Original Article

Salivary cortisol and dehydroepiandrosterone as oral biomarkers to determine stress in patients with recurrent aphthous stomatitis

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Abstract

Background: Recurrent aphthous stomatitis (RAS) is one of the most common oral ulcerative diseases with a multifactorial etiology. Although psychological stress is an exacerbating factor, the role of salivary stress hormones, cortisol, and dehydroepiandrosterone (DHEA) in this oral disease has not been extensively reported. The study aimed to estimate and compare the salivary cortisol and DHEA levels in RAS patients and healthy control group with the aid of ELISA microplate reader.

Subjects and Methods: Sixty patients were enrolled in our study, which included 30 patients with clinically diagnosed RAS and 30 healthy controls. Two mL of unstimulated whole saliva was collected and salivary cortisol and DHEA levels were measured using ELISA kit, and the values were read by microplate ELISA reader and recorded in both groups.

Results and Conclusion: The mean salivary cortisol and DHEA levels were elevated in the RAS patients compared to the healthy controls and were statistically significant. Salivary cortisol and DHEA can serve as oral biomarkers to determine stress in patients with RAS. However, the present study necessitates further studies with larger sample size and an improved protocol to ascertain the actual role of these presumed oral biomarkers as well as anxiety and stress as triggers in the pathogenesis of RAS.

Keywords: Dehydroepiandrosterone, ELISA, recurrent aphthous ulcer, salivary cortisol, stress

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INTRODUCTION

Recurrent aphthous stomatitis (RAS) is defined as "a chronic inflammatory disease characterized by recurrent bouts of one or several shallow, rounded or ovoid, painful ulcers of oral mucosa that recur at intervals of a few days or up to 2–3 months and is of unknown etiology."[1] The etiology and pathogenesis of RAS so far have remained elusive and the potential trigger factors reported are as follows: local trauma, anxiety and stress, vitamin and microelement deficiencies, increased oxidative stress, viral and bacterial infections, genetic predisposition, food allergies and hormonal and systemic diseases.[2] Psychological stress has been frequently observed during stressful situations such as school examination periods, family problems, new job, dental treatments or periods of significant changes in life.[3]

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During a stress reaction, the hypothalamic–pituitary–axis activity is increased. The stressor activates corticotrophin-releasing hormone and arginine vasopressin secretion. These hormones stimulate the frontal lobe of the pituitary gland, which releases the adrenocorticotropic hormone, which, in turn, stimulates cortisol synthesis and secretion in adrenal cells.[4] Dehydroepiandrosterone (DHEA) is a major secretory product of the adrenal glands which is released along with cortisol and can be estimated in extracellular fluids such as blood, urine and saliva. Cortisol and DHEA have closely related metabolic pathways and are involved actively in the growth and development, immune response, stress resistance and cardiovascular function of an individual. Hence, altered levels of cortisol and DHEA may indicate changes in adrenal function that can immensely affect the energy levels, disease resistance, emotional state and general sense of well-being of an individual.[5] However, only few reports regarding the role of salivary DHEA in oral diseases have been reported in comparison with salivary cortisol. Although literature mentions anxiety and stress as potential etiologic factors for the development of aphthous stomatitis, this association still remains controversial.

SUBJECTS AND METHODS

The present study was conducted at the Outpatient Departments of Oral Pathology and Microbiology and Oral Medicine and Radiology, Meenakshi Ammal Dental College and Hospital, Chennai, India. The selection of participants was based on the patient's history and a thorough clinical examination. Thirty participants in the age group of 15–60 years with RAS were categorized as Group I. Participants with a history of systemic disease and disorders that could alter the levels of salivary cortisol and DHEA such as type II diabetes, Parkinson's disease, cardiovascular disease, Cushing syndrome, AIDS, bleeding dyscrasias, participants under corticosteroid medication for any systemic disease and participants with other inflammatory oral lesions such as periodontitis, Sjögren's syndrome and Burning mouth syndrome were excluded from the study.

