



Cortisol and dehydroepiandrosterone salivary levels, stress and anxiety in patients with recurrent aphthous stomatitis

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Abstract

Objective: In the present study we investigated salivary concentrations of the hormones cortisol and dehydroepiandrosterone (DHEA), stress and anxiety levels in patients with recurrent aphthous stomatitis (RAS).

Methods: The sample consisted of 60 individuals of both sexes, aged between 18 and 50 years: 30 patients with RAS and 30 controls. Stress and anxiety were assessed using the Lipp's Adult Stress Symptom Inventory and the Beck's Anxiety Inventory, respectively. The saliva samples were collected in the morning and in the afternoon on the same day. In the RAS group, the samples were collected in the presence of active lesions and after remission. The salivary levels of cortisol and DHEA were determined by radioimmunoassay.

Results: RAS patients exhibited significantly higher anxiety scores ($P=0.001$) and prevalence of stress ($P=0.004$). There was no significant difference in DHEA and cortisol levels between RAS and control groups. Cortisol levels ($P=0.008$ and $P=0.001$) and the cortisol/DHEA ratio ($P=0.054$ and $P=0.007$) were significantly increased in the RAS patients with active lesions comparing with the remission phase ($P=0.007$).

Conclusion: The present study demonstrates an association of stress and anxiety with RAS. However cortisol and DHEA did not differ between patients with RAS and controls.

Key words: Aphthous stomatitis; Cortisol; Dehydroepiandrosterone; Stress; Anxiety

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Níveis salivares de cortisol e desidroepiandrosterona, estresse e ansiedade em pacientes com estomatite aftosa recorrente

Resumo

Objetivo: Neste estudo foram investigadas as concentrações salivares dos hormônios cortisol e desidroepiandrosterona (DHEA), níveis de estresse e ansiedade de pacientes com ulceração aftosa recorrente (UAR).

Métodos: A amostra consistiu de 60 indivíduos de ambos os sexos, com idades entre 18 e 50 anos: 30 pacientes com UAR e 30 pacientes-controle. O estresse e a ansiedade foram avaliados por meio do Inventário de Sintomas de Stress de Lipp e do Inventário de Ansiedade de Beck, respectivamente. Amostras de saliva foram coletadas pela manhã e à tarde no mesmo dia. No grupo-UAR as amostras foram coletadas na presença de lesões ativas e após sua remissão. Os níveis salivares de cortisol e DHEA foram analisados por radioimunoensaio.

Resultados: Os escores de ansiedade ($P=0.001$) e prevalência de estresse ($P=0.004$) foram significativamente superiores no grupo-UAR. Não houve diferença significativa entre os grupos quanto aos níveis de cortisol e DHEA. Os níveis de cortisol ($P=0.008$ e $P=0.001$) e o *ratio* cortisol/DHEA ($P=0.054$ e $P=0.007$) foram significativamente superiores nos pacientes com lesões ativas quando comparados à fase de remissão.

Conclusões: O presente estudo demonstra uma associação de estresse e ansiedade com UAR. Entretanto, o cortisol e a DHEA não diferiram entre pacientes com UAR e controles.

Palavras-chave: Estomatite aftosa; Cortisol; Dehidroepiandrosterona; Estresse; Ansiedade

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Introduction

Recurrent aphthous stomatitis (RAS) is one of the most frequent diseases of the oral mucosa and its prevalence in the general population ranges from 5 to 66% [1,2]. The lesions are found, specially, in non-keratinized mucosa and are characterized by painful single or multiple ulcerations, round or oval shaped with an erythematous halo [2]. Aphthae etiology is complex, multifactorial, and the mechanism that triggers the lesion development remains unknown. Genetic predisposition, hypersensitivity to certain foods, immunologic, hormonal, microbiological, traumatic, psychological factors, as well as nutritional deficiency are the main causes investigated [1,2]. Its development involves dysregulation in the oral mucosa response mechanisms against exogenous or endogenous antigens, occurring an immune reaction that involves the T cells. There is an imbalance of the TCD4+ cells, with later TCD8+ lymphocyte proliferation, cytotoxic reaction mediators that cause tissue damage [3]. Some studies report T-Helper cytokines imbalance with the increase of Th1 activity in relation to Th2 in patients with aphthae [4,5].

There is evidence that anxiety and stress predispose to the occurrence of RAS once these psychological disorders can modify and deregulate immune functions [1,6]. Stress stimulates the central nervous system, activating the HPA [Hypothalamus-Pituitary-Adrenal] axis and releasing cortisol. This glucocorticoid hormone is secreted through the adrenal cortex and acts on the inflammatory and immunological responses. High salivary levels of cortisol have been found in individuals with depression, stress and anxiety, being considered a biomarker of these psychological disorders [7]. High serum and salivary levels of that steroid were previously found in individuals with RAS [1,8].

