

# Stress as a Cause of Recurrent Aphthous Stomatitis and Its Correlation with Salivary Stress Markers

Kirthana Kunikullaya U.<sup>1</sup>, Arun Kumar M.<sup>1</sup>, Vasanthi Ananthkrishnan<sup>1</sup>,  
and Goturu Jaisri<sup>2</sup>

<sup>1</sup>Department of Physiology, M S Ramaiah Medical College and Hospitals, MSR Nagar, MSRIT Post, Bangalore, Karnataka 560054

and

<sup>2</sup>Department of Physiology, International Medical School, MSR Nagar, MSRIT Post, Bangalore Karnataka 560054, India

## Abstract

Stress causes an increase in cortisol and amylase. Recurrent aphthous stomatitis (RAS) results due to a multitude of causes, amongst which stress is one of the most important. Aim of the study was to estimate the level of stress, serum cortisol, salivary cortisol, amylase and electrolytes in subjects with RAS. Thirty-four subjects with RAS (cases) were compared with 34 controls. Stress was measured using state trait anxiety inventory (STAI). Serum cortisol (radioimmunoassay), salivary cortisol, amylase (enzyme-linked immunosorbent assay, ELISA) and electrolytes (flame photometry) were measured. Statistical analysis was done using SPSS 18.0 version software. The mean STAI scores were  $48.71 \pm 4.6$  in cases and  $46.74 \pm 6.4$  in controls ( $P = 0.13$ ). The mean salivary cortisol concentration was  $3.35 \pm 1.8$  ng/dl in cases and  $3.65 \pm 2.5$  ng/dl in controls ( $P = 0.78$ ). The mean salivary amylase was  $155.09 \pm 116.1$  U/ml in cases and  $128.74 \pm 86.3$  U/ml in controls ( $P = 0.49$ ). The salivary sodium ( $0.24 \pm 0.4$  in both groups) and potassium ( $0.65 \pm 0.5$  in cases and  $0.82 \pm 0.4$  in controls;  $P = 0.07$ ) was not different in the two groups (electrolytes in mEq/dl). No correlation was seen between the salivary stress markers and STAI scores. Though stress was higher in RAS group none of the measured parameters were different from the control group. Stress may cause RAS but, in this study, there was no change in the salivary homeostasis.

**Key Words:** recurrent aphthous stomatitis, salivary amylase, salivary cortisol levels, STAI questionnaire, Stress

## Introduction

The common causes of oral ulcers are trauma, recurrent aphthous stomatitis (RAS), microbial infections, mucocutaneous disease, systemic disorders, squamous cell carcinoma and drug therapy. Of all non-traumatic recurrent mouth ulcers, RAS or canker sores are the most common (7).

RAS was first described by Hippocrates in 360

B.C. It is one of the most common oral mucosal diseases, affecting one out of five in a population worldwide. It is characterized by small (usually 1-2 mm wide) painful ulcers which typically have red borders and yellow-gray centers. It has further been classified into three types, minor, major, and herpetiform. Minor aphthous ulcers are recurrent, small painful ulcers with necrotic centers, lasting less than ten days. Major aphthous ulcers are similar to

the minor but are larger, deeper, often giving rise to scars and lasting for weeks to months. Herpetiform ulcers are the least common and appear as small, numerous ulcers which can coalesce (7).

Several factors have been identified, which can trigger the onset of RAS. These include stress, genetic factors, immunological and nutritional deficiencies (thiamine, folate, Vitamin B<sub>12</sub>, iron), and infectious agents (human herpes virus 6). Precipitating factors like smoking, local trauma, drug allergy, food sensitivity (e.g. benzoic acid or cinnamaldehyde), and hormonal imbalance have been identified as less common triggering factors. However, as observed in previous literature, stress is one of the precipitating causes of RAS (2, 11, 18).

