Original Article

Comparison of salivary epidermal growth factor in patients with recurrent aphthous stomatitis, smokers, and healthy individuals

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Abstract

Background: Recurrent aphthous stomatitis (RAS) is one of the most common oral ulcerative diseases with unknown etiology. Epidermal growth factor (EGF) has been suggested to play a similar role in RAS. Therefore, this study investigated the salivary EGF level in patients with RAS, the patients without RAS, and smokers. **Materials and Methods:** A total of 91 samples were recruited in this case-control study: 30 RAS patients, 30 controls, and 31 smokers. Age and gender were matched in the groups. In the case group, the salivary sample was taken during the infection and remission periods. Salivary EGF concentration was measured by Crystal Dibiotic assay (made in China) using ELISA technique. Finally, the data were analyzed by SPSS software (Version 18.0, Inc., Chicago, IL, USA). **Results:** The results of paired *t*-test showed no statistically significant difference in salivary EGF between the infection and remission periods ($P = 0.987$). ANOVA test showed a statistically significant difference in EGF between the study groups ($P < 0.001$), as the mean salivary EGF was significantly lower in the smokers than the case and control groups during the infection and remission periods. **Conclusion:** The present study showed a lower level of salivary EGF in the smokers without a history of RAS. There was no statistically significant difference between the infection and remission periods in salivary EGF in the patients with RAS. Furthermore, salivary EGF showed no statistically significant difference between the patients with RAS and the controls.

Keywords: Epidermal growth factor, recurrent aphthous stomatitis, saliva, smoking

Introduction

Recurrent aphthous stomatitis (RAS) is the most prevalent cause of the painful oral ulcer. RAS affects approximately 20% of general population. RAS is characterized by recurrent ulcers. Each lesion lasts about 1–2 weeks and is typically multiple, small, round, or oval with a defined margin, a red halo, and a yellow or gray background.[1] RAS is classified to minor ulcer (less than diameter), major ulcer (over 1 cm), and herpetiform ulcers.

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RAS is a multifactorial illness with unknown etiology.[2] Genetics, nonregulation of immune system, malnutrition, stress, topical trauma, hormonal disorders, infection, oral health, weak anemia due to iron deficiency, folic acid and deficient absorption of B12 vitamin, periodic neutropenia, and celiac disease have been proposed as the factors affecting the incidence of this disease in specific subgroups of people.[3] Recently, free radicals have been suggested to be involved in the etiology of this illness by inducing oxidative stress.[4] When oxygen-free radicals are produced beyond their physiologic limit, or when the antioxidant defense mechanism of the body is reduced, oxidative stress occurs, which can be a life-threatening issue and can lead to histological damage in some cases.[5] Some studies have supported the effect

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of smoking on the reduced incidence of aphthous lesions but the reason of that is not clear.[6]

Salivary epidermal growth factor (EGF) plays a pivotal role in maintaining the oral health and ameliorating the oral ulcers. Saliva plays a key role in the oral health so that it can preserve the integrity of the oral mucosal membrane through liquefaction and amelioration of the soft tissue.[7] EGF is a single polypeptide with 53 amino acids.[8] Numerous growth factors such as EGF, insulin-like growth factor (IGF), neural growth factor (NGF) and transforming growth factor (TGF) synthesis are secreted through saliva, the most important of which is EGF. Salivary EGF induces the mitotic response of the cell, thereby playing a role in the activation of RNA and synthesis of DNA, protein, and extracellular macromolecules.[8]

As for the effect of EGF on aphthous ulcers, it is assumed that salivary growth factors such as EGF play a key role in maintaining the oral health, healing the oral ulcers, and preserving the oral mucosa health.[9] Considering the efficacy of salivary EGF in maintaining oral health and healing oral ulcers, the researchers in this study made an attempt to determine whether salivary EGF level is different in the infection and remission periods and whether it is different in the healthy people and patients. Also, since aphthous has been reported to reduce in the smokers, we made an effort to see whether smoking affected salivary EGF or not.

Materials and Methods

A total of 91 samples, including 30 participants without RAS, 30 patients with minor RAS (less than 1-cm lesion) and 30 smokers without RAS (smoking at least six cigarettes daily and a history of six months) were recruited in this case-control study. They were selected by a convenience sampling method. The indices required for sample size estimation were taken from the study of Wang *et al*.[10] as follows:

μ_1=0.58, μ_2 = 0.52, S_1 = 0.07, S_2 = 0.07.

The following formula was used to calculate the sample size. Furthermore, the confidence level of 95% and test power of 90% were taken into account.

$$n = \frac{\left(Z_{1-\frac{\alpha}{2}} + Z_{1-\beta}\right)^2 \left(S_1^2 + S_2^2\right)}{\left(\mu_1 - \mu_2\right)^2} = 30$$

Sample size calculation was performed by PASS software (Version 11).

In this case-control study, salivary EGF was compared between the study groups. The first group included healthy individuals (control group), the second group comprised of the patients with minor RAS (case group), and the third group consisted of the smokers (control group for RAS). The second group was evaluated in the infection period and almost ten days after. After taking written informed consent from the participants, they were included in the study. All the consent forms were approved by the ethics committee of the Kermanshah University of Medical Sciences. As stated in the consent forms, participation or nonparticipation in this study was voluntary and had no effect on the treatment process.

