Original Article

Decreased Salivary Cortisol in Recurrent Aphthous Stomatitis Treated with Topical Steroids

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Abstract

Background and Aim: Stress has been associated with recurrent aphthous stomatitis (RAS). The common treatment of RAS is a topical steroid. This study aimed to investigate the difference of salivary cortisol between RAS patients treated with a topical steroid and those without treatment.

Materials and Methods: Thirty-two female patients with RAS participated in this case-control study and were randomly divided into two groups: the case group (n=16) treated with a topical steroid and the control group (n=16) without any treatment. The inclusion criteria comprised of non-pregnant women with RAS. The exclusion criteria consisted of having other diseases, taking medications, and smoking. All subjects were examined for pre- and post-treatment salivary cortisol at the onset of the ulcers in the case group and when the ulcers were healed in the control group. Data on the characteristics of the subjects were collected and presented descriptively, and the difference in salivary cortisol was analyzed using Mann-Whitney-U test and independent t-test with a 95% confidence interval (CI) in SPSS 17.00 software.

Results: The mean salivary cortisol in the pre-treatment group (10.51±5.15 ng/ml) was higher than that in the post-treatment group (9.30±3.77 ng/ml). The mean salivary cortisol at the onset of RAS (9.55±4.03 ng/ml) was lower than when RAS was healed (13.07±3.82 ng/ml) in the control group. There was a significant difference in the mean of pre- and post-treatment salivary cortisol levels between the case and the control groups ($P<0.05$).

Conclusion: Topical steroids may not only reduce inflammation of oral ulcers but also may reduce salivary cortisol in RAS.

Key Words: Recurrent Aphthous Stomatitis, Saliva, Cortisol, Topical Steroid

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Introduction

Recurrent aphthous stomatitis (RAS) is a disease of the oral mucosa with the prevalence of about 5%-25% in the general population. The prevalence is slightly higher in women [1-5]. There are three types of RAS based on the clinical signs: minor RAS (the ulcer size is less than 10 mm), major RAS (the ulcer size is more than 10 mm), and herpetiform RAS (the ulcer size is 2-3 mm with clustered multiple ulcers) [1,6]. The clinical signs of RAS may comprise single or multiple ulcerative lesions with a round or oval, yellowish base and an erythematous halo at the margin accompanied by pain. The ulcer is recurrent, heal within 7-14 days, and may last up to 21 days in each episode [1,6]. The etiology is still unclear although it may be related to several factors. Age, gender, hypersensitivity associated with foods and drugs, bacteria, family history, trauma, and nutritional deficiency may be the predisposing factors of

RAS; moreover, hormonal factors may predispose women to RAS [1]. Studies also have revealed that RAS is associated with stress [2,7,8]. RAS may be caused by the abnormality of the mucosal immune response which causes tissue damage mediated by T cell lymphocyte CD8 [1,9]. Stress may be involved in RAS pathogenesis by changing the immune response of the oral mucosa but the exact mechanism is still unclear [5]. It is well-known that stress may induce the release of cortisol which is a hormone produced in the adrenal cortex in response to stress through the Hypothalamus-Pituitary-Adrenal (HPA) axis. The production of cortisol by the adrenal cortex is initiated by the production of corticotropin-releasing hormone (CRH) in the hypothalamus, which in turn, activates the secretion of adrenocorticotrophic hormone (ACTH) from the pituitary gland. The ACTH stimulates the adrenal cortex to secrete glucocorticoids (cortisol) [10]. In acute stress, there will be an adaptive mechanism of the HPA axis to the stress, but in chronic stress, the production of cortisol decreases, which in turn, fails to activate the negative feedback of cortisol to the HPA axis and results in the dysregulation of cortisol production [11].