Stress is a biological reaction due to various causes. It is a complex process, the complete mechanism of which has not been entirely understood. However, various attempts have been made in order to quantify the level of stress by means of questionnaires, immunological markers, heart rate variability, psychological tests and other physiological parameters. Blood biomarkers such as catecholamines, cortisol, amylase, interleukins and many more have been investigated to quantify the level of stress. Recently saliva has been recognized as a good biological fluid for the detection of stress markers due to the ease of its collection and at the same time, it is not painful for the subjects due to non-invasive method of collection and it does not require any expertise. But, detection of saliva biomarkers can be equally sensitive as blood biomarkers (24).

The hypothalamo-pituitary-adrenal system (HPA) and the sympatho-adrenomedullary system (SM) are the two neuroendocrine systems that get activated when one is exposed to stress. It is the activation of the HPA axis that increases cortisol levels (6, 17). Cortisol can be measured in the various biological fluids such as plasma, serum, urine and saliva. However, blood sampling increases the stress of venipuncture. When acute changes in cortisol levels are measured, using urine samples, the results may be inconclusive, this is a distinct disadvantage. Thus, saliva cortisol is clearly a better approach as it is stress free, correlates well with blood cortisol levels and accurately reflects HPA activity (12). Although, saliva is a good marker for measuring HPA activity (cortisol), it has been observed that a similar measurement of salivary catecholamines may not indicate the SM activity with the same accuracy (26).

The salivary glands receive sympathetic and parasympathetic innervation. Parasympathetic stimulation causes release of profuse amount of saliva that is watery and poor in organic constituents. Sympathetic stimulation results in release of small

amount of saliva at reduced salivary flow rate that is hypertonic and rich in organic constituents, potassium (K<sup>+</sup>), but relatively depleted of sodium (Na<sup>+</sup>) and chloride (Cl<sup>-</sup>) (10). The major enzyme that is released on sympathetic stimulation is alpha ( $\alpha$ ) amylase. Thus it is presumed that, changes in salivary alpha amylase indicate the activity of the SM system during stress (5). Further, both salivary cortisol and alpha amylase have been used by a number of researchers recently to quantify stress or as biomarkers of stress (14, 19, 27). Nevertheless, it has also been put forth that salivary amylase activity is an index of plasma norepinephrine levels under a variety of stressful conditions (25).

We hypothesized that RAS is a multifactorial condition of which; increased stress is one of the precipitating factors. Stress in turn may present with enhanced salivary amylase, cortisol levels and K<sup>+</sup> ions due to activation of HPA and SM systems. The present study was aimed to evaluate the levels of stress by using state trait anxiety inventory (STAI), a standardized stress questionnaire, in subjects with RAS. Subsequently, the biological stress was quantified using serum cortisol and salivary stress markers such as amylase, cortisol and electrolytes.

## Materials and Methods

### Study Design

A case-control study was conducted on 68 subjects divided into two equal groups based on presence (n = 34) or absence of RAS (n = 34). The subjects in the control group were age and sex matched. Inclusion criteria were all healthy subjects who are mentally and physically fit, presenting with active RAS, aged between 18 to 30 years. The exclusion criteria were fever, history of diabetes mellitus, hypertension, nutritional deficiency, traumatic ulcers, HIV infection, Behcet's disease, Crohn's disease, Coeliac disease, Addison's and Conn's disease, infectious disease, history of drug intake like steroids, nicorandil, anti-inflammatory drugs, oral nicotine replacement therapy and smoking. We also excluded subjects on any other drug that is known to disturb the autonomic function or cortisol and amylase levels.

### Basis of Sample Size

Thirty four students were taken in each group based on the study by Farmaki *et al.*, with salivary cortisol levels in students with RAS ( $1.44 \pm 0.58$   $\mu\text{g/dl}$ ) and in controls ( $0.91 \pm 0.56$   $\mu\text{g/dl}$ ). The alpha error was considered as 5% and the beta error as 20% with clinically significant difference (minimum expected difference) of 0.4  $\mu\text{g/dl}$  between the two

groups (1).

