the somatic anxiety. The individuals were rated on a five point scale for each of the 14 items ranging from 0 (not present) – 4 (severe). The total anxiety score ranges from 0 – 56. The patients with a total score of 18 comes under mild anxiety group and patients having score between 19 to 25 as moderate anxiety group, and score 30 is considered as severe anxiety group.

T-test was used to compare the anxiety and salivary cortisol levels between patients with OLP and the control group. Pearson's correlation analysis was used to study the correlation among anxiety and salivary cortisol levels in patients with OLP. Logistic regression analysis was used to assess the variables related with OLP. Logistic regression analysis was used for calculation of each variable's independent contribution to dependent variable. Dependent variable must always be dichotomous, as group membership. OLP was a dependent variable, and salivary cortisol and anxiety levels were independent variables in this model.

RESULTS

The mean age and SD of 20 OLP patients and 20 control patients was 36.6 ±13.29 yrs. Results suggested that salivary cortisol and anxiety levels were significantly higher in OLP group as compared to control group as indicated in [Table/Fig-1]. There was a highly significant positive correlation (p<0.001) between salivary cortisol levels and anxiety.

A logistic regression model in which the OLP was taken as dependent variable and salivary cortisol and anxiety levels were taken as independent variables were performed. Salivary cortisol levels and anxiety scores were found significantly related with OLP. Group membership of each individual can be predicted correctly with 76.1 % probability in this model [Table/Fig-2].

The anxiety levels and cortisol levels were compared between males and females. It was found to be statistically not significant in our study with a p-value of 0.18.

DISCUSSION

OLP is a fairly common disease of adults and has a worldwide distribution [2]. Although, aetiology of OLP is unknown, cell -mediated immune system plays an important role in OLP pathogenesis. Various studies have shown that factors such as stress and psychological problems, especially depression and anxiety, have been mentioned as etiologic factors in lichen planus, but there is still controversy concerning the role of stress as a major or minor etiologic factor in the pathogenicity of lichen planus [14].

The assessment of cortisol in saliva has gained interest in studies for evaluating anxiety [9]. Various studies have been conducted to assess the relationship of anxiety and salivary cortisol levels in OLP; but with different inconsistent results. Psychological assessment of OLP patients were also done by many studies using various scales; which were either self reported or assessed by psychiatrist [1-3,6,8,15].

One study with different psychiatric tests such as "General Health Questionnaire", "Hamilton Anxiety Scale", "Melancholia scale, depression", Hamilton Depression Scale" demonstrated that OLP patients had higher depression and anxiety scores [16]. Another study showed 53% depression in OLP patients and 20% in control group by "Beck Depression Score". This study confirmed importance of depression assessment in skin diseases like lichen planus and psoriasis [17]. Hampf et al., [18] showed OLP lesions became worse during times of mental stress, but most patients did not feel any need for psychiatric treatment. Rojo-Moreno et al.,[19] in a controlled study on 100 patients using different psychometric tests found greater anxiety and depression in OLP patients than the controls. On the contrary, Allen et al.,[20] in a controlled study using the State Trait Anxiety Inventory (STAI), found no significant difference between the patients with lichen planus and control subjects. Chaudhary [15] showed significantly higher stress, anxiety, and depression levels in OLP patients than the general population, suggesting probable role of stressors in OLP. Our study used Hamilton's anxiety scale which measures both psychic and somatic anxiety; and showed that there is a statistically significant raise in anxiety levels in OLP patients as compared to controls.

The rate of salivary cortisol can be an indicator of higher level of stress. Salivary cortisol and its correlation to OLP have been evaluated. A study by Koray et al.,[6] showed the salivary cortisol and state and trait anxiety levels in OLP group were significantly higher than healthy group that concluded that oral lichen planus is closely related with stress. Shah et al., [2] also showed that salivary cortisol, anxiety, depression, and stress levels in OLP patients were higher than healthy group suggesting a positive correlation between psychiatric disorders and salivary cortisol levels in OLP patients. In contrast to these studies Rodstrom et al.,[8] conducted a study in OLP patients by measuring the amount of salivary cortisol levels for assessment of temporary stress and concluded that OLP patients did not have higher level of stress. Girardi et al.,[21] measured salivary cortisol and dehydroepiandrosterone (DHEA) levels in OLP patients. Although the results suggested an association of OLP with anxiety, DHEA and cortisol levels did not differ between different groups, which do not support any neuroendocrine aetiology for OLP. Our study in contrast to these studies has shown a significant raise in salivary cortisol and anxiety levels in OLP patients compared to healthy controls. It also showed a positive correlation between salivary cortisol levels and anxiety levels in OLP patients suggesting that stress may play a role in etiopathogenesis of OLP.

This present study includes a very limited number of study population, hence a study is required with larger group of study

population. Male and female patients were not equal in number in this study; hence a study is required with equal number of male and female population.

CONCLUSION

In conclusion, though there have been many studies conducted to evaluate the role of stress in etiopathogenesis of OLP, the results have been conflicting. The present study results matches with majority of the studies, showing a positive association between salivary cortisol and anxiety levels in OLP patients, suggesting psychiatric treatment along with traditional treatment can be effective in reducing the size of the lesions. With regards to the discussion above, it seems logical to claim that psychiatric evaluation and appropriate treatment of the patients along routine treatment of OLP lesions should be recommended.

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