The inclusion criteria in this study were as follows:
1. Patients with RAS (with a minimum history of thrice a year in the case group);
2. Patients with RAS after improvement of the lesion;
3. People with no history of RAS in the control group; and
4. Smokers (at least six cigarettes a day for six months) with no history of oral aphthous.

The exclusion criteria in this study comprised of the following:
1. History of systemic diseases and pregnant women;
2. Periodontal diseases;
3. History of any drug consumption in the past three months;
4. Presence of any oral pathologic lesion;
5. Smoking less than the amount determined in this study; and
6. The case and control groups being matched in terms of age, gender, and oral health.

After washing their mouth for 2 min to decrease bacterial contamination, saliva samples (5 cc) were collected from all participants by spitting during 10:00–12:00 am when the samples had not drunk or eaten anything 2 h before saliva collection.[11] All samples were kept at -20°C for analysis. The samples were then centrifuged with 100 cc diluted phosphate buffered saline at 400 g for 5 min. Salivary EGF concentration was measured by Crystal Dibiotic assay (made in China) by the ELISA method.[8]

To collect the data, first, the consent forms were approved by the ethics committee of the Kermanshah University of Medical Sciences (no: IR.KUMS.REC.1396.382). As shown in the consent forms, participation in the study was completely voluntary and had no effect on the treatment process. After homogenization, the two groups were tested.

Data analysis was done through descriptive and inferential statistics. For descriptive statistics, central tendency and dispersion parameters along with tables and graphs were reported. For inferential statistics, the normality of data was analyzed by the Kolmogorov–Smirnov test. Given the normality of data, the *t*-test was used to compare the salivary EGF between the infection and remission periods, and the ANOVA test was run for multiple comparisons between groups. Moreover, the Tukey post-hoc test was used for the pair comparison. Data were analyzed by SPSS software (Version 18.0, Inc., Chicago, IL, USA). $P < 0.5$ was considered significant.

As for ethical considerations, written consent was taken from all participants. They could withdraw from the study at any stage they wished. No risk threatened the patients.

The limitations of this study included patient cooperation (they were motivated to take part in the study) and small sample size (a limited time was set to complete the samples).

Results

A total of 91 participants were included in this study: 30 patients with RAS, 31 smokers, and 30 healthy controls.

The results of the Kolmogorov–Smirnov test showed the data followed a normal distribution pattern ($P > 0.1$) [Table 1].

The findings of the paired *t*-test indicated no statistically significant difference between the infection and remission periods in salivary EGF [Table 2].

The results of ANOVA test showed a statistically significant difference in salivary EGF among the study groups ($P < 0.001$) as the mean salivary EGF was significantly lower in the smokers than the control and infection groups [Table 3].

The results of ANOVA test indicated a statistically significant difference among the study groups in salivary EGF ($P < 0.001$) as mean salivary EGF was significantly lower in the smokers than control and remission groups [Table 4].

Table 1: Results of Kolmogorov-Smirnov for the normality of data

Group	Test	*P* value
Infection	Kolmogorov-Smirnov *Z*	0.642
	P	0.805
Remission	Kolmogorov-Smirnov *Z*	0.848
	P	0.468
Control	Kolmogorov-Smirnov *Z*	0.787
	P	0.565
Smoker	Kolmogorov-Smirnov *Z*	1.090
	P	0.186

Table 2: Mean and standard deviation of salivary EGF in the infection and remission periods along with their pair comparison

	Infection	Remission	*P*
Mean	984.46	982.27	0.987
Standard deviation	547.42	418.49	

Table 3: Mean and standard deviation of salivary EGF in the smokers and infection and control groups

	Infection	Control	Smoker	*P*‡
Mean	984.46[b]	969.75[b]	378.92[a]	<0.001
Standard deviation	547.42	326.24	104.78	

Means with the same superscript letters are not significantly different ($P>0.05$), ‡ANOVA test, followed by the Tukey test

Table 4: Mean and standard deviation of salivary EGF in the smokers and infection (after remission) and control groups

	Remission	Control	Smoker	*P*
Mean	982.27[b]	969.75[b]	378.92[a]	<0.001
Standard deviation	418.49	326.24	104.78	

Means with the same superscript letters are not significantly different ($P>0.05$), ‡ANOVA test, followed by the Tukey test

Discussion

Bacterial infection, immunologic disorders, and increased viscosity of the submucosal extracellular matrix are factors that affect the incidence of RAS. Furthermore, various studies have reported psychological factors, hormonal changes, trauma, dietary sensitivity and allergy, blood disorders, and nutritional deficiency as the risk factors of aphthous. In fact, RAS is a cellular immune response in which T lymphocytes, cytokines, and tumor necrosis cause epithelial cell death and ulcer.[12,13] Natural healing of ulcers and oral lesions is the result of a complex interaction among various types of cells at the ulcer area and their ability in producing a series of growth factors and responses. These factors regulate growth, cell migration and proliferation, extracellular matrix production, enzymatic activity, and higher growth factor production. Therefore, it is believed that the growth factors, which have a topical function, partly regulate the remission process.[14,15]

