Dental Research Journal

Original Article

Evaluation the relationship between psychological profile and salivary cortisol in patients with recurrent aphthous stomatitis

Marjan Mirzaei[1], Mahdieh Zarabadipour[2], Monirsadat Mirzadeh[3]

[1]Department of Oral and Maxillofacial Medicine, Student Research Committee, Qazvin University of Medical Sciences, [3]Department of Community Medicine, Metabolic Diseases Research Center, Research Institute for Prevention of Non-Communicable Diseases, Qazvin University of Medical Sciences, [2]Department of Oral and Maxillofacial Medicine, Dental Caries Prevention Research Center, Qazvin University of Medical Sciences, Qazvin, Iran

Received: 26-Dec-2019
Revised: 28-Jun-2020
Accepted: 20-Sep-2020
Published: 22-Jun-2021

Address for correspondence:
Dr. Mahdieh Zarabadipour, Department of Oral and Maxillofacial Medicine, Dental Caries Prevention Research Center, Qazvin University of Medical Sciences, Bahonar Blvd., Qazvin, Iran.
E-mail: mzarabadipour@qums.ac.ir

ABSTRACT

Background: Recurrent aphthous stomatitis (RAS) is one of the most common oral mucosal diseases which are diagnosed with recurrent and painful ulcers. The possible association between psychological factors and salivary stress related factors in patients with aphthous ulcers has been discussed in various studies. The aim of this study was to evaluate salivary cortisol level, anxiety, and depression in patients with RAS.

Materials and Methods: In this case control study, 30 patients with RAS and 30 healthy individuals were enrolled by matching their age and sex. Anxiety and depression were assessed by beck anxiety inventory and beck depression inventory. Unstimulated saliva of both groups were collected and then tested by DiaMetra kit and the ELISA method. Data were analyzed by SPSS using an Independent *t*-test and Mann–Whitney test. A statistical significance level of <0.05 was considered.

Results: The mean salivary cortisol in the case group was 5.35 ng/ml and in the control group was 4.73 ng/ml which was not statistically significant ($P > 0.05$). There was no significant difference in anxiety and depression level between the two groups ($P > 0.05$). According to Spearman correlation coefficient, there was an average, negative, and significant correlation between salivary cortisol and anxiety and depression scores in the case group ($P < 0.05$).

Conclusion: This study shows that, although the mean salivary cortisol was slightly higher in patients with aphthous lesions, anxiety, and depression were not possible factors for RAS. There was a moderate, negative correlation between salivary cortisol level and stress and anxiety in patients with RAS.

Key Words: Anxiety, aphthous, cortisol, depression, saliva, stomatitis

INTRODUCTION

Recurrent aphthous stomatitis (RAS) is one of the most common oral mucosal diseases with a prevalence of 5%–66%[1-4] and the most common nontraumatic agent of recurrent oral ulcers.[5,6] These lesions are commonly occurring on nonkeratinized mucosa such as labial and buccal mucosa, ventral surface of the tongue, floor of the mouth, and soft palate. They are characterized as one or more round or oval painful ulcers with erythematosus halo.[7,8]

Access this article online

Website: www.drj.ir
www.drjjournal.net
www.ncbi.nlm.nih.gov/pmc/journals/1480

How to cite this article: Mirzaei M, Zarabadipour M, Mirzadeh M. Evaluation the relationship between psychological profile and salivary cortisol in patients with recurrent aphthous stomatitis. Dent Res J 2021;18:50.

RAS is described as recurrent and self-limiting ulcers,[9] which is divided into three types of minor, major, and herpetiform.[3,10] Minor type is the most common form of the disease, which constitutes a clinical presentation in about 80% of the patients.[3] Minor aphthous ulcers are recurrent, small painful ulcers with necrotic centers. The ulceration generally heals without scarring after 10–14 days.[3,6,7,11] Major aphthous ulcers are similar to the minor but are larger, deeper, often giving rise to scars and lasting for weeks to months.[5] Herpetiform ulcers are the least common and appear as small, numerous ulcers which can coalesce.[3,5,7,9]

The etiology of RAS is unknown,[8,9,11] although 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_{12}, and iron), and infectious agents (human herpes virus 6).[5]

