Original Article

Relationship of salivary cortisol and anxiety in recurrent aphthous stomatitis

Lakshmi Kavitha Nadendla, Venkateswarlu Meduri, Geetha Paramkusam, Koteswara Rao Pachava

Departments of Oral Medicine and Radiology, Kamineni Institute of Dental Sciences, Sreepuram, Narketpally, Nalgonda District, Andhra Pradesh, India

ABSTRACT

Background and Objectives: Recurrent aphthous stomatitis (RAS) is one of the most frequently encountered oral mucosal disorders. Despite extensive amount of research, the etiology of RAS remains unclear. Psychological-emotional factors were considered as one of the major predisposing factors. The aim of the study was to assess the levels of anxiety and salivary cortisol levels in patients with RAS and also to determine the association and relationship of salivary cortisol levels to variations of stress. **Materials and Methods:** A total of 30 patients suffering with RAS, along with the same number of age and sex matched healthy controls were included in the study. Saliva was collected from all the subjects at 9.00 am to avoid diurnal variations of cortisol levels. Salivary cortisol levels were measured by competitive enzyme linked immunosorbent assay. Anxiety levels of both groups 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 RAS group showed a very highly significant difference ($P = 0.000$) from the controls. The mean anxiety scores of the RAS group showed a very highly significant difference ($P = 0.000$) from the controls. The values of Pearson correlation coefficient between anxiety and salivary cortisol was 0.980 and one with a P value of 0.000 showing that there is a highly positive correlation between anxiety and salivary cortisol. **Conclusion:** Results suggest that anxiety may be involved in the pathogenesis of RAS. Thus besides traditional treatment of RAS patients, our findings suggest that psychological support is also needed.

Key words: Anxiety, enzyme linked immunosorbent assay, recurrent aphthous stomatitis, salivary cortisol

Introduction

Recurrent aphthous stomatitis (RAS) is the most common type of ulcerative disease of the oral mucosa, and it affects approximately 20% of the general population[1] with a range from 5% to 66%.[2] Highest prevalence of 66% was found by Ship *et al.* on dental and medical students.[3] Three forms of RAS have been recognized. Minor RAS, which makes up more than 80% of all RAS cases, is a small (up to 1 cm in diameter), shallow, painful, well-circumscribed, and round-shaped ulceration that is covered with a yellow-grayish pseudo membrane and surrounded by an erythematous halo. The ulceration generally heals without scarring after 10-14 days. Major RAS is characterized by ulcers that are typically larger and deeper than minor RAS. Furthermore, they heal more slowly and often cause scarring. Herpetiform ulcers manifest as multiple recurrent clusters of small ulcers (less than 4 mm in diameter) that are scattered throughout the oral mucosa. These ulcers may further coalesce into larger ulcerations.[4]

The hypotheses of RAS pathogenesis are numerous. Stress has been postulated as a precipitating factor in RAS.[5] Previous studies have suggested that psychological disturbances such as stress and anxiety could play a role in the onset and recurrence of RAS lesions.[4] The worldwide distribution, high frequency and decreased quality-of-life generated by RAS have resulted in a great deal of research into the etiology and efficient therapy of this disease. However, the etiology of RAS still remains unclear, and

Access this article online	
Quick Response Code:	Website: www.ijem.in
	DOI: 10.4103/2230-8210.131768

Corresponding Author: Dr. Lakshmi Kavitha Nadendla, Department of Oral Medicine and Radiology, Kamineni Institute of Dental Sciences, Sreepuram, Narketpally, Nalgonda District, Andhra Pradesh, India. E-mail: drkavitha_p@yahoo.co.in

the currently available therapy remains inadequate.[4] Several studies have reported a relationship between RAS and various causes, but the results are conflicting.[2]

It is suggested that stress with its presumed effects on the immune system, constitutes one of the major causative agents of RAS.[2] Previous studies report increased frequency of oral ulcer manifestation in intensely stressed populations.[6] Thus the psychological-emotional factors seem to be major predisposing factors for RAS. An insight into a patient's psychological status can be estimated from both serum free and salivary cortisol levels.[5] Cortisol, also called as a stress hormone, has been used as an indicator in the stress evaluation studies.[7] The assessment of cortisol in saliva has gained interest in studies for evaluating anxiety. 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.[8] Salivary cortisol exhibits a clear diurnal variation and circadian rhythmicity with a time course closely parallel to that of plasma cortisol.[8]