Dehydroepiandrosterone (DHEA), the most abundant circulating steroid in the human body, is a precursor for potent androgens and estrogens. DHEA is released by the adrenal gland cortex, by the gonads and by the central nervous system [9]. Its mechanism of action in the human body is not completely understood and remains controversial. That steroid seems to modulate the immune response, and the decrease of its serum concentrations is associated with the deterioration of several physiological functions [10]. Low concentrations of this steroid were found in patients with rheumatoid arthritis, systemic lupus erythematosus [11], panic syndrome and burning mouth syndrome [12]. In normal conditions DHEA is released synchronically with cortisol and it antagonizes several deleterious effects of that hormone [9]. However, several disorders such as burns, anorexia nervosa, Cushing's syndrome, schizophrenia, depression, anxiety, Alzheimer's disease, diabetes, systemic lupus erythematosus are characterized by increased levels of cortisol and a reduction in DHEA [9,13,14]. De Peretti and Forest [14] report the response to stress as an example of increased cortisol/DHEA ratio. On the other hand there are studies showing that acute stress elevates DHEA as well as cortisol levels [15]. There are no earlier studies in

the international literature on the DHEA levels in patients with RAS.

As RAS is in immunologic disorder and since there are reports of its association with psychological variables, in the present study we analyzed salivary cortisol and DHEA concentrations, stress and anxiety levels, trying to establish possible associations between RAS, psychological disorders and hormone levels.

Methods

This study was approved by the Research Ethics Committee of Pontifical Catholic University of Rio Grande do Sul (PUCRS). Patients were selected consecutively from the Oral Medicine Division of São Lucas Hospital (PUCRS) and from a Dental Service of the State of Rio Grande do Sul (Brazil). Each of the participants in the study signed an informed consent form. The sample comprised 60 individuals from both sexes, between 18 and 50 years old, divided in two groups matched by sex and age;

- RAS group: 30 patients with minor RAS, with less than 72 hour evolution, were included. These patients should have had at least three outbreaks of the disease in the last year.
- Control group: 30 healthy patients with no RAS history.

Patients with erosive, ulcerated or infectious lesions in the oral mucosa were excluded from the study as well as patients with systemic lupus erythematosus, Behcet's syndrome, Crohn's disease, celiac disease, Sjögren's syndrome, cyclical neutropenia, AIDS or HIV seropositives, with the burning mouth syndrome, smokers, patients using antidepressant, anxiolytic, corticosteroid drugs or hormone replacement. Also excluded from this study were patients who presented changes in the blood count, in the serum levels of glucose, iron, folic acid and vitamin B₁₂ or ANF [anti-nuclear factor] reacting with titles equal or above 1:160.

The patient's medical history and medications used were recorded. The individuals with RAS were questioned about the evolution time of the disorder, lesion[s] duration, frequency of outbreaks and whether she/he associated it with some cause. The number and location of lesions were recorded in the intraoral examination. For the RAS diagnosis the criteria established by Jurge et al. [2] were used. All the patients in this study were asked for blood count test, glucose, anti-HIV, ANF, serum concentrations for iron, folic acid and vitamin B₁₂.

Beck's Anxiety Inventory (BAI) [16] was used – in the Portuguese version – to assess anxiety symptoms. For stress assessment the Lipp's adult Stress Symptom Inventory (LSSI) [17] was used. In the RAS group psychometric instruments were applied in the first assessment, that is, up 72 hours after lesion development.

Saliva sample collection

For the RAS group the saliva was collected in the presence of active lesions (observing the 72-hour evolution limit) and one week after its remission. The saliva was

collected in the same day between 07 h and 09 h (before breakfast) and between 11 h and 13 h (before lunch).

Patients were advised not to ingest any alcoholic beverage in the 24-hour before the collection; not to ingest any food or beverage neither put any substance in their mouth, including not to brush their teeth one hour before collection; not to apply any medicine or cosmetics on the lips. They were asked to rinse the mouth with water before collection, sit down with eyes open and deposit the saliva into the collection flask according to its accumulation in the mouth (for the time necessary to reach the 1.5 mL mark). The flasks were stored at -20⁰ C until the time of analyses of the hormones.

Analysis of DHEA and cortisol salivary levels

The samples for each collection were analyzed separately. 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™-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™ Cortisol kit (Siemens Medical Solutions Diagnostics – Los Angeles, California, USA) was used to determine the salivary levels of cortisol. The radioactivity in the tubes was measured in a Gamma C12™ counter (EURO-DPC). The specific equipment software calculated the DHEA and cortisol levels in each saliva sample.