Stress is a biological reaction due to various causes. Several attempts have been made 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.[5]

Cortisol is a glucocorticoid hormone secreted through the adrenal cortex and acts on inflammatory and immunological responses.[2] Cortisol production has an ACTH-dependent circadian rhythm with peak levels in the early morning and a minimum at night. The factors controlling this circadian rhythm are not completely defined. The circadian rhythm of ACTH/cortisol secretion matures gradually during early infancy.[1]

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, salivary cortisol is clearly a better approach as it is stress free, correlates well with blood cortisol levels. 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 individuals due to noninvasive method of collection and it does not require any expertise.[5]

Previous studies have suggested that psychological disturbances such as stress and anxiety could play a role in the onset and recurrence of RAS lesions (4.9). However, the obtained results were rather varied and conflicting,[9] some studies have shown that salivary cortisol is significantly higher in RAS patients compared to those without RAS. In contrast, some studies did not find any significant difference between RAS and non-RAS patients regarding salivary cortisol level.[10]

The aim of this study was to investigate the relationship between psychological profile and salivary cortisol in patients with RAS.

MATERIALS AND METHODS

In this case–control study, 60 individuals were selected from patients referred to Qazvin University of Medical Sciences. They were divided into two equal groups that matched for age and sex. The study group consisted of 30 patients with active lesion of RAS, that their lesions appeared during the last 24–48 h and no treatment was performed for the elimination of them. The control group was selected from among the individuals who had no previous history of RAS and oral pains and referred to the Oral Medicine Department of our Dentistry school for routine dental examination. The diagnosis of RAS was made by oral and maxillofacial specialist based on clinical examination.

The study was approved by the Institutional Ethics Committee of Qazvin University of Medical Sciences (IR.QUMS.REC.1397.333). The objectives and methods of the study were explained to the patients and entered into the study after agreeing to obtain consent.

The inclusion criteria for patients were as follows: having aphthous lesions at least 3 times a year in study group, absence of systemic and psychiatric diseases in both groups, no history of taking any steroid, contraceptive pills, saliva reducing drugs and cigarette smoking in both groups, absence of any other oral soft-tissue lesions in both groups, no treatment for the elimination of aphthous lesions in study group (eight-item blood test was performed for all patients to determine the cause of the lesion which consist: Folic acid, CBC, Vitamin B_{12}, Vitamin D, Ferritin, Zinc, Serum Iron, and Total Iron Bonding Capacity), women were not during pregnancy or menstruation. The exclusion criteria for the

participants were as follows: incomplete completion of questionnaires and not willing to complete the questionnaires or participate in the study.

First, the patients were asked to answer beck anxiety inventory (BAI) and beck depression inventory (BDI) questionnaires to assess the level of anxiety and depression. The BAI contains 21 questions, each answer being scored on a scale value of 0 (not at all) to 3 (severely). Higher total scores indicate more severe anxiety symptoms. Each test item describes one of the most common symptoms of anxiety (mental, physical, phobic), the standardized cutoffs are:

- 0–7: minimal anxiety
- 8–15: mild anxiety
- 16–25: moderate anxiety
- 26–63: severe anxiety.

The BDI is designed for individuals aged 13 years and over and consisted of 21 questions about how the subject has been feeling in the last week. Each question had a set of at least four possible responses, ranging in intensity. When the test is scored, a value of 0–3 is assigned for each answer and then the total score is compared to a key to determine the depression's severity. The standard cut off scores were as follows:

- 0–9: indicates minimal depression
- 10–18: indicates mild depression
- 19–29: indicates moderate depression
- 30–63: indicates severe depression.

In the case group, the intensity of pain was measured by visual analog scale (VAS) before the saliva collecting. All participants were advised not to consume any food or drink 1 h before the saliva collection, avoid from taking any medicines or cosmetics on lips and wash their mouth properly before sample collection. All individuals were also asked to stop any physical activity for 30 min before collecting salivary samples, then sit in a relaxed position for 30 min, finally, their nonstimulated saliva was collected into the plastic tubes until 5 ml between 8 and 9 am.

The samples were transferred to the laboratory, and then stored at −20°C until testing. The temperature of all samples was changed to 37°C during testing and centrifuged for 15 min at 3000 rpm to separate the debris. Finally, the measurement of salivary cortisol was obtained by ELISA (enzyme-linked immunosorbent assay) using the DiaMetra kit (Made in Italy).

