Original Article

Evaluation of Salivary Cortisol Changes and Psychological Profiles in Patients with Recurrent Aphthous Stomatitis

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Abstract

Background and Objective: Some studies suggest that psychological condition and stress can play role in the development of recurrent aphthous stomatitis (RAS). The purpose of this study was to evaluate salivary cortisol changes and psychological conditions in patients with RAS. **Materials and Methods:** Twenty-seven patients (13 males and 14 females, mean age of 32.8 (±10.2) years) with minor RAS and 27 age- and sex-matched controls without RAS participated in this study. The concentration of cortisol (nanomole/L) was measured in samples of unstimulated saliva from patients and controls two times; once during the presence of active lesions and once again when the lesions had healed by immunologic assay. The Hospital Anxiety and Depression Scale was employed to determine psychological condition. Visual analog scale for pain severity was recorded for patients with active lesions episode. Data were analyzed by the SPSS software (version 18.0) using paired and unpaired *t*-tests and Pearson correlation coefficient. **Results:** Salivary cortisol level was lower in patients during active lesions (12.4 ± 5.1) and healing (10.5 ± 3.9) episodes compared to the controls (13.1 ± 3.6) ($P = 0.583$, $P = 0.015$; respectively). There was no significant difference in salivary cortisol between active lesions and healing episodes ($P = 0.943$). Anxiety and depression represented no significant differences between active lesions and healing episodes ($P > 0.05$). Anxiety and depression levels in patients were significantly higher than in controls ($P < 0.05$). Pain severity in active lesions was not significantly correlated to salivary cortisol level, and anxiety or depression scores ($P > 0.05$). **Conclusion:** The findings showed that occurrence of RAS was associated with anxiety and depression but not with alterations of salivary cortisol level.

Keywords: *Cortisol, recurrent aphthous stomatitis, saliva, the hospital anxiety and depression scale*

Introduction

Recurrent aphthous stomatitis (RAS) is a common oral mucosal disorder which affects 5%–25% of the general population. RAS has three types: minor, major, and herpetic form. Minor aphthous ulcer is the most common form of the disease which constitutes a clinical presentation in about 80% of the patients. This minor form is characterized by repeated oral mucosal lesions with solitary or multiple painful ulcers. It is a self-limited disease and heals after 10–14 days.[1]

The etiology of RAS is unknown, although some precipitating factors including genetic factors, food allergies, local traumas, hormonal alterations (e.g., during menstrual period), cessation of smoking, some chemical products, microbial agents, and stress have a role in the development of RAS.[2] Some studies have advocated the relationship between RAS and psychological stress. In Gallo *et al*. study, RAS patients had a higher level of psychological stress in acute episode of the disease in comparison to control group.[3] Zadik *et al*. reported that feelings of anger and anxiety were more common in patients with active RAS compared to those without a history of RAS and RAS patients had higher level of stress.[4] Some reports studying RAS patients have shown that stressful life events have role in new episodes of RAS but not related to the duration of the disease. Of these events, the influence of psychological stresses is more prominent than physical stresses.[5,6] Acute stress is characterized by alterations in catecholamine level. In chronic stress, alterations in cortisol level are more prominent.[7] Cortisol, or stress hormone, is the most important synthesized glucocorticosteroid in the cortex of the adrenal gland. To assess anxiety, measurement of salivary cortisol is superior

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to serum cortisol level. This is because when salivary cortisol level is measured, unbound cortisol is assayed. In addition, collecting salivary sample is noninvasive, does not need trained staff, is relatively nonstressful, and changes in environmental conditions such as temperature, movement, and growth of microorganisms do not change salivary cortisol level.[8,9] So far, limited studies have been done about the relationship between salivary cortisol, as a marker for stress, and RAS. There is controversy among various studies on this topic. Some studies have shown that salivary cortisol is significantly higher in RAS patients compared to those without RAS.[10,11] In contrast, some studies did not find any significant difference between RAS and non-RAS patients regarding salivary cortisol level.[12]

In most previous studies, salivary cortisol was compared between patients and controls. Based on the literature search, no study has compared salivary cortisol level in recurrence and healing periods. The objective of this study was to assess changes of salivary cortisol in RAS patients. Furthermore, the psychological status of the patients was assessed.

Materials and Methods

This study was approved by the Research Deputy of Kermanshah University of Medical Sciences, Kermanshah, Iran. In this study, two groups were included (study group and 2 control groups). The study group consisted of patients with active lesion of minor RAS in oral cavity (the most common type) and control groups consisted of the same patients after healing the lesions and age- and gender-matched healthy people (individuals without the history of RAS). Inclusion criteria of RAS group were having RAS for at least three times in the year. Those who had systemic diseases were taking steroid medications and oral contraceptive pills, pregnant women, and smokers were excluded.