The treatment of choice for RAS is steroidal anti-inflammatory drugs; the steroid may be used topically or systemically [12,13]. Usually, RAS is treated using topical steroids [9]. The use of topical steroids may reduce the inflammation, the duration, and the pain of the ulcers. By the use of topical steroids on RAS, the negative side effects of systemic steroids can be avoided. The inflammation and pain in the oral mucosa induced by RAS may cause stress for the patients [1,9]. The stress may reflect as behavioral changes in RAS patients, such as refraining from eating certain foods and limiting the activities which may aggravate the pain [1,6]. A study has revealed that RAS negatively impacts the quality of life of the patients [14]. One of the important factors that may have a negative effect on the quality of life of RAS patients is the pain originated from oral ulcers [14]. Therefore, stress may not only be associated with the occurrence of RAS, but it also may result from RAS [15-17]. Stress may be reflected by the increase of salivary cortisol in RAS patients. Studies have shown that cortisol level in RAS patients is higher than that in healthy controls, which may indicate that a stressful condition may induce an increase in cortisol production by the adrenal cortex [15,18]. The cortisol, which is secreted by the adrenal cortex, may also be present in saliva. Studies have shown that the serum level of cortisol has a strong correlation with the level of cortisol in saliva [19]. Although the increase of the salivary cortisol may be induced by long-standing stress, salivary cortisol may also increase during RAS [15,16] and may decrease with RAS treatment. Salivary cortisol has been used in several studies as a marker of stress in RAS; however, the results of these studies are inconsistent [15,16,18]. It is unclear whether RAS patients in those studies were treated or not. None of the studies investigated the effect of topical steroids on salivary cortisol in RAS patients. Therefore, the aim of the present study was to investigate the effect of topical steroid treatment on salivary cortisol in RAS patients.

Materials and Methods

The subjects of this case-control study were selected from among those who had been diagnosed with RAS at the Department of Oral Medicine of Dental Hospital of the Faculty of Dentistry, Universitas Gadjah Mada, Yogyakarta, Indonesia. They were informed about the study and were asked to participate. The inclusion criteria comprised RAS patients having oral ulcers with the age range of 18 to 30 years old. The exclusion criteria consisted of the presence of systemic diseases or conditions, oral or topical consumption of medications including multivitamins, and smoking. This study was approved by the Ethical Committee for Research of the Faculty of Dentistry of Gadjah Mada University, Yogyakarta, Indonesia (00752/KKEP/FKG-UGM/EC/2016). Informed consent was obtained from all patients. Additionally, information about age, gender, ethnicity, marital status, education level, and predisposing factors of RAS were obtained using a questionnaire. The pain score of RAS was determined using a 10-point visual analog scale (VAS) at the baseline/pretreatment status in both groups and after the treatment (the case group) or when the ulcers were healed (the control group). In the case group, subjects received 0.1% topical

triamcinolone acetonide (TA) in orabase for oral ulcer treatment, which was used four times daily until the oral ulcers were healed. Subjects in the control groups did not receive any medication.

All participants underwent a whole saliva examination to determine salivary cortisol level using the enzyme-linked immunosorbent assay (ELISA) method (salivary cortisol ELISA, DRG Instruments GmbH, Marburg, Germany) [18]. The normal levels of salivary cortisol are 1.2-14.7 ng/ml. The saliva samples were obtained between 9.00 a.m. and 10.00 a.m. in the first visit from subjects with RAS before receiving medication for oral ulcers (the case group) as well as from subjects with RAS who did not receive medications for oral ulcers (the control group). The saliva samples were collected again when the ulcers were healed in both groups.

Statistical analysis:

The demographics of RAS patients were analyzed using descriptive statistics. Shapiro-Wilk test was used to test whether the data were normally distributed. The difference in salivary cortisol between patients with RAS treated with topical steroid and those without treatment was analyzed by independent t-test for normally distributed data and by Mann-Whitney-U test for non-normally distributed data. Paired t-test was used to analyze the difference in salivary cortisol between baseline/pre-treatment and post-treatment (use of topical steroids) states in the case group as well as the difference in salivary cortisol at the onset of ulcers and after the ulcers were healed in the control group. The statistical analyses were performed with a 95% confidence interval (CI) using SPSS 17.00 (SPSS Inc., Chicago, IL, USA).

Results

Thirty-two RAS patients participated in this study. Most of the subjects in the case group were single college students (100%), and most of the subjects in the control group were single Javanese people (93%). Stress and hormonal factors were the predisposing factors in both groups. Subjects in the control group had less than 10 oral ulcers (94%), and the size of the ulcers was less than 10 mm (100%) in both groups. The buccal and labial mucosae were the most common sites of the oral ulcers. All subjects reported oral ulcers with mild pain (Table 1). The level of pre-treatment salivary cortisol in the case group was higher than that at post-treatment, and the level of salivary cortisol at the onset of the ulcers was lower than when the ulcers were healed in the control group (Table 2).