Data were analyzed by SPSS software version 24 (SPSS Inc., Chicago, IL, USA) and Independent *t*-test and Mann–Whitney tests. The statistical significance level was considered to be $P < 0.05$.

RESULTS

This study was performed on people referring to Qazvin Dental School. Thirty patients eligible for aphthous ulcers were included in the study group and 30 patients without aphthous lesions in the control group. There were 16 male (53.3%) and 14 female (46.7%) in the study group and the number of males and females were equal in the control group. Both the groups were similar in sex and no significant difference was found between them ($P > 0.05$). Mean distribution of age in in case group was 36.83 ± 10.78 and in control group was 35.43 ± 9.35. There was no significant difference between case and control groups ($P > 0.05$)

Table 1 shows the salivary cortisol levels in both groups. The results show that there was no significant difference between case and control groups ($P > 0.05$).

The mean level of anxiety was similar between the two groups, but the mean level of depression was higher in the case group. However, finally, according to Mann–Whitney test, no significant difference was found between anxiety and depression in both groups ($P > 0.05$) [Table 2].

According to Spearman correlation coefficient [Table 3], there was an average, negative, and significant correlation between salivary cortisol and anxiety and depression scores in the case group, which means that if anxiety and depression increased, salivary cortisol decreased, but this association is not strong.

DISCUSSION

RAS is a common ulcerative disease which causes a change in the quality of life because of pain and discomfort. The etiology of RAS is still unknown but many studies have shown a correlation between RAS and psychological conditions such as stress, anxiety, and depression. However, there are contradictory comments regarding this connection.[9]

In this case–control study, salivary cortisol levels, degree of anxiety, and depression in patients with RAS and healthy subjects were compared.

Table 1: Distribution of mean concentration of salivary cortisol in case and control groups

Variable	Group	Mean±SD	*P*
Cortisol	Case	5.35±3.71	0.67
	Control	4.73±2.91	

SD: Standard deviation

Table 2: Mean distribution of anxiety and depression in two groups of case and control groups

Variable	Group	Mean±SD	*P*
Anxiety	Case	8.67±7.94	0.95
	Control	8.67±7.23	
Depression	Case	8.80±6.93	0.44
	Control	8.50±8.66	

SD: Standard deviation

Table 3: Relationship between salivary cortisol level and anxiety and depression scores in case and control groups

Variable	Control		Case	
	r	*P*	*r*	*P*
Anxiety score	−0.19	0.31	−0.45	0.01
Depression score	0.01	0.95	−0.37	0.03

According to inclusion criteria, our study was performed on 60 subjects, 30 of whom were in the case group and 30 in the control group. The case group consisted of 16 men and 14 women and the mean age was 36.83 years. The control group consisted of 15 men and 15 women and the mean age was 35.43 years.

According to the results of this study, the mean salivary cortisol level in the case group was 5.35 and in the control group was 4.73 ng/ml. These values were not statistically significant, which means cortisol levels in saliva is a little higher in case group in comparison with control group and it is similar to the studies of Rezaei *et al*., Kunikullaya *et al*., Eguia-del Valle *et al*., and Michel *et al*.[2,5,10,12] Contrary to the results of the present study, Nadendla *et al*., Albanidou-Farmaki *et al*., and Karthikeyan and Aswath studies found a significant relationship between salivary cortisol levels in both case and control groups.[1,3,13,14] In Nadendla *et al*.'s study, the number of women was more than men, and the mean age of the individuals was 29.2 years, which was lower than the mean age in the present study. Furthermore, in their study, subjects were sampled when the lesions were improved, which is likely to be the cause of the difference in the results of studies due to differences in age, sex and time of sampling. Unlike the present study, Albanidou-Farmaki *et al*. used stimulated saliva and the luminescent immunoassay method to measure cortisol. According to studies, the chemical nature of the stimulatory and nonstimulatory saliva is different and expectation of feedback is not the same. Aghahosseini reported in a study that stimulated saliva could not be a reliable measure for the possible diagnostic evaluation of diseases, and it would be better to consider unstimulated saliva.[15] In addition, according to the previous studies, the chemical nature of nonstimulatory saliva in comparison with the stimulatory saliva was closer to the serum factors of the individual body, therefore, in the present study, nonstimulatory saliva was used. Karthikeyan and Aswath measured serum cortisol, saliva, and urine in patients with aphthous and oral lichen planus by Electrochemiluminescence immunoassay, which in addition to differences in race, may differ in measurement method causes of the difference in results.