Considering this issue, the present study investigated the salivary EGF in the healthy people, patients with RAS, and smokers in order to shed more light on the effect of this topical growth factor on the prevention or rapid improvement of the ulcer as well as the lesions caused by RAS. Kim *et al.* reported that EGF increased the production of protein and RNA in the epidermal cells.[16] Jiang *et al.* also indicated the role of EGF in the restoration of corneal injuries and gastric ulcer and stated that this factor inhibited the proliferation of gastric cancer cells.[17] Moreover, Hashimoto *et al.* reported that this factor had a mitogenic effect and increased the proliferation of keratinocyte, fibroblast, and epithelial cells *in vitro* and *in vivo*.[18]

In a clinical study titled "diabetes mellitus type 2 and prevalence of endodontic and periodontic diseases" in a Brazilian population, Marrotta recruited 30 patients with type-2 diabetes and 60 nondiabetic samples. They reported that salivary EGF played a critical role in improving the complications of oral lesions and periodontal disease, oral infections, enlargement of salivary glands, and dry mouth.[19-22] In a study on the salivary EGF in Behcet's disease and RAS, Adisen *et al.* stated that salivary EGF protected the patients against injuries and helped to maintain the integrity of the digestive system mucosa. Their results showed Behcet's disease and RAS reduced the salivary EGF level even in the absence of oral ulcer.[15]

Gu *et al.* evaluated the salivary EGF concentration changes in the patients with RAS. They recruited 33 healthy controls and 27 patients with RAS and homogenized them in terms of age and gender. In the case group, the saliva samples were taken in the infection and remission periods. Their findings showed EGF concentration was lower in the case group than the control group,

indicating a significant difference between them. However, there was no statistically significant difference between the infection and remission periods in EGF concentration in the case group.[19] In the present study, the paired *t*-test showed a statistically significant difference between the infection and remission periods in the salivary EGF. Hence, it seems that salivary EGF remains constant in these people, which may be a reason for the periodic recurrence of RAS in these patients. Also, improvement occurs only normally, and lesion recurrence is possible in the case of intensification of any etiologic factors. Furthermore, there is no inherent mechanism in the oral cavity and saliva to prevent or improve these lesions rapidly.

In a case-control study, Brozovic *et al.* investigated vascular endothelial growth factor (VEGF) in the patients with minor and major aphthous (case group) and individuals with no history of RAS (control group). The study sample included 27 controls and 30 patients who were homogenized with regard to age and gender. The results indicated a statistically significant difference between the two groups in VEGF; the lower was the VEGF level, the higher was the RAS.[20] Girdler *et al.* assessed the effects of EGF mouthwash on the cytotoxicity induced by oral aphthous. They evaluated the effect of this mouthwash on the improvement and initiation of oral ulcer in 12 patients under cancer chemotherapy. They found no significant difference between the controls and patients in the speed of ulcer healing but reported a small delay in the initiation and severity of the ulcer.[21]

Wung *et al.* investigated the salivary EGF in the patients with RAS and controls and reported EGF as a very important cell support in the saliva. They classified the progress of RAS into three stages, including proactive stage (red mucosa), active stage (mucosal ulcer), and remission stage. Their results showed salivary EGF reduced significantly in the active stage of ulcer in comparison with the control group. In general, they reported a significant association between the salivary EGF and improvement of the ulcer induced by RAS.[10]

On the other hand, various studies have pointed out that smoking decreases the incidence of RAS. Quitting smoking has been reported as a factor accelerating aphthous in some patients.[23,24] In an epidemiologic study, all the tobacco smokers showed a lower incidence of RAS than nonsmokers. In this study, tobacco increased mucosal keratinization, which in turn caused the mucosa to be less prone to developing the ulcer. Tobacco reuse after a nonuse period improved the preexisting ulcers during few days.[24]

The findings of the present study showed a statistically significant difference among the study groups in salivary EGF; as salivary EGF was significantly lower in the smokers than the control and infection groups. Moreover, mean salivary EGF was significantly lower in the smokers than the control and remission groups.

Conclusion

The results of the current study indicated no statistically significant difference between the infection and remission periods in the salivary EGF in the patients with RAS. Furthermore, there was no statistically significant difference between the controls and patients with RAS in the salivary EGF. Moreover, salivary EGF concentration was significantly lower in the smokers without aphthous than the control and case groups. Some studies have shown that smoking reduces the risk of RAS. On the other hand, epidermal growth has an effective topical impact on the restoration and prevention of various lesions and ulcers. Hence, it was hypothesized that increased EGF would be an intermediate ring for the effect of smoking on the reduction of RAS, which was rejected based on the results obtained in this study. Furthermore, smoking did not increase the EGF but reduced the concentration of this factor. Accordingly, future studies are suggested to evaluate the role of other growth factors, cytokines, inflammatory factors, etc., in the patients with RAS (during infection and remission periods), healthy people, and controls to shed more light on all dimensions of this illness.

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Conflicts of interest

There are no conflicts of interest.

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