Discussion

This is the first study that investigated the difference in salivary cortisol level between RAS patients treated with topical steroids and those without treatment. RAS is an oral mucosal disease that may show some variations in the clinical signs. The characteristics of RAS may vary regarding the number and the size of ulcers. Higher numbers and larger sizes of ulcers may cause more severe symptoms in RAS patients [20]. Our study revealed that most subjects have multiple small ulcers, i.e., minor RAS [1,6,7,9,21]. This indicates that the severity of the RAS symptoms in both groups was moderate according to the VAS score of pain at the baseline/pre-treatment status. Pain was the most common symptom of RAS patients. The pain was caused by the inflammation of oral ulcers. The pain originated from ulcers may induce stress and result in a poor quality of life for RAS patients [1,14]. None of our subjects had oral ulcers larger than 10 mm or with high pain scores. This is also supported by the relatively similar mean duration of the ulcers in both groups. The oral ulcers in our study healed approximately during 10 days. Although studies have shown that oral ulcers treated by topical steroids heal faster (in less than 7 days) than oral ulcers in control groups [12], several factors may contribute to the delayed healing of oral ulcers in our case group. The important factors that may affect the healing of the ulcers in this study were local trauma and the stress induced by oral ulcers [22]. However, the wound healing duration of oral ulcers in this study was in a normal range. Normally, oral ulcers may take 7-14 days to heal [1,6,9,21].

The present study indicated that the mean VAS scores of pain in both groups were relatively similar at the baseline/pre-treatment status. After the oral ulcers were treated using topical steroids, we found that the VAS score of pain reduced significantly in both groups. A lower VAS score of pain after treatment may be caused by topical steroids which reduce the inflammation of oral

Table 1. Demographics of subjects with recurrent aphthous stomatitis (RAS) and the predisposing factors of RAS

Variables	Case (n=16)	Control (n=16)	P-value
Age (year; mean±SD)	21.44±2.13	21.38±1.99	
Education level: n (%)			
High education	16(100)	12(75)	
Ethnicity: n (%)			
Javanese	9(56)	15(93)	
Marital status: n (%)			
Single	16(100)	16(100)	
Predisposing factors: n (%)			
Stress	4(25)	7(43)	
Hormonal factors	5(31)	1(6)	
Stress and hormonal factors	7(43)	7(43)	
Unknown	0(0)	1(6)	
Oral ulcer's characteristics			
Number of ulcers: n (%)			
<10	11(69)	15(94)	
>10	5(31)	1(6)	
Size of ulcers (mm): n (%)			
<10	16(100)	16(100)	
>10	0(0)	0(0)	
Duration of ulcers (days; mean±SD)	10.19±2.48*	10.56±2.71*	0.686
Location of ulcers: n (%)			
The floor of the mouth			
Tongue	2(6)	1(3)	
Gingiva	5(16)	2(6)	
Labial mucosa	2(6)	4(13)	
Buccal mucosa	10(31)	8(25)	
Soft palate	10(31)	10(31)	
Vestibulum	2(6)	2(6)	
	0(0)	0(0)	

N=number of subjects, mm=millimeter; SD=Standard Deviation,

***: Independent t-test results with non-significant differences (P>0.05)**

Table 2. The difference of salivary cortisol in recurrent aphthous stomatitis (RAS) between the case and the control groups

Variables	Case (n=16) Mean±SD	Control (n=16) Mean±SD	P-value
VAS pain score			
Pre-treatment	5.13±1.59*	5.44±2.58*	0.683***
Post-treatment	1.38±0.88*	2.75±2.17*	<0.05***
Salivary cortisol			
Pre-treatment	10.51±5.15	9.55±4.03*	0.564***
Post-treatment	9.30±3.77	13.07±3.82*	<0.05***
Pre- and post-treatment VAS score difference	3.81±1.72	2.69±1.96	0.095***
Pre- and post-treatment salivary cortisol difference	-1.21±6.90	3.52±5.44	<0.05**

***Intragroup results of paired t-test, significant at P<0.05,**
****Results of Mann-Whitney-U test in the comparison between the case and the control groups,**
*****Results of independent t-test in the comparison between the case and the control groups,**
VAS=Visual Analog Scale, SD=Standard Deviation

ulcers [1]. However, the inflammation may persist longer in the control group and may result in a small change in the VAS score of pain. This is also supported by a systematic review which showed that topical steroids may reduce the pain of RAS oral ulcers compared to the group treated by a placebo [12].