The present study used Beck Depression Anxiety and Depression Inventory to assess the level of anxiety and depression. Finally, no significant difference was found between anxiety and depression between the two groups, which is similar to the study of Kunikullaya *et al*., Dhopte *et al*., Zwiri and Pratibha *et al*.,[5,16-18] in these Studies have also shown no association between anxiety and depression and aphthous lesions.

Unlike the present study, Albanidou-Farmaki *et al*., Nadendla *et al*., Michel *et al.,* and Kumar *et al*. reported in their studies that anxiety and depression can be associated with the incidence of aphthous lesions.[1-13] This difference in results can be caused by differences in the studied populations or the test used to clarified anxiety and depression.

According to the study by Al-Omiri *et al*., anxiety and stress are more associated with the occurrence of aphthous lesions,[19] therefore in this study, BAI was used.

In the present study, similar to the study of Rezaei *et al*., no significant relationship was found between the pain of lesions and degree of anxiety, depression, and cortisol concentration ($P > 0.05$).[10] Unlike the results of our study, Gavic *et al.* expressed a significant relationship between pain severity and anxiety ($P < 0/000$).[20] Since pain intensity expression (VAS) is a patient-dependent factor, the response of the patient to the pain severity

questionnaire may vary in different communities and circumstances, so the statistical results in different situations may not be the same.

Unlike the results of the present study, the level of anxiety and depression of women and men was not statistically significant, in the studies of Al-Omiri *et al*. and the Sanatkhani, the level of anxiety and depression in women was higher than men.[19,21]

The relationship between salivary cortisol level and psychological status of the patients with aphthous lesions was average and negative in this study. Data analysis shows that the increase in stress or anxiety of the patient cause salivary cortisol to be decreased. Rezaei *et al*.[10] and Kunikullaya *et al*.[5] reported in their studies that there is not a significant relation between salivary levels of cortisol and stress, but Albanidou-Farmaki *et al*.,[1] Michel *et al*.,[2] and Nadendla *et al*.[3] show in their studies that salivary cortisol level is correlated to psychological profile of the patients. These controversies may be related to the difference in population, study design and methodology of the study.

The main strength of the study was arranging a case–control-based method to compare the data with normal individuals in the same population. However, the study also have some limitations such as patient's cooperation, limited time for examination of the lesion (first 24–48 h of lesion appearance) that may exclude many cases and even limitations in time and budget.

The study describes the emotional and biochemical effects of oral aphthous lesions that affect many patients at least one time in their lives. It also expresses these findings that although oral aphthous stomatitis could change the patient's quality of life, but psychological and biochemical changes are not much more annoying.

Authors suggested the future studies for these items based on a larger sample size, and design the study which compares the geographic situations, jobs, educations, and cultures to find a new concept for etiology of the disease.

CONCLUSION

Based on the results of this study, although the mean cortisol of saliva was higher in the case group but anxiety and depression are not possible factors for the incidence of RAS. In addition, there was no difference in anxiety and depression between women and men. There was a moderate, negative correlation between salivary cortisol level and stress and anxiety in patients with RAS.

Acknowledgments

We would like to thank the respectable staff of the Baran Lab, Qazvin that helped us in preparing this article.

Financial support and sponsorship

Nil.

Conflicts of interest

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or non-financial in this article.