### Methodology

The study was cleared by the institutional ethical and scientific review board. After explanation of the study protocol, written informed consent was obtained from all the subjects. General socio-demographic details of the subjects were collected and recorded on to a pre-structured pre-tested proforma. The general health status of all subjects was assessed.

### STAI Self Evaluation Questionnaire

Stress levels were measured using Spielberger STAI self-evaluation questionnaire on both groups of subjects. STAI is a self-evaluation reliable questionnaire used to measure the level of stress (8, 13).

### Serum and Saliva Collection

Serum and saliva samples were collected between 8 – 10 a.m. in order to avoid diurnal variations. Collection of serum and measurement of cortisol is as explained in Arun Kumar *et al.*, (3). Then the samples were centrifuged at 3000 rpm for 15 min and supernatant was stored at -20°C until further analysis. Saliva electrolytes were estimated using flame photometry. Saliva Cortisol was measured using enzyme-linked immunosorbent assay (ELISA, DRG International, Inc., Springfield NJ, USA). The interassay and intraassay coefficient of variation were <8% (n = 12) and <5% (n = 20) respectively. Salivary alpha amylase was also measured using a commercially available ELISA kit (LDN diagnostica, Nordhorn, Germany). The intraassay and interassay coefficient of variation was <1.5%.

### Statistical Analysis

Descriptive statistics of salivary cortisol, amylase, electrolytes and stress scores were analyzed and expressed as mean and standard deviation (SD). Independent *t*-test was used to compare all the above parameters between 2 groups. Pearson correlation coefficient was used to find the relationship between stress score and salivary stress markers. *P* value of  $\leq 0.05$  was considered as significant.

## Results

The mean (SD) age of subjects with RAS was 23.29 (4.4) and that of controls was 22.36 (3.3), and the difference was comparable (*P* = 0.237). There were 16 males and 18 females in group 1 and 12 males and 22 females in group 2 (*P* = 0.460). There

**Table 1. Comparison of serum cortisol, salivary stress markers and stress score between RAS cases and controls represented as mean (SD)**

Parameters	RAS Cases (N = 34)	Controls (N = 34)	<i>P</i>
Salivary amylase	155.07 (116.1)	128.74 (86.3)	0.292
Salivary cortisol	3.35 (1.8)	3.65 (2.5)	0.581
Salivary sodium	0.34 (0.3)	0.30 (0.2)	0.641
Salivary potassium	0.65 (0.5)	0.82 (0.4)	0.102
Serum cortisol	114.97 (75.2)	295.78 (192.7)	0.001*
STAI score	48.71 (4.6)	46.74 (6.4)	0.127

RAS: Recurrent aphthous stomatitis; STAI: State trait anxiety inventory; SD: Standard deviation; N: Number of subjects; *P*: Probability value; \*: *P* value statistically significant.

**Table 2. Correlation of salivary parameters with serum cortisol in RAS and control group**

Salivary stress markers	RAS cases		Control group	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
Salivary sodium	0.051	0.776	-0.035	0.846
Salivary potassium	0.294	0.091	-0.075	0.673
Salivary cortisol	0.112	0.527	0.276	0.114
Salivary amylase	-0.114	0.521	0.014	0.938

STAI: State trait anxiety inventory; *P*: Probability value; *r*: correlation co-efficient

was a statistically significant difference between the two groups with respect to serum cortisol levels with higher levels in the control group (*P* = 0.001). Further, there was statistically no significant difference in the mean levels of Salivary cortisol (*P* = 0.782), sodium (*P* = 0.641). The stress levels and salivary amylase levels were higher and salivary potassium levels were lower in RAS subjects, though the difference was statistically not significant.

None of the salivary parameters were correlating with serum cortisol or with each other, except for the salivary cortisol with stress scores (STAI) among controls, but this correlation is not strong enough ( $r^2 = 16\%$ ) [Not shown in the table].

## Discussion

In this study we evaluated the levels of stress, salivary stress markers such as salivary cortisol, serum cortisol, alpha amylase and electrolytes among

a group of RAS cases and compared these with controls without any oral lesions.