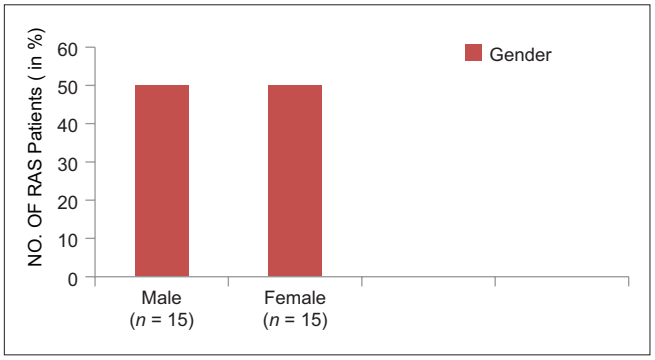
Thirty healthy individuals in the age group of 15–60 years were categorized as Group II. Individuals with a history of previous oral ulcerations were excluded. The clearance for conducting the study was obtained from the Ethical Committee, MAHER University, following the regular protocol and the written consent of the patient was obtained. A detailed case history was recorded, and the clinical photographs were obtained to complete the case records.

The participants in the experimental group were asked to rinse their mouth with water and unstimulated whole saliva of approximately 2 mL was collected in sitting position in the sterile containers of 150 mL capacity. Samples were collected between 10.00 a.m. and 1.00 p.m. to prevent diurnal variation. The collected saliva samples were labeled and stored at −80°C. Estimation of salivary cortisol and DHEA levels was done by solid-phase competitive ELISA technique using salivary cortisol and salivary DHEA enzyme immunoassay kits. To compare the levels of salivary cortisol and DHEA between disease group and healthy control group and to analyze their role as potential biomarkers to determine stress in patients with the disease, the values were analyzed statistically to meet the objectives of the study. The results were obtained and the mean ± standard deviation and the levels of significance of the parameters were determined to correlate the levels of cortisol and DHEA in the test and control groups on the basis of age and gender of the patient.

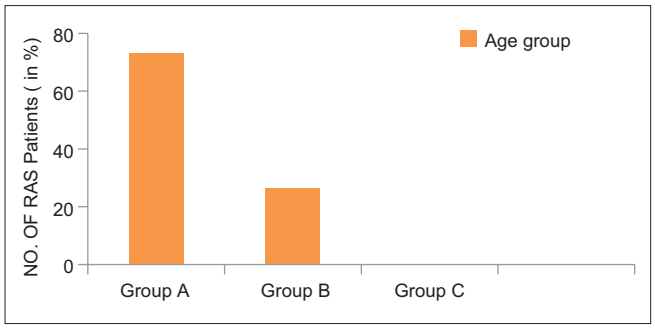
RESULTS

The distribution of the RAS group based on their gender indicated that males and females were equally affected [Graph 1]. The distribution of RAS group based on their age showed that Group A (15–30 years) was more affected (73.3%) compared to Group B (31–45 years) (26.6%), whereas patients in Group C (46–60 years) were not affected (0%) [Graph 2].

The distribution of salivary cortisol levels in RAS patients showed that 83.3% of the patients had salivary cortisol levels >3 ng/mL, whereas 16.7% had normal salivary cortisol levels of 1.2–3 ng/mL (minimum and maximum values were 2.5 ng/mL and 4.9 ng/mL, respectively). All 30 controls had normal range of cortisol levels with a mean value of 1.96 pg/mL. The 95% confidence limit between the two groups and statistically significant value of P (<0.001) obtained from the Student's t-test clearly indicated that the patients with RAS have high cortisol levels compared to the control group.



