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**Stress and recurrent aphthous stomatitis**

Isil Cakmak Karaer<sup>1</sup>, Ayca Urhan<sup>2</sup>, Ismail Reyhani<sup>3</sup>

<sup>1</sup>Malatya Training and Research Hospital, Ministry of Health , ENT Clinic, Malatya, Turkey

<sup>2</sup>Ministry of Health, Malatya Training and Research Hospital, Clinic of Biochemistry, Malatya, Turkey

<sup>3</sup>Inonu University Faculty of Medicine, Department of Health Services, Programme of Child Development, Mus, Turkey

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**Abstract**

Recurrent aphthous stomatitis (RAS) is a commonly-encountered oral lesion. The aim of this study was to identify the levels of stress, anxiety and depression in patients with RAS. Forty (40) patients with RAS and 40 age-matched controls were enrolled in this study. Blood and salivary levels were analyzed electrochemiluminescence technique. The Beck Depression Rating Scale (BDRS) and Beck Anxiety Scale (BAS), Perceived Stress Scale (PSS), and State-Trait Anxiety Inventory (STAI-S and STAI-T) were applied to all participants. There was no statistically significant differences levels between two groups regarding to BDRS, BAS, PSS, STAI-S and STAI-T level. Blood cortisol levels were statistically significantly higher in patients with RAS than controls ( $12.45 \pm 0.74 \mu\text{g/dL}$  vs.  $9.8 \pm 0.68$ , respectively,  $p=0.01$ ), whereas there was no statistically significant difference between women with RAS than controls regarding to salivary cortisol levels ( $0.33 \pm 0.03 \mu\text{g/dL}$  vs.  $0.34 \pm 0.03$ , respectively). Patients with RAS have not higher depression and anxiety levels compared to the control.

**Keywords:** Aphthous stomatitis, anxiety, stress, depression, free cortisol, salivary cortisol

**Introduction**

Recurrent aphthous stomatitis (RAS) is a painful situation occurring as oral mucosal ulcers which negatively affect quality of life. It has been reported that the prevalence of RAS is around 20% [1]. Classically, self-limiting ulcers are observed in non-keratinizing oral mucosa. After 24-48 hours of prodromal findings of pain and burning, the clinical scene begins to progress [2]. There are three clinical subtypes based on the shape and spread of the ulcers: minor, major and herpetiform RAS [3]. In the literature, though many factors have been shown as a cause of RAS such as genetics, nutrition, local trauma, allergies, endocrine disease, changes in oral microbiota (disruption of flora), and quitting smoking, the definite cause is not fully known [4-6]. The general opinion is that RAS is triggered by stress and anxiety [7].

The Beck depression rating scale (BDRS) and Beck anxiety scale (BAS) are tests commonly used around the world to determine the presence and severity of anxiety and depression [8-10]. To measure for anxiety levels, Spielberger's state-trait anxiety inventory (STAI-S and STAI-T) comprises the STAI-T providing a general view of personality constructs and the STAI-S assessing anxiety in specific situations. The perceived stress scale (PSS) is the most widely used psychological instrument for measuring the perception of stress. It is a measure of the degree to which situations in one's life are appraised as stressful [11].

Cortisol is released from the adrenal cortex and is accepted as a stress marker; another name for it is the stress hormone. In humans, stress and anxiety levels can be identified with free blood and salivary cortisol levels. The literature shows that salivary cortisol is more valuable than free cortisol in blood as a stress indicator [12]. In addition to, blood is invasive to collect and may be painful when compared to saliva.

In addition to, the sampling is easier than blood sa .

The aim of this study was to determine whether a association

\*Corresponding Author: Isil C. Karaer, Malatya Training and Research Hospital, Hastane street no:44, postal code:44623 Department of Otolaryngology, Malatya, Turkey, E-mail: [isilkaraer@gmail.com](mailto:isilkaraer@gmail.com)

between between RAS and the levels of depression, anxiety, stress, blood cortisol and salivary cortisol.

