



Comparing salivary level of alpha-amylase in patients with recurrent aphthous stomatitis and healthy individuals

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Original Article

Abstract

BACKGROUND AND AIM: Recurrent aphthous stomatitis (RAS) results due to a multiple of causes, amongst which stress is one of the most important factors. On the other hand, salivary alpha (α)-amylase (SAA) is a secretory protein that increases in stress conditions. This study evaluated SAA level in subjects with RAS.

METHODS: In this case-control (descriptive-analytical) study, unstimulated saliva samples were collected from 27 patients with RAS and 29 healthy controls. SAA activity was determined by spectrophotometric method using commercially available kit according to manufacturer procedure. Data were analyzed using SPSS software with t test ($P < 0.05$ was considered significant).

RESULTS: SAA level in patients with RAS was 80.78 ± 4.69 U/ml and 65.61 ± 27.52 U/ml during recurrence and recovery, respectively ($P = 0.005$). SAA level in control group was 19.99 ± 4.65 U/ml. There was a significant difference in the SAA level between RAS and control groups.

CONCLUSION: SAA level has been increased in patients with aphthous ulcer during recurrence, which may indicate an association between aphthous ulcer and stress.

KEYWORDS: Aphthous Stomatitis; Salivary Alpha-Amylases; Stress

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One of the most common oral ulcers is recurrent aphthous stomatitis (RAS). These ulcers are characterized by a painful ulcer with a yellowish gray pseudomembranous center and an erythematous ulcer halo.¹

Most patients with aphthous ulcer have multiple episodes of this disease during a year. The accurate mechanism of aphthous ulcer formation is still not clear; the causative agents of aphthous ulceration include trauma, stress, nutritional deficiencies (vitamin deficiency, especially B12, zinc, folic acid, iron), inheritance, and genetic factors.^{1,2}

Stress is assumed to be a stimulating factor in the onset of RAS. Previous studies suggest

that psychological disorders such as stress and anxiety can contribute to the onset and recurrence of RAS lesions.³

Saliva biomarkers are collected quickly and non-invasively compared to blood and urine samples. As a result, the patient is more satisfied.

The salivary alpha (α)-amylase (SAA) enzyme is created by the salivary glands and its primary role is to begin the digestion of carbohydrates. The sympathetic autonomic nervous system (SANS) controls its release and this enzyme plays an important role in psychological-biological stress. Therefore, one of the signs of stress and anxiety is SAA.^{4,5}

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RAS is caused by a variety of causes, among which stress is one of the most important factors; also, SAA is a secretory protein that increases under stress conditions. This study evaluated SAA levels in subjects with RAS.

Methods

This case-control study was performed in School of Dentistry, Zahedan University of Medical Sciences, Zahedan, Iran. 27 patients with RAS and 29 healthy controls were enrolled after obtaining informed consent from them. The study protocol was confirmed by the Ethics Committee of Zahedan University of Medical Sciences (code: IR.ZAUMS.REC.1392.6270).

Inclusion criteria included active minor aphthous ulcer (for case group), age of 20-40 years, no other oral ulcers or history of systemic disease, or drug and tobacco use. The control group was matched according to age and sex with the case group.

The recognition of RAS was based on the principles explained by Preeti et al. RAS diagnosis is made when there are four major criteria and one minor. Major criteria include the appearance of the lesion, recurrent history, spontaneous recovery, and painful lesion. Minor criteria include family history, location and duration of the lesion, etc.⁶

People should have avoided eating, drinking, brushing, and exercising 90 minutes before sampling. All samples were collected from 9 to 11 AM. Unstimulated saliva was collected from all subjects in the case and control groups by spitting method. In the spitting method, the individual collects saliva in the mouth and then spits into a pre-weighed tube, frequently once every 60 seconds for 5-15 minutes.¹ Then, collected saliva samples were transferred to test tubes and in order to remove debris, they were centrifuged at 3500 revolutions per minute (rpm) for 20 minutes. Upon transfer to the biochemistry laboratory of Zahedan School of Medicine, supernatants were stored at -20 °C until analysis.

To measure the activity of SAA, the frozen

supernatants were melted at laboratory temperature for about half hour and diluted 1:100 with physiological saline solution. SAA activity was determined using commercially-available kit (Pars Azmoon Company, Iran), according to manufacturer procedure. This method detects activity of α -amylase through two reactions using 4,6-ethylidene-(G7)-p-nitrophenyl-(G1)- α -D-maltoheptaoside (EPS-G7) as substrate. α -amylase will cleave the substrate EPS-G7 to produce smaller fragments that are eventually modified by α -glucosidase, causing the release of a p-nitrophenol which can be spectrophotometrically measured at 405 nm. The rate of SAA present in the specimen is directly proportional to the increase in absorbance at 405 nm.