Graph 1: Recurrent aphthous stomatitis group: Gender-wise distribution



Graph 2: Recurrent aphthous stomatitis group: Age-wise distribution

The distribution of salivary DHEA levels in RAS patients showed that 50% had normal DHEA level of 48–61 pg/ml, 27% had less than the normal range, and the remaining 23% had higher range of DHEA (minimum and maximum values were 42 pg/mL and 117 pg/mL, respectively). The distribution of salivary DHEA in healthy controls showed that all the 30 participants had <48 pg/ml. The *t*-test was applied to compare the two mean values and the statistically significant value of *P* (<0.001) confirmed that the mean DHEA levels were higher in the RAS patients than the healthy controls.

DISCUSSION

Due to the association of RAS with psychological variables, salivary cortisol and DHEA levels were estimated in the RAS patients and healthy controls by the ELISA method as recommended by Gozansky *et al*. in 2005.[6]

In the current study, comprising 30 RAS patients, the individuals in the age group of 15–30 years (Group A) were more commonly affected (73.3%, *n* = 22) than the other age groups. This is similar to the studies conducted by Rivera-Hidalgo *et al*., in 2004,[7] who reported that in the US adult population, individuals <40 years of age had almost twice the prevalence of RAS than those >40 years. Abdullah, in 2013,[8] suggested 20–29 years as the most common age of occurrence of RAS in the Iraqi population and Vivek and Nair, in 2011,[9] stated that the peak onset of RAS ranged between 10 and 19 years in the South Indian population. However, Ali and Abdul, in 2011,[10] studied RAS patients in Sudan, Northern Africa, and reported 40 years as the mean age of occurrence. Since individuals <30 years are more prone to physical, biological, and psychological stress, there could have been an increased tendency for RAS occurrence in this age group in the present study. Furthermore, in the age group of 15–30 years, occupational status and educational level seem to have a greater impact on the prevalence of RAS. In the present study, males (50%) and females (50%) were equally affected with RAS. This is in contrary to the numerous studies (Ship, 1972; Donatsky, 1973; Fahmy, 1976; Axell and Henricsson, 1985; Pongissawaranun and Laohapand, 1991; Field *et al*, 1992; Kleinman *et al*, 1994, Kovac-Kovacic and Skaleric, 2000 and Davatchi F, 2008)[11-18] where females were predominantly affected. Some women experience recurrent oral ulcerations during the luteal phase of the menstrual cycle and also a decline in its incidence during pregnancy suggesting an association between RAS and progesterone levels. Thus, the increased incidence of RAS in women is attributed to hormonal changes. However, according to McCartan *et al*.,[19] based on the study conducted at Dublin, Ireland, there was no association between aphthous stomatitis and premenstrual period or pregnancy or menopause. Chattopadhyaya, in 2007,[20] and Mathew, in 2008,[21] cited an increased frequency of RAS in men in the US adult population and South Indian population, respectively. The equal incidence among men and women in our study suggests that hormonal levels have a limited role in the multifactorial etiology of RAS.

In the present study, salivary cortisol levels in controls were 0.13–0.26 μg/dL. Eguia-del Valle *et al*., in 2013,[22] Albanidou-Farmaki *et al*., in 2008,[23] and Mello *et al*., in 2011,[24] in their studies reported salivary cortisol levels of 0.25–1.09 μg/dL, 0.35–1.47 μg/dL, and 0.30–3.20 μg/dL, respectively, in controls. This variation could be attributed to the varying populations and methodologies used.

In our study, the mean levels of the salivary cortisol in RAS patients was 0.385 ± 0.067 μg/dL and in the controls was 0.196 ± 0.038 μg/dL. The salivary cortisol level in RAS patients was two times higher than in the controls. Similar results were obtained by Albanidou-Farmaki *et al*., in 2008,[23] who reported approximately two times higher cortisol levels in saliva of the patients with RAS (mean salivary cortisol levels: 1.44 ± 0.58 μg/dl) compared to the controls (mean value of 0.91 ± 0.56 μg/dL). Nadendla *et al*.[25] also observed a highly significant difference in salivary cortisol levels between the RAS group (mean value: 1.73 ± 0.319 μg/dl) and healthy control group (mean value: 0.504 ± 0.129 μg/dL).