## Materials and Methods

A prospective case-control study was carried out in the Department of Otolaryngology, Malatya Training and Research Hospital. Data were collected between September 2016 and September 2017 after obtaining informed consent from the subjects. A total of 80 patients were included in the study.

### Study population

The study involved two groups. Group 1 was the RAS group, which comprised 40 patients (16 males and 24 females) with a mean age of  $34.7 \pm 2$  (min 15- max 63) years(y). The control group comprised 40 patients (14 males and 26 females) with a mean age of  $36.08 \pm 2.1$  (min 15-max 62) y. All study participants had minor RAS form (superficial, oval ulcers in buccal mucosa, smaller than 10 mm, localized to the tongue and pharynx). Only patients with RAS complaint with incidence of at least two per month during at least two years were included in the study.

The exclusion criteria is major and herpetiform RAS , cigarette smoking smokers, those using any medication like steroids or oral contraceptives, with systemic and immunological disorders (such as Behçet disease) , any vitamin deficiency or anemia. The controls comprised individuals with no active RAS and no history of RAS, who were healthy with no systemic disease and no medication use.

### Psychoanalytic Tests

Beck Depression Rating Scale (BDRS) and Beck Anxiety Scale (BAS), Perceived Stress Scale (PSS), and State-Trait Anxiety Inventory (STAI-S and STAI-T) were applied to all study participants. Study participants were given information about the study protocol and provided informed consent before being given the form prepared by the researchers and requested to complete it fully. In this way, the sociodemographic characteristics of cases were determined.

With the aim of determining the anxiety levels of cases, the Spielberger State-Trait Anxiety Inventory (STAI-T) and Spielberger Perceived Stress Scale (STAI-S) were used, each scala comprising 20 questions [8]. For both scales, 4 answer choices were present with weight varying from 1 to 4. The scales included 2 types of statement; direct or plain statements and inverse statements. The state anxiety scale includes 10 inverse statements, while the trait anxiety scale includes 7 inverse statements. Points for direct and inverse statements are collected separately and the total weighted points for inverse statements are subtracted from the total weighted points obtained from direct statements. This number has a fixed value added, previously determined as 50 for the state anxiety scale and 35 for the trait anxiety scale. The value is the individual's anxiety points which determine the anxiety level.

With the aim of determining the depression levels of cases, the Beck Depression rating scale (BDRS) was used. The BDRS is a self-assessment scale comprising 21 items which measures symptoms seen in depression. Each question has four answer choices with weight degrees from 0 to 3. According to cut-off points of 17 for the scale, those with points above this value are assessed as being at risk of clinical depression [9]. The Basel Beck Anxiety scale

(BAS) was developed by Beck et al. with the aim of determining the incidence of anxiety symptoms experienced by individuals and is a self-assessment scale. It comprises 21 items with Likert-type responses and points from 0-3 [10,11].

### Salivary and Serum Cortisol analysis

All patients had blood and saliva samples taken during the active period of the disease. Salivary and serum samples of study participants were obtained in the early morning (9 to 10 a.m.). Saliva samples were collected by special saliva tubes (Salivette Cortisol, Sarstedt, Sevelen, Switzerland). All samples were collected at least 8-10 hours fasting. At the time of saliva collection, the couples were warned to avoid drinking, eating, and tooth brushing in the first 30 min, and avoid drinking milk or coffee in the first 60 min after waking up.. All saliva samples were stored at  $-80^{\circ}\text{C}$  until analysis. Salivary and serum cortisol levels were analyzed by the electrochemiluminescence technique (Cobas 6000, E601 analyzer, Roche Diagnostic, Germany). Cortisol levels were expressed as microgram per deciliter ( $\mu\text{g}/\text{dL}$ ). The measurement ranges are 0.054-63.4  $\mu\text{g}/\text{dL}$  according to the manufacturer's protocol.