Various scales were used in the psychological assessment of patients in previous studies, some were self-reported and others assessed by a psychiatrist.[9] Though stress and anxiety have been mentioned as possible factors related to the development of RAS; this association somewhat remains controversial.[2] The aim of this cross sectional study was to conduct an investigation in assessing the relationship between anxiety and salivary cortisol in patients with RAS by using both psychological testing instrument (Hamilton's anxiety scale [HAS]) and physiological testing instrument (salivary cortisol).

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. Thirty patients suffering from RAS (17 females 57%, 13 males 43%) were taken for the study after an informed consent. Age and sex matched 30 healthy individuals were taken as the control group.

The inclusion criteria for patients were as follows: Minor form of RAS, non-smokers, and a minimum of 2 years of RAS history to rule out from other types of acute recurrent ulcers like recurrent herpes. Patients suffering from systemic diseases including endocrine and metabolic diseases, hematinic deficiencies, patients using steroids/oral contraceptive pills, pregnant patients, smokers were excluded from the study. The diagnosis of RAS was done mainly by history and clinical examination.

Saliva samples were obtained from the study group when aphthous stomatitis was absent after healing. Control and study group samples were collected between 9 and 9:15 am, before a meal without stimulation by passive drooling directly into a sterile glass tube until 5 ml is collected. All participants were asked to wash their mouth properly before sample collection. The collected salivary samples were centrifuged for 15 min at 3000 rpm and frozen at -20°C until shortly before assay. During assay, the samples were thawed at 37°C. The supernatant fluid, thus obtained, was used for cortisol estimation.

Salivary cortisol was measured by competitive enzyme linked immunosorbent assay 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 ranges from 8.2 to 27.59 nmol/l (0.3-1 μg/dl) at morning time and 1.65-6.89 nmol/l (0.06-0.25 μg/dl) at evening collected samples.

After saliva collection, the patients were subjected to psychological evaluation. Anxiety levels were measured by using "HAS" that provides the measures of overall anxiety, psychic anxiety (mental agitation and psychological distress) and somatic anxiety (physical complaints related to anxiety). This scale consists of 14 questions, among which seven address the psychic and the remaining seven addresses the somatic anxiety. The individuals are rated on a five point scale for each of the 14 items ranging from 0 to 4. The total anxiety score ranges from 0 to 56, where <17 indicates mild severity, 18-24 mild to moderate severity and 25-30 moderate to severe.

T-test was used to compare the anxiety and salivary cortisol levels between patients with RAS and the control group as the base line data was normally distributed. Pearson's correlation analysis was used to study the correlation among anxiety and salivary cortisol levels in patients with RAS. Logistic regression analysis was used to assess the variables related with RAS. 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. RAS was a dependent variable, and salivary cortisol and anxiety levels were independent variables in this model.

Results

For this trail 60 patients were enrolled. The RAS group comprised of 13 males and 17 females and the mean age was 29.2 years (range 18-60 years). The control group comprised of the same number of age and sex matched individuals (mean age 29.2 years). The sex and age do not significantly differ between the two groups.

The mean salivary cortisol levels were 47.73 ± 8.80 nmol/l (1.73 ± 0.319 μg/dl) in RAS patients and 13.905 ± 3.55 nmol/l (0.504 ± 0.129 μg/dl) in controls with a *P* value of 0.000. The mean anxiety levels in RAS group were 27 ± 4.76 and 10.2 ± 3.27 in the control group (*P* = 0.000) as indicated in Table 1.

We found that salivary cortisol and anxiety levels were significantly higher in RAS group as compared to control group. There was a highly significant positive correlation (*P* = 0.000) between salivary cortisol levels and anxiety.

A logistic regression model in which the RAS 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 RAS [Table 2].