REFERENCES

1. Albanidou-Farmaki E, Poulopoulos AK, Epivatianos A, Farmakis K, Karamouzis M, Antoniades D. Increased anxiety level and high salivary and serum cortisol concentrations in patients with recurrent aphthous stomatitis. Tohoku J Exp Med 2008;214:291-6.
2. Michel AR, Luz C, Wudarcki S, Cherubini K, de Figueiredo MA, Salum FG. Cortisol and dehydroepiandrosterone salivary levels, stress and anxiety in patients with recurrent aphthous stomatitis. Revista Odonto Ciência 2015;30;120-5.
3. Nadendla LK, Meduri V, Paramkusam G, Pachava KR. Relationship of salivary cortisol and anxiety in recurrent aphthous stomatitis. Indian J Endocrinol Metab 2015;19:56-9.
4. Aytuğar E, Borahan BO, Pekiner FN. Comparison of depression and anxiety levels in patients between Behçet's disease and recurrent aphtous stomatitis. Marmara Dent J 2013;1:39-41.
5. Kunikullaya UK, Kumar MA, Ananthakrishnan V, Jaisri G. Stress as a cause of recurrent aphthous stomatitis and its correlation with salivary stress markers. Chin J Physiol 2017;60:226-30.
6. Gökler ME. Prevalence of oral apthous ulceration and health related quality of life among adults in Middle Anatolia, Turkey. Research 2015;2:1288.
7. George S, Joseph BB. A study on aphthous ulcer and its association with stress among medical students of an Indian medical institution. Month 2016;3:1692-5.
8. Mehyari M, Mortazavi N, Sayyadi F, Mohsenitavakoli S, Zahedpasha S, Mortazaviamiri S, *et al*. A review on the oxidative stress in recurrent aphtous stomatitis. J Dent Med 2015;27:89-279.
9. Gallo Cde B, Mimura MA, Sugaya NN. Psychological stress and recurrent aphthous stomatitis. Clinics (Sao Paulo) 2009;64:645-8.
10. Rezaei F, Aminian M, Raygani AV. Evaluation of salivary cortisol changes and psychological profiles in patients with recurrent aphthous stomatitis. Contemp Clin Dent 2017;8:259-63.
11. Zain RB. Classification, epidemiology and aetiology of oral recurrent aphtous ulceration/stomatitis. Ann Dent (ADUM) 1999;6:34-7.
12. Eguia-del Valle A, Martínez-Conde-Llamosas R, López-Vicente J, Uribarri-Etxebarria A, Aguirre-Urizar JM. Salivary cortisol

determination in patients from the Basque Country with recurrent aphthous stomatitis. A pilot study. Med Oral Patol Oral Cir Bucal 2013;18:e207-11.
13. Kumar A, Ananthakrishnan V, Goturu J. Stress in academia manifested as aphthous ulcers. Int J Basic Appl Med Sci 2014;4:273-7.
14. Karthikeyan P, Aswath N. Stress as an etiologic co-factor in recurrent aphthous ulcers and oral lichen planus. J Oral Sci 2016;58:237-40.
15. Aghahosseini F, Dizgah E, Amirkhani S. Stimulated and unstimulated whole saliva compositions of dental female students, Tehran University of Medical Sciences in 2005. J Islamic Dent Assoc Iran 2005;17:23-8.
16. Zwiri AM. Anxiety, depression and quality of life among patients with recurrent aphthous ulcers. J Contemp Dent Pract 2015;16:112-7.
17. Dhopte A, Naidu G, Singh-Makkad R, Nagi R, Bagde H, Jain S. Psychometric analysis of stress, anxiety and depression in patients with recurrent aphthous Stomatitis-A cross-sectional survey based study. J Clin Exp Dent 2018;10:e1109-e1114.
18. Pratibha P, Prerna J, Meena A, Bhat K, Chakravarthy K, Bhat G. Association of recurrent aphthous ulcers with stress among students in an Indian dental institution. Nat J Integr Res Med 2012;3:141-7
19. Al-Omiri MK, Karasneh J, Lynch E. Psychological profiles in patients with recurrent aphthous ulcers. Int J Oral Maxillofac Surg 2012;41:384-8.
20. Gavic L, Cigic L, Biocina Lukenda D, Gruden V, Gruden Pokupec JS. The role of anxiety, depression, and psychological stress on the clinical status of recurrent aphthous stomatitis and oral lichen planus. J Oral Pathol Med 2014;43:410-7.
21. Sanatkhani M, Manteghi A, Pakfetrat A, Mohammadian F. Psychiatric evaluation of patients with recurrent aphthous ulceration referred to Oral Disease Department of Mashhad Dental School-Iran (2006-2007). J Mashhad Dent Sch 2010;34:219-30.