### Statistical analysis

All analyses were conducted using SPSS 15.0 (SPSS® for Windows 15.0, Chicago, USA). The data were distributed not normally according to the one-sample Kolmogorov-Smirnov test. The variables were expressed as the mean  $\pm$  SD. Comparisons of all parameters were carried out using the Mann-Whitney U test. Multiple comparisons were carried out using Spearman's test. A two-tailed  $p < 0.05$  was considered statistically significant.

## Results

The mean age of the patients with RAS was  $34.7 \pm 2.06$  (min 15-max 63) years(y), whereas the mean age of controls was  $34.2 \pm 2.5$  (min 15-max 62) y. When the mean ages of patients with RAS were compared with controls, there was no statistically significant difference ( $p = 0.61$ ).

The mean BDRS points was  $14.7 \pm 1.6$ , in patients with RAS and mean  $11 \pm 1.3$ , in controls ( $p = 0.187$ ). BAS points were mean  $15.1 \pm 1.9$ , in Group RAS and mean  $10 \pm 1.7$ , in the controls ( $p = 0.106$ ). The PSS points were mean  $19.2 \pm 1.05$ , in Group RAS and mean  $17.5 \pm 1.05$ , in the controls ( $p = 0.390$ ). The STAI-S points were mean  $39.5 \pm 1.49$ , in Group RAS and mean  $37.9 \pm 1.4$ , in the controls ( $p = 0.461$ ). The STAI-T points were mean  $47 \pm 1.2$ , in Group RAS and mean  $46.4 \pm 1.5$ , in the controls ( $p = 0.760$ ). Between both groups, there were no statistically significant differences identified for BDRS, BAS, PSS, STAI-S and STAI-T test results. Laboratory and psychoanalysis findings for both groups are presented in Table 1.

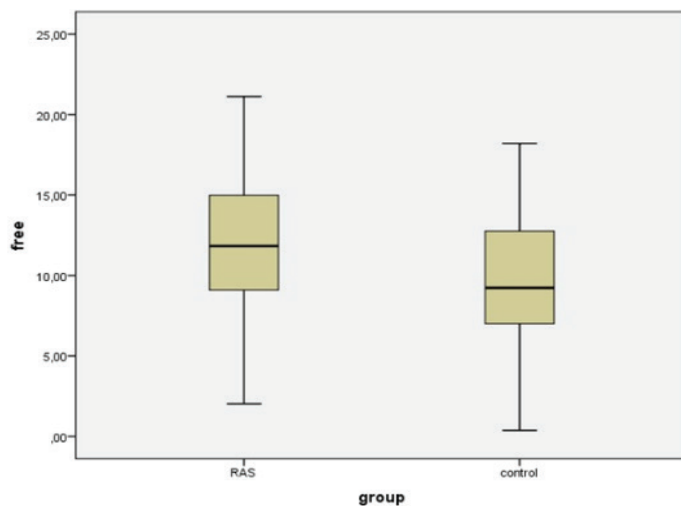
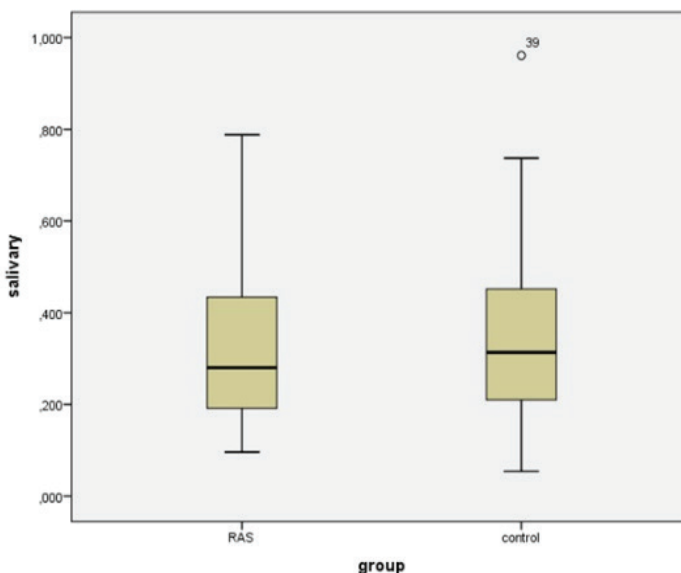
The mean free blood cortisol value was  $12.45 \pm 0.74$   $\mu\text{g}/\text{dL}$  in group RAS, while the mean free blood cortisol values were  $9.8 \pm 0.68$   $\mu\text{g}/\text{dL}$ , in controls. The mean salivary cortisol value was  $0.33 \pm 0.31$   $\mu\text{g}/\text{dL}$ , in group RAS. The mean salivary cortisol value was  $0.34 \pm 0.35$   $\mu\text{g}/\text{dL}$ , in controls (Figure 1 and 2). There was a statistically significant difference in the free cortisol values in group RAS and controls ( $p = 0.011$ ). On the contrary, there was no statistically significant difference between patients with RAS and the controls regarding to salivary cortisol levels ( $p = 0.82$ ).