RAS is thought to be a stress related response. The sympathoadrenal system or the hypothalamo-pituitary axis that gets stimulated during periods of stress, fails to return towards basal levels in RAS. During stressful conditions, the body's immune mechanisms are hampered to a certain extent (4). This causes the stress hormones and biomarkers to increase. The stress levels of the patients was higher than controls in this study (statistically insignificant) and the increase in stress levels might precede and persist even after the levels of the biomarkers have almost returned to normal (6, 12, 25).

Cortisol is related to the HPA axis and this along with adrenocorticotrophic hormone (ACTH), varies both in acute and chronic stress. During stress, this change in the cortisol level is sustained and it is directly proportional to the severity of stress. Serum cortisol is directly related to salivary cortisol, especially during stress (8). Stress and anxiety are thought to precipitate the occurrence of RAS with elevations in salivary cortisol levels (9, 15). Psychological stress can be measured using questionnaires like STAI. Further, STAI was shown to be significantly associated with serum and salivary cortisol levels among RAS cases (1). In this study a significant decrease in serum cortisol levels was observed. The salivary cortisol remained unchanged among RAS cases. This could be due to the changes in the saliva being slower than the changes that occur in the blood.

Hormones related to the SM system are epinephrine and norepinephrine. Estimation of their levels in response to stress has its own limitations like shorter half life of these hormones. Hence salivary gland activity is measured as an indirect measurement of SM system in response to stress. When salivary glands are maximally stimulated the saliva is rich in enzymes with change in electrolyte composition as explained before. Recently a few studies have used salivary alpha amylase as a reliable biomarker of stress (14, 19, 21, 23, 27, 28). Alpha amylase is peculiar, as it does not diffuse into saliva from the blood but is produced in the acinar cells of the salivary glands, its secretion is independent of flow rate and does not correlate to changes in cortisol (22). It correlates well with the sympathetic activity and concentration of norepinephrine (4, 20).

As the saliva flows from the acini *via* the ducts, its composition is varied. This process is influenced by neural and other chemical stimuli. Sodium and chloride ions are reabsorbed and potassium and bicarbonate ions are secreted. When there is maximal stimulation of salivary glands, the saliva is rich in sodium and chloride concentration, while with slower

salivary secretion rates the concentration of salivary amylase, potassium and bicarbonate increases. In this study the change found in salivary sodium and potassium indicated increased salivary flow, though statistically insignificant.

Saliva protects the oral mucosa due to presence of lysozymes and immunoglobulins. Alpha amylase is the salivary enzyme that initiates carbohydrate digestion. It is important to note that the salivary enzymes which when secreted without food in the oral cavity can lead to adverse changes in the mucosa. It could be presumed that even a slight increase of this enzyme along with imbalance in the protective immune mechanisms could trigger the event of RAS (ulcer) initiation. Further, sustained rise in cortisol levels with amylase could trigger the pathophysiological sequence of ulcer progression.

Contrary to our hypothesis, there was no significant difference between salivary cortisol, amylase, sodium or potassium levels among subjects with RAS when compared with controls. There was a mild increase in alpha amylase level, but this is possibly due to the transient rise during the collection procedure. The failure to find a rise in the salivary stress markers could also be that there is a reactive increase in the salivary flow rate during active RAS (16). However, the flow rate was not measured in this study and thus a conclusive cause may not be commented upon. Other techniques for measuring the actual sympathetic activity such as heart rate variability would have been better in rightly interpreting our results. One of the reasons for failure to find any rise in salivary stress markers among RAS cases in this study could be because of the variation in the severity of RAS as salivary markers vary with the degree/extent of RAS. However, stress levels were higher among the cases along with serum cortisol values in our study similar to previous literature.

This study has shown that, though stress levels are mildly higher among patients with RAS, the salivary biomarkers were not significantly elevated. RAS may be precipitated by stress, but since salivary homeostasis remained unchanged, it could be caused by various other factors as explained. Thus future studies, looking into the limitations of the present study are encouraged.

### Conflict of Interest

The authors declared no conflicts of interest.

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