RAS patients were recruited from patients who presented to the Oral Medicine Department of our Dentistry School. The control individuals were recruited from dentistry students. The diagnosis of minor RAS was made by a specialist in the diagnosis of oral diseases based on clinical examination. The objectives and methods of the study were explained to the patients, and if agreed, were included.

First, using the hospital anxiety and depression scale (HADS), the psychological status of the samples was determined. This scale has two subscales (anxiety and depression); each has 7 four-item questions. For each item, score range of 0–3 is considered. Based on this, the total score for each subscale has a range from 0 to 21. Higher scores reflect more severe anxiety or depression.[13-15] Kaviani *et al*. study showed that the HADS and its subscales (anxiety and depression) have appropriate validity, reliability, and internal consistency in Iranian population.[16] HADS scale employed during both healing and active lesion period in patients and compared with control group.

Then, the individuals were asked to completely wash their mouth with water before collecting salivary samples. In all individuals, unstimulated saliva samples (5 mL) were collected from 9:00 to 9:15 am before eating breakfast. The saliva samples were collected once in control group and twice in RAS group (once during active RAS lesions and once during healing period at least 14 days after lesion healing).

The samples were centrifuged for 15 min at 3000 rpm to separate all debris and were kept at −20°C until assayed. When assay was done the temperature of the sample was changed to 37°C. The supernatant inside the tube was separated for measuring cortisol. Cortisol level was assayed by immunologic method and using Salimetrics® ELISA kit.

In RAS group, after collecting saliva samples, during the presence of active lesions the severity of pain was measured by a visual analog scale.

Statistical analyses

The gathered data were analyzed by the SPSS software (version. 18.0, IBM, Chicago, Illinois, USA). In RAS group, to compare salivary cortisol level and the HADS score between active stomatitis and when the lesions had healed (healing episode), the paired *t*-test was used. Salivary cortisol level and the HADS score were compared between RAS and control groups by unpaired *t*-test. To assess the correlation between pain severity with cortisol level, total HADS score, depression, score, and anxiety score the Pearson correlation coefficient was calculated. The significance level was set at 0.05.

Results

There were 27 patients in RAS group including 13 males (48.1%) and 14 females (51.9%) with age range of 17–57 years and mean (standard deviation [SD]) age of 32.8 (10.2) years. In control group, there were 27 patients including 13 males (48.1%) and 14 females (51.9%) with age range of 18–59 years with mean (SD) age of 32.8 (10.5) years. Table 1 presents salivary cortisol level in patients and control group. Salivary cortisol level in a patient with active ulcer was (12.4 ± 5.1) and in healing, episode was (10.5 ± 3.9) that was not significantly different (P = 0.943). Furthermore, salivary cortisol level between the patients with active ulcer (12.4 ± 5.1) and control group (13.1 ± 3.6) was not significantly different (P =0.0.583). However, salivary cortisol level in healing episode of the patient (10.5 ± 3.9) was significantly lower than control group (13.1 ± 3.6) (P = 0.943) [Table 1].

Table 2 presents the HADS score as well as its subscales (anxiety and depression) and their comparisons between RAS and control groups. In addition, comparisons between active ulcer episode and healing episode in RAS groups

Table 1: Comparison of salivary cortisol level between recurrent aphthous stomatitis patients and controls and between active ulcers and healing episode

Group	Salivary cortisol level (nmol/L)	*P**
Patient	12.4±5.1 (active ulcers) 10.5±3.9 (healing episode)	0.943
Control	13.1±3.6	
*P***	0.583 0.015	

*Paired *t*-test; **Unpaired *t*-test

Table 2: Comparison of the Hospital Anxiety and Depression Scale total score and anxiety and depression scores between recurrent aphthous stomatitis patients and controls and between active ulcers and healing episode of recurrent aphthous stomatitis group

Group	Scores	*P**
HADS total score		
Patient	18.6±4.2 (active ulcers) 18.7±2.7 (healing episode)	0.925
Control	13.2±4.3	
*P***	<0.001 <0.001	
Anxiety score		
Patient	2.3±9.6 (active ulcers) 9.8±1.6 (healing episode)	0.646
Control	6.7±1.9	
*P***	<0.001 <0.001	
Depression score		
Patient	8.9±2.7 (active ulcers) 8.8±1.8 (healing episode)	0.757
Control	6.5±3.5	
*P***	0.006 0.003	

*Paired *t*-test; **Unpaired *t*-test. HADS: Hospital Anxiety and Depression Scale

are presented in Table 2. HADS score in patients with active ulcer (18.6 ± 4.2), and healing episode (18.7 ± 2.7) was not statistically significant (P =0.0.925). HADS score in patients with active ulcer (18.6 ± 4.2), and healing episode (18.7 ± 2.7) was significantly higher than control group (13.2 ± 4.3) ($P < 0.001$).