There was not statistically correlation free cortisol and salivary cortisol level between psychoanalytic tests.

**Table 1.** Clinical characteristics , laboratory profile and Psychoanalytic Tests results of study participants

	SAD	SP	OCD
Age(y)	34.7 ± 2.0	36.08 ± 2.1	0.61
Female/Male	24/16(60%/40%)	26 /14 (%65/%35)	
Free cortisol (µg/dL)	12.4± 0.74	9.8 ± 0.6	0.011*
Salivary cortisol (µg/dL)	0.33± 0.03	0.34± 0.03	0.827
BDRS	14.7± 1.6	11 ± 1.3	0.187
BAS	15.1± 1.9	10 ± 1.7	0.106
PSS	19.2± 1.05	17.5 ± 1.04	0.390
STAI-S	39.5± 1.49	37.9 ± 1.4	0.461
STAI-T	47± 1.20	46.4 ± 1.5	0.760

Beck Depression Rating Scale ( BDRS ), Beck Anxiety Scale (BAS), Perceived Stress Scale (PSS), State-Trait Anxiety Inventory (STAI-S and STAI-T) results shown that mean± standard deviation , \* p<0.05

**Figure 1.** Blood Free cortisol levels (µg/dL) in patients with Recurrent Aphthous Stomatitis (RAS) and in control group**Figure 2.** Salivary cortisol levels (µg/dL) in patients with Recurrent Aphthous Stomatitis (RAS) and in control group

## Discussion

In this study, there was no statistically significant difference between BDRS, BAS, PSS, STAI-S and STAI-T value in both groups.. When the patients with RAS compared to controls, there was no statistically significant difference between any of the psychoanalytic test results. The blood cortisol levels of patients with RAS was statistically significant higher than controls. However, there was no statistically significant difference between two groups regarding to salivary cortisol levels.

It is considered that RAS activation is associated with stress and anxiety, with more frequent recurrence in stressful situations [13-15]. Gallo et al. applied a psychoanalytic test created by the Psychology Institute of Sao Paulo University (Symptoms of Stress List; SSLVAS questionnaire) to RAS patients. Compared to the controls , patients with RAS were found to experience higher psychological stress in the acute period. [16] Zadik et al. compared patients with aphthous stomatitis without RAS history with patients with RAS they found that patients with RAS had higher both anger and anxiety level [17]. A study by Huling et al. applied the Recent Life Changes Questionnaire (RLCQ) with mental and physical stressor components to 160 patients with RAS . This study found that patients with experienced RAS attack when faced with mental stress rather than physical stress. However, they stated the presence of stress did not affect the duration of active period of RAS [7]. Similarly, Keennan et al. applied the Recent Life Changes Questionnaire (RLCQ) to patients with RAS .they stated that mental stressors were more effective on RAS activation than physical stressors; however, the stress severity did not affect the duration and frequency of RAS [18].