Statistical Analysis

The software *Statistical Package for the Social Sciences* (SPSS) version 11.0 was used in the statistical analysis. The variables were analyzed by means of descriptive statistics. In order to compare the anxiety scores and the presence of stress between the groups, the tests Mann-Whitney and Fisher’s Exact were applied, respectively. The Mann-Whitney test

was also used for comparing the groups regarding the DHEA and cortisol salivary levels, and cortisol/DHEA ratio. Values of P≤0.05 were considered significant.

Results

In both groups, 22 (73.3%) individuals were females and 8 (22.7%) were males, ranging from 18 to 50 years old. The mean age of the RAS group was 31.8 (±11.06) years and for the control group was 32.1 (±11.04). The most affected sites were the tongue (35.3%), labial mucosa (33.3%), alveolar mucosa (23.5%), buccal mucosa (3.9%) and floor of mouth (3.9%). With regards to the time of development, 17 (56.7%) patients reported to have had the disease since childhood, seven (23.3%) since adolescence and six (20%) reported to have had the first outbreak in adult life. The frequency of outbreaks ranged from three times a month to three times a year. When questioned about the possible cause agent associated with the outbreak of the ulcerations, 30% of the patients mentioned stress, 30% did not associate the lesions with any specific cause, 20% related with food, 10% with traumatic agents and 10% with food and stress.

Anxiety and stress symptoms

The Beck’s Anxiety Inventory (BAI) scores were significantly higher in RAS patients when compared with the control individuals (P<0.001) (Table 1). A significant difference was also found between the groups (P=0.004) regarding the presence of stress, as this psychological disorder was present in 19 patients with RAS and in seven individuals from the control group (Table 2).

Cortisol and DHEA levels

There were no significant differences in salivary cortisol (P=0.258 and P=0.083) or DHEA (P=0.420 and P=0.620) levels between RAS group with active lesions and control group (Table 3), although the levels of these hormones have been higher in patients with RAS. When assessing the cortisol/DHEA ratio no significant difference was observed between those groups (P=0.988, and P=0.442) (Table 4).

Table 1. Anxiety scores of RAS and control groups obtained through BAI inventory

	RAS group		Control group		P
	Median	P 25-75	Median	P 25-75	
BAI Scores	8.0	3.0 – 10.3	2.0	1.0 – 5.3	<0.001

Mann-Whitney Test.

Table 2. Distribution of RAS and control patients with regards to the presence of stress

Presence of Stress	RAS group n (%)	Control group n (%)	Total n (%)
Without stress	11 (36.67)	23 (76.67)	34 (56.67)
With stress	19 (63.33)	7 (23.33)	26 (43.33)
Total	30 (100)	30 (100)	60 (100)

Fischer’s exact test; P = 0.004.
n = frequency; % = percentage.



Tabela 3. Salivary levels of cortisol and DHEA (nmol/L) in the RAS group with active lesions and control group

	RAS group (active lesions) Median (P25-P75)	Control group Median (P25-P75)	P
Cortisol (BB)	15.80 (11.70-21.83)	14.50 (12.20-17.30)	0.258
Cortisol (BL)	8.05 (6.85-9.18)	7.25 (6.48-8.65)	0.083
DHEA (BB)	0.70 (0.55-1.03)	0.66 (0.55-0.83)	0.420
DHEA (BL)	0.47 (0.38-0.67)	0.45 (0.36-0.53)	0.620

BB = before breakfast samples (saliva collected between 07h and 09h).
BL = before lunch samples (saliva collected between 11h and 13h).
Mann-Whitney Test.

Table 4. Cortisol/DHEA ratio (nmol/L) in the RAS group with active lesions and control group

	RAS group (active lesions) Median (P25-P75)	Control group Median (P25-P75)	P
Cortisol/DHEA (BB)	20.75 (18.78-28.13)	21.55 (18.89-25.47)	0.988
Cortisol/DHEA (BL)	16.95 (15.07-20.87)	16.32 (14.06-18.75)	0.442

BB = before breakfast samples (saliva collected between 07h and 09h).
BL = before lunch samples (saliva collected between 11h and 13h).
Mann-Whitney Test.

Table 5. Salivary levels of cortisol and DHEA (nmol/L) in the RAS group with active lesions and after remission

	RAS group		P
	Active lesions Median (P25-P75)	Active lesions Median (P25-P75)	
Cortisol (BB)	15.80 (11.70-21.83)	12.95 (9.40-16.10)	0.008
Cortisol (BL)	8.05 (6.85-9.18)	6.00 (5.20-7.43)	0.001
DHEA (BB)	0.70 (0.55-1.03)	0.68 (0.57-0.81)	0.365
DHEA (BL)	0.47 (0.38-0.67)	0.43 (0.38-0.54)	0.144

BB= before breakfast samples (saliva collected between 07h and 09h).
BL= before lunch samples (saliva collected between 11h and 13h).
Mann-Whitney Test.