Prolonged stress leads to a persistent increase in the cortisol levels, which tend to dysregulate the various homeostatic mechanisms in the body by altering the local immune response in the oral mucosa. Although cortisol decreases the recruitment and activation of B lymphocytes, its main effects are exhibited on cell-mediated immunity. It causes suppression of immune system, decreases

lymphocyte infiltration, lymphocyte and thymocyte populations (anti-mitotic), reduces differentiation and proliferation of local mast cells and production of nitric oxide and platelet-activating factor, stabilizes lysosomes and impairs phagocytosis. Cortisol also suppresses synthesis and release of arachidonic acid (the key precursor for several inflammatory mediators) and decreases the production of gamma interferon and interleukins (ILs) (the critical mediators of immune response). The susceptible individuals might overreact to precipitating factors like mucosal microtrauma or food allergens, which could progress to ulcer development in RAS.

Eguia-del Valle *et al*., in 2013,[22] noted that the mean salivary cortisol levels were not statistically increased in patients with RAS compared to the healthy control group (0.64 μg/dl for patients with RAS and 0.57 μg/dl for controls). According to him, there is increased stress and increase in tumor necrosis factor-alpha, which is a proinflammatory cytokine in the saliva of participants suffering from active RAS. The important pathogenesis in RAS seems to be the inability to suppress the inflammatory reaction initiated by trauma or any inciting stimuli probably due to a functional deficiency of IL-10 in the oral mucosa.

In the present study, 83.3% of the patients with RAS (25/30) had a high level of salivary cortisol. However, only 15% of the patients with RAS (3/20) presented with increased levels of salivary cortisol in the study conducted by Eguia-del Valle *et al*. in 2013.[22]

In this study, the salivary DHEA levels of the controls were in the range of 20–40 pg/ml. In the present study, 23.3% of the patients with RAS (7/30) had a high level of salivary DHEA. The mean levels of the salivary DHEA in RAS patients were 59.16 ± 18.15 pg/mL and in the controls, the mean DHEA value was 29.23 ± 6.66 pg/ml. The salivary DHEA level in RAS patients was also two times higher than in the controls.

Although salivary cortisol and DHEA levels show similar proportional increase between the RAS patients and controls, the percentage of patients showing the significant difference is higher for cortisol than DHEA. This indicates that salivary cortisol is a better oral biomarker in RAS than DHEA. To the best of our knowledge, this is the first study to investigate salivary DHEA levels in patients with RAS, hence, making it difficult to compare our results. However, conflicting DHEA levels in oral diseases such as oral lichen planus and periodontitis have been reported. Ansai *et al*., in 2009,[26] reported significantly elevated levels of cortisol (mean value – 2.06 ng/mL) and DHEA (mean value – 60.24 pg/mL) in the saliva of Japanese adults with periodontitis. In contrast, Girardi *et al*., in 2011,[27] reported that the salivary cortisol and DHEA levels did not differ between controls and oral lichen planus patients.

Although the present study shows a positive association between salivary cortisol and DHEA levels and RAS patients, due to the insufficient and inconsistent studies of cortisol and DHEA pattern in RAS patients in diverse populations, with different methodologies and stress scales followed, it is difficult to compare the present study with the available studies. Within the limitations of the present study (small sample size, inconsideration of the stage of the ulcer and the lack of using stress measuring scales to determine the degree of stress in the RAS patients), the results obtained indicate that though salivary cortisol and DHEA can serve as oral biomarkers to determine stress in patients with RAS, salivary cortisol proves to be a better biomarker than DHEA. It is also suggested that along with conventional treatment methods, stress management interventions may also be beneficial in RAS patients.

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Conflicts of interest
There are no conflicts of interest.

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