Kandagal et al. monitored 278 patients with anxiety and 398 patients with depression. In this study, the Hamilton anxiety and depression scale were applied. The patients' results were compared with 676 controls. In the 6-month follow-up duration, 20% of patients with anxiety and 9% of patients with depression experienced RAS. The RAS rate in the control group was stated to be 5%. In conclusion, they stated that anxiety and depression had a definite place in the etiology of RAS [19]. Contrary to this study , the study by Varkal et al. applied the STAI-S, STAI-T and Beck depression inventory to RAS patients. The STAI-S test results were higher for patients

with RAS; however, there was no difference identified in terms of depression among patients with RAS [20]. In agreement with this study, other studies found no significant association between depression and RAS [12,13].

A study by Karthikeyan et al. investigated the blood, salivary and urinary cortisol levels in 30 patients with RAS and 30 patients with oral lichen planus. The blood, salivary and urinary cortisol levels were higher in patients with RAS than controls [21]. Albanidou-Farmaki et al. found that Patients with RAS had higher both blood and saliva cortisol levels than controls [22]. However, Valle et al. found no statistically significant difference between patients with RAS and controls regarding to salivary cortisol levels [23]. A study by Nadendla et al. investigated the salivary cortisol and Hamilton anxiety scale points. They identified both salivary cortisol levels and anxiety levels in patients with RAS were higher compared to the controls [24]. Another study compared the salivary cortisone levels, Hamilton anxiety rating scale (HARS) and Hamilton depression rating scale (HDRS) and compared psychoanalytical test results with the control group. The results of the study did not identify a statistically significant difference between salivary cortisol levels and HARS results in RAS patients compared to the control group. However, the HDRS index in RAS patients was found to be higher than the control group. In this study, only depression levels were found to be high in RAS patients [25]. McCartan et al. studied two groups. The first group comprised 12 RAS patients with oral lesions which did not respond to vitamin treatment. The second group comprised 12 patients with RAS diagnosis who responded to vitamin treatment. The salivary cortisone levels, HARS and HDRS psychometric analyses were compared in patients in these two groups. In conclusion, the first group of patients who did not respond to treatment had much higher both cortisone levels and anxiety levels compared to the other group [12]. However, in the literature there are publications stating there is no correlation between stress levels and anxiety with RAS. These authors stated that psychological factors had no effect on the etiology and severity of RAS [26-28].

The strong aspects of this study are that all blood and saliva samples taken from patients with RAS were taken in the active RAS period and from 9-10 AM. Psychoanalytic tests were again applied in the active period. The laboratory findings of all patients were normal and no anemia or vitamin deficiency was identified. Patients were chosen from the minor RAS group. All examinations were performed by the same otolaryngologist. Different to previous studies, broader psychoanalysis was performed with the BDRS, BAS, PSS, STAI-S and STAI-T. Psychoanalytical tests were assessed by the same specialist psychologist blind to the study. A limitation of the study is that salivary cortisol was only assessed once.

## Conclusions

This study identified that the idea that RAS patients have higher depression levels, higher stress perception levels and greater anxiety was not correct compared to a control group. Identification of high blood cortisol levels in RAS patients may be due to pain or sympathetic activation. The results of this study found that the place of stress, anxiety and depression in RAS etiology is suspect. Further studies with larger numbers of cases will be beneficial.

## Competing interests

*The authors declare that they have no competing interest.*

## Financial Disclosure

*There are no financial supports*

## Ethical approval

*This study was approved by the Institutional Ethics Committee and conducted in compliance with the ethical principles according to the Declaration of Helsinki.*

*Isil Cakmak Karaer ORCID: 0000-0002-9429-2248*

*Ayca Urhan ORCID: 0000-0002-50359711*

*Ismail Reyhani ORCID: 0000-0002-2573-0996*

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