Discussion

The impact of oral disorders on quality of life has been increasingly recognized as an important outcome measure for clinical trials, especially since oral disorders frequently have detrimental effects on speech, nutrition, physical appearance, self-esteem and social interaction. RAS frequently affects patient quality of life as a result of long lasting and recurrent episodes of burning pain.[4] The etiopathogenesis of RAS appears to be complex; interactions with genetic, nutritional and hematological factors are reported.[10] Much has now been clarified about the mechanisms involved, interesting new associations such as the involvement of T-cell mediated immunologic reaction have emerged.[2] Stress has been postulated as a precipitating factor in RAS. Several reviews have, however, reported little objective evidence to support such an association.[11-13] Nevertheless, many patients make an association between RAS and what they term stress.[5] An insight into patient's psychological status can be estimated from cortisol levels. There is good evidence that stress and anxiety are related to increased resting levels of cortisol.[14] The present study was undertaken in an attempt to gain a better understanding of the role that has been attributed to stress, anxiety and salivary cortisol in the development of RAS and also to assess the relationship of salivary cortisol levels with stress and anxiety in patients with RAS.

Table 1: Comparison of salivary cortisol levels and anxiety scores (mean±SD) of patients

Variables	Study group RAS patients (*n*=30)	Control group (*n*=30)	*t* value*	*P* value
Salivary cortisol	47.73±8.80 nmol/l	13.90±3.55 nmol/l	−19.64	0.000
Anxiety	27±4.76	10.2±3.27	−15.84	0.000

**t*-test, RAS: Recurrent aphthous stomatitis, SD: Standard deviation

Table 2: Logistic regression analysis, in which the group membership (RAS or control) was dependent variable

Variable	B	Wald	df	*P* value
Salivary cortisol	−1.64	9.20	1	0.00
Anxiety	−0.03	2.80	1	0.01

RAS: Recurrent aphthous stomatitis, DF: Degree of freedom

A previous study McCartan *et al*.[5] investigated the possible association between anxiety, measured by Hospital Anxiety and Depression scale, and salivary cortisol in patients with RAS; and concluded that stress may play a role in the etiology of RAS. Albanidou-Farmaki *et al*.[2] conducted a case control study and compared the association between state and trait anxiety, measured by Spielberger's State-Trait anxiety inventory; and serum and salivary cortisol levels in patients with RAS; concluding that stress may be involved in the pathogenesis of RAS. Gallo Cde *et al*.[4] conducted a case control study to assess the influence of psychological stress on manifestations of RAS; by means of a questionnaire developed by the Psychology Institute of Sao Paulo University (Symptoms of Stress List; VAS Visual Analog scale questionnaire) and concluded that stress may play a role in the manifestation of RAS.

Present study results also showed statistically significant increase in salivary cortisol and anxiety levels in RAS patients during inactive stage compared to control group. Our study in contrast to previous studies (2 and 5) used HAS, which provides the measures of overall anxiety, psychic anxiety (mental agitation and psychological distress) and somatic anxiety (physical complaints related to anxiety). The study also showed a positive correlation between salivary cortisol levels and anxiety.

Stress and anxiety may play a significant role in the onset and recurrence of RAS lesions.[15] RAS is typically observed during stressful situations such as school exam periods, dental treatments and periods of significant changes in life.[4] Stress alters the regulation of both the sympathetic and parasympathetic branches of the nervous system, with consequential alterations in HPA-hypothalamic-pituitary-adrenal axis.[15] Autonomic activation and elevation of hormones, including those produced by HPA axis play pivotal roles in regulating immune surveillance mechanisms.[15] This immune regulatory activity with an increased number of leukocytes at the sites of inflammation induced by psychological stress is characteristic, often observed during the pathogenesis of RAS.

In conclusion, the present study showed a positive association between salivary cortisol levels and anxiety in

RAS patients during inactive stage. Therefore, measurement of the salivary cortisol and anxiety which reflect response to stress seems a promising parameter in the investigation of RAS. In conclusion, we suggest that stress management interventions may be beneficial along with conventional treatment methods in RAS patients.

Acknowledgment

I would like to thank Dr. Shruthi Mohanty and Dr. Neeta for their valuable help in salivary cortisol analysis.

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Cite this article as: Nadendla LK, Meduri V, Paramkusam G, Pachava KR. Relationship of salivary cortisol and anxiety in recurrent aphthous stomatitis. Indian J Endocr Metab 2015;19:56-9.

Source of Support: Nil, **Conflict of Interest:** None declared.