Anxiety level in patients with active ulcer (9.6 ± 2.3) and healing episode (9.8 ± 1.6) was not statistically significant (P =0.0.646). Anxiety level in patients with active ulcer (9.6 ± 2.3) and healing episode (9.8 ± 1.6) was significantly higher than control group (6.7 ± 1.9) (P <.0.001). Depression level in patients with active ulcer (8.9 ± 2.7) and healing episode (8.8 ± 1.8) was not statistically significant ($P = 0.757$). Depression level in patients with active ulcer (8.9 ± 2.7) and healing episode (8.8 ± 1.8) was significantly higher than control group (6.5 ± 3.5) ($P < 0.05$) [Table 2].

In RAS group, pain severity did not have significant correlation with salivary cortisol level ($r = -0.117$, $P = 0.562$), the HADS score ($r = 0.068$, $P = 0.735$), depression score ($r = -0.032$, $P = 0.875$), and anxiety score ($r = 0.163$, $P = 0.416$).

Discussion

Studies have shown relationship between RAS and psychological status.[6,17] In the current study, salivary cortisol level was used as a biologic marker of stress and the HADS was used as a tool to assess psychological status of RAS patients.

In this study, cortisol was studied as a biologic marker for stress. Cortisol is a key hormone in reaction to physical and sociopsychological stresses which secreted in high amounts if repeated stressful situations occur. This high cortisol level will result in adverse effects on health.[18]

Here, it was observed that salivary cortisol level elevated after healing of the ulcers, but not significantly different from the active episode. McCartan *et al*. reported significant elevation of salivary cortisol in RAS patients with healed ulcers compared to those who had active ulcers.[19] There are differences in the methodology of these two studies. Here, we included a single group of patients and active episodes, as well as healing episode, were compared within this group. However, McCartan *et al*. compared these factors between two groups of patients.

The current findings showed that salivary cortisol level was not significantly different between active episode in RAS group and control group. Similarly, in a former study, the authors reported that there was no considerable difference regarding cortisol level between active stomatitis and control groups.[12] In contrast, some studies[10,11,20] showed that salivary cortisol was higher in active episode of RAS than in control group. The exact cause for this discrepancy cannot be explained. However, differences in methods of various studies, limited sample size, and the effect of various biologic and environmental factors in RAS development can affect the results.

In the presented study, the anxiety score was higher in RAS patients group. Similarly, Al-Omiri *et al*. reported that RAS patients experienced a higher level of anxiety than control group did.[14] Also, in another study, higher levels of anxiety were seen in patients with oral lesions, including RAS.[13] In addition to more severe anxiety in RAS patients, development of RAS is more common in anxious patients (12%) than in controls (2.2%).[20] Hence, it seems that anxiety can be a risk factor for oral lesions including RAS. Oral diseases can be affected by

emotional or psychological changes directly or indirectly.[21] Psychological factors can change nervous system markers (catecholamines, adrenaline, noradrenaline, and dopamine), hormonal system markers (cortical, aldosterone), and immune system (T cells, B cells, natural killer cells, and immunoglobulins) and cause initiation and progression of oral diseases.[22] However, the negative impacts of oral diseases on mental health should also be considered. Oral diseases such as RAS, oral lichen planus, candidiasis, and temporomandibular disorders all can have a severe impact on quality of life of patients.[23,24]

Here, depression score was higher in RAS group than in control group. According to a previous study, RAS in depressed individuals (4.02%) was two times more common compared to control group (2.2%).[20] Psychological problems can cause RAS through increasing the immune system activity.[25-28] In contrast to what we observed, Al-Omiri *et al*. reported that the prevalence of RAS was comparable between depressed patients and controls.[14]

According to the current findings, pain severity was not correlated significantly with salivary cortisol level, anxiety severity, or depression severity. Likewise, Al-Omiri *et al*. reported that, in addition to pain severity of lesions, other factors such as number, anatomic location, and duration of lesions are not correlated with the HADS score.[14] Plus, Sherman *et al*. reported that no relationship existed between pain severity of RAS with psychological characteristics of the patients.[29]

Pain is a subjective feeling correlated to some factors such as personal experience, age, and social and racial factors as well as perceptual abilities.[30] In contrast to the current study, Gavic *et al*. reported that a significant relationship existed between RAS lesion pain severity and anxiety.[31] This discrepancy can be attributed to the fact that they used another scale (State-Trait Anxiety Inventory) for assessment of anxiety.

Conclusion

The current study showed that alterations in salivary cortisol during healing of RAS lesions were not significant. Anxiety and depression were greater in patients who suffered from minor RAS so they may be the precipitating factors.

Limitation

Some of the patients with RAS did not come back for gathering the samples after healing of their lesions. Hence, we recruited new patients.

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Conflicts of interest

There are no conflicts of interest.

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