Data analysis was performed utilizing SPSS software (version 20, IBM Corporation, Armonk, NY, USA). Kolmogorov-Smirnov test (K-S test) was utilized to assess normal distribution of the variables. The independent t-test compared SAA levels in the two groups (case and control). Paired-samples t-test was used to compare SAA level during recurrence and recovery in patient group. $P < 0.05$ was considered significant.

Results

In the RAS group, 13 (48.10%) subjects were women and 14 (51.90%) were men. In the healthy group, 13 (44.83%) subjects were women and 16 (55.17%) were men. The mean age of RAS group was 29.11 ± 4.08 years and of healthy group was 27.52 ± 3.70 years. The control group was coordinated according to age and sex with the patient group. The normal distribution of data was assessed by the K-S test. The SAA level had a normal distribution in both groups. SAA level in patients with RAS was 80.78 ± 4.69 U/ml and 65.61 ± 27.52 U/ml during recurrence and recovery, respectively. A significant difference was in the SAA level during recurrence and recovery (Table 1).

SAA level in subjects with RAS was 80.78 ± 4.69 U/ml and at healthy group was 19.99 ± 4.65 U/ml.

Table 1. Comparison of alpha (α)-amylase level in patients with recurrent aphthous stomatitis (RAS) during recurrence and recovery phases

| Variable | Patients with RAS | | P* |
|------------|----------------------------------|--------------------------------|-------|
| | Recurrence phase (mean \pm SD) | Recovery phase (mean \pm SD) | |
| SAA (U/ml) | 80.78 \pm 4.69 | 65.61 \pm 27.52 | 0.005 |

*Paired samples t-test

RAS: Recurrent aphthous stomatitis; SAA: Salivary alpha-amylase; SD: Standard deviation

There was a significant difference in SAA level between RAS and healthy groups (Table 2).

Discussion

The present study investigated SAA activity in subjects with RAS and healthy individuals. SAA level was significantly higher in subjects with RAS than in healthy individuals ($P = 0.001$).

The predisposing factors related to RAS include genetic agents, hematologic or immunologic abnormalities, heredity, and local factors such as trauma. Although the specific defect remains unknown, there is much research stating that immune dysfunction is related to RAS. Other factors that are associated with RAS include anxiety and periods of psychological stress.¹

Increase of SAA during psychosocial stress may be explained by physiological reaction to stress. Shirasaki et al. reported the correlation of SAA levels with pain scale in chronic pain of patients.⁷ Increased specific activities occur in the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic-adrenal-medullary system (SAM) in reaction to psychosocial stress. Increased SAA secretion can be seen with SAM activity. Many research reported that SAA revealed the adrenergic activity and therefore may be used as an acceptable criteria of the SAM activity during stress.^{4,5}

Hormones related to the SAM system are epinephrine and norepinephrine. These hormones have a short half-life; therefore,

salivary gland activity is measured as an indirect measurement of SAM system in response to stress. Recently, some studies have used SAA as a reliable biomarker of stress.^{8,9} Rashkova et al. showed a two-fold increase in SAA concentration in stress and its reduction in a stress-free condition.¹⁰

Many studies have reported the association of RAS and stress.

Gallo et al. suggested that psychological stress might play a role in RAS; they pointed out that it might be as a trigger or a modifying aspect instead of being a cause of RAS.¹¹ Nadendla et al. reported a significant increase in salivary cortisol and anxiety levels in subjects with RAS compared to control group.³

Kunikullaya et al. noted that the salivary enzymes when secreted without food in the oral could lead to adverse effect in the mucosa.¹² They believed that even a slight increase of α -amylase along with imbalance in the protective immune mechanisms could trigger the event of RAS initiation. In the study of kunikullaya et al., although the SAA level was not significantly different in patients with RAS compared to the healthy group, it was higher in the patient group.¹²

The results of kunikullaya et al.'s study¹² are contradictory to the present study, which may be due to differences in the sampling method of SAA measurement or the differences in social status of the cases studied.

Similar studies to this study were limited, so comparison with other studies was not possible.

Table 2. Comparison of alpha (α)-amylase level in patients with recurrent aphthous stomatitis (RAS) and control group

| Variable | Patients with RAS (mean \pm SD) | Control group (mean \pm SD) | P* |
|------------|-----------------------------------|-------------------------------|-------|
| SAA (U/ml) | 80.78 \pm 4.69 | 19.99 \pm 4.65 | 0.001 |

*Independent samples t-test

RAS: Recurrent aphthous stomatitis; SAA: Salivary alpha-amylase; SD: Standard deviation

Another limitation of our study was the small sample size, which was difficult to find patients who met our inclusion criteria. Also, some patients had no referral after aphthous ulcer healing.

Conclusion

SAA level increased in patients with aphthous ulcer during recurrence, which may indicate an association between aphthous ulcer and stress.

Conflict of Interests

Authors have no conflict of interest.

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