This study used salivary cortisol as an indicator of stress induced by RAS. Salivary cortisol has been used to assess stress in different conditions including RAS [18,23]. Studies have revealed higher salivary cortisol in acute RAS compared to healthy controls [15,23]. The results of those studies may explain that the high level of salivary cortisol may be caused by acute oral ulcers. On the other hand, RAS oral ulcers may cause stress as well [17]. Therefore, it may be stated that the treatment of RAS may not only reduce the inflammation of ulcers and accelerate wound healing but also probably indirectly reduces the stress related to RAS. This is supported by the evidence suggesting that stress may cause several diseases mediated by inflammation [24]. Not all our subjects claimed stress as a predisposing factor of their RAS; there were subjects who claimed hormonal factors as the predisposing factor. However, it is possible that hormonal changes during the menstrual cycle affect the stress response and elevate cortisol in women as all subjects in this study were women [25]. This is supported by the evidence suggesting that stress is one of the predisposing factors for RAS [1,6,9,17,23,26]. Hence, it may be stated that repeated stress may induce the recurrence of RAS. Also, inflammation of oral ulcers may cause psychological distress [17,27]. In this study, we found that the VAS score of oral ulcers was reduced followed by a reduced salivary cortisol level only in the case group. In the control group, the reduced VAS score of RAS oral ulcers was followed by a slight increase in salivary cortisol. These conditions may explain that untreated oral ulcers in the control group may have more inflammation which induces stress in RAS patients, compared to oral ulcers treated with topical steroidal anti-inflammatory drugs.

Topical steroids are the most common choice for the treatment of RAS [21]. Steroids can be used topically as anti-inflammatory drugs for oral ulcers. TA in orabase was the topical steroid used in the present study. TA is a medium-potency steroid which is used as the drug of choice for topical treatment of RAS oral ulcers [28,29]. The mechanism by which steroidal anti-inflammatory

drugs reduce salivary cortisol is still unclear. The possible mechanism is that, similar to steroidal anti-inflammatory drugs, triamcinolone also suppresses the immune system by inducing apoptosis by T lymphocytes, neutrophils, basophils, and eosinophils and suppresses transcription factors such as nuclear factor kappa beta (NF-kβ), pro-inflammatory cytokines such as interleukin (IL)-1β, IL-1α, IL-2,-4,-5,-6,-8,-12, tumor necrosis factor-alfa (TNF-α), interferon-gamma (IFN-γ), and granulocyte-macrophage colony-stimulating factor (GM-CSF) [30,31]. All these pro-inflammatory cytokines (TNF-α, IL-1β, and IL-6) increase in stressful conditions [24].

The activity of topical steroids in reducing cortisol, as an indicator of stress, also can be explained by the evidence suggesting that the inhibition of IL-1β reduces the anxiogenic behavior or stress in RAS. Moreover, the inhibition of IL-1β may reduce depressive behavior caused by TNF-α. The inhibition of IL-6 may inactivate the HPA axis which is responsible for the release of cortisol [24,32].

The present study has some limitations. All the subjects were women; therefore, it was unclear whether a similar result may also be found in men with RAS since different hormonal changes in males and females may influence the results. The second limitation was that we did not assess the stress of the participants using a validated questionnaire. The last limitation was the lack of information regarding the results of blood examinations to assess other systemic conditions, such as certain deficiencies, which may interfere with wound healing or other predisposing factors of RAS.

Conclusion

According to the results, topical steroids may not only reduce inflammation of oral ulcers but also may reduce salivary cortisol in RAS.

Conflict of interest

The authors declare that they have no conflict of interest with regard to this study. Funding has been made available from the authors' institution.

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