Table 6. Cortisol/DHEA ratio (nmol/L) of the RAS group with active lesions and after remission

	RAS group		P
	Active lesions Median (P25-75)	Remission of lesions Median (P25-75)	
Cortisol/DHEA (BB)	20.75 (18.78-28.13)	20.24 (14.59-24.20)	0.054
Cortisol/DHEA (BL)	16.95 (15.07-20.87)	14.68 (12.49-16.68)	0.007

BB= before breakfast samples (saliva collected between 07h and 09h).
BL= before lunch samples (saliva collected between 11h and 13h).
Mann-Whitney Test.

When cortisol levels were compared between RAS patients with active lesions and after remission, significantly higher values were shown by patients with lesions (P=0.008 and P=0.001). There was no significant difference in the DHEA salivary levels (P=0.365 and P=0.144) (Table 5). The cortisol/DHEA ratio was significantly higher in patients with active lesions (P=0.054 and P=0.007) (Table 6).

No significant correlation was observed between cortisol and DHEA levels and anxiety scores. Cortisol and DHEA salivary levels have not shown any association with stress either (data not shown).

Discussion

Previous studies have shown that patients with RAS may have increased concentrations of cortisol [1,8], meanwhile DHEA levels were not further analyzed in patients with this

disorder. The literature demonstrates that some disorders are characterized by increased cortisol and a reduction in DHEA levels [9,13,18]. This steroid is an important modulator of the immune response, it regulates the production of cytokines, increasing the production of IL-2 by Th1 cells and decreasing 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 [19]. In the present study, DHEA levels varied parallel to the cortisol ones and no significant difference was found between the groups with regards to DHEA. Girardi et al. [20] analyzed salivary levels of this steroid in patients with oral lichen planus and also found no significant difference compared to control group.

In spite of being higher in patients with active lesions, cortisol levels did not differ significantly when compared

to control group. The salivary samples were collected up till three days after ulceration development, at this time cortisol levels might not have been so high. Patients would have started stress adjustment phase, which explains the absence of a significant difference with regards to the control individuals. Cortisol salivary levels in patients with RAS have also been investigated by other authors. Corroborating the results of the present study Eguia-del Valle et al. [21] did not find significant difference in salivary cortisol levels between patients with RAS and controls. On the other hand, McCartan et al. [8] have observed that patients with RAS presented higher levels of anxiety, stress and salivary cortisol in comparison with the patients with the disease, but which exhibited hematinic deficiencies. In that study, however, there were no control patients. Albanidou-Farmaki et al. [1] have found higher levels of anxiety as well as serum and salivary cortisol in patients with RAS, when compared with control patients.

In the RAS group, salivary analysis was carried out in the presence of active lesions, and one week after remission, which made it possible to research the hormone levels in two different physiological conditions. In this group, the cortisol salivary levels were increased in the presence of RAS compared with the lesion remission phase. It is possible that stress and anxiety may have elevated cortisol levels, which preceded the development of RAS. The increase in the cortisol levels after stimulus, gives a negative feedback, which is characterized by the decrease in ACTH discharge at hypothalamus, thus reducing cortisol production [22]. Probably for this reason, the cortisol levels were lower after lesion remission.

The frequency of patients with stress and the anxiety scores were both higher in the RAS group compared with the control group, paired by sex and age. In addition, during the interview, 40% of patients associated the outbreak of the RAS with stress. These results corroborate the literature, evidencing a possible association of the psychological disorders investigated with RAS [1,8,23]. Gallo et al. [6] suggest that stress acts as an initiator in individuals prone to the occurrence of aphthae. Stress, anxiety and other psychological disorders can change immune functions, causing their dysregulation, which predisposes to the development of some diseases [24]. Stress promotes the increase in both, the mononuclear cells chemotaxis and the expression of the cell adhesion molecules, which contribute to the increase of the immune system cells migration and recruitment of [25]. According to Elenkov [26], acute or chronic stress can affect the system immune response increasing cortisol levels and causing cytokines Th1/Th2 imbalance. Borra et al. [4] have demonstrated that there is an imbalance of these cytokines in aphthae development, with an increase in Th1 activity.

Conclusion

Patients with aphthae have their quality of life affected, as lesions can be extremely painful and relapsing. In the

present study, RAS patients have presented higher levels of anxiety and higher stress frequency than the control individuals, proving a possible association between these psychological disorders with the disease. Cortisol salivary levels were higher in patients with lesions but did not differ significantly in relation to the control patients. This has been the first study to investigate DHEA salivary levels in patients with RAS, as this steroid seems to be related with psychological disorders and with the immune response modulation. However, patients with aphthae have not shown different levels of DHEA in the presence of lesion or in their remission, neither when compared with the individuals without the disease.

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