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Salivary Level of Epidermal Growth Factor in Recurrent Aphthous Stomatitis*

Stężenie nabłonkowego czynnika wzrostu w ślinie w aftach przewlekle nawracających

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Abstract

Background. Recurrent aphthous stomatitis (RAS) represents a very common oral lesion, affecting 5–60% of the general population. Different mechanisms might be involved in the etiopathogenesis of RAS, but to date, no single definite cause has been confirmed.

Objectives. The aim of this study was to compare salivary epidermal growth factor (EGF) levels in two phases of RAS (ulcerative and remission phases) and in healthy controls.

Material and Methods. In this case-control study, the salivary epidermal growth factor concentration (pg/mL) was measured in 18 consecutive dental patients with RAS compared to 18 healthy controls. The two groups were matched in terms of age, sex, and salivary flow rate. Unstimulated whole saliva samples were collected in the first 3 days after mucosal ulceration and two weeks after resolution of RAS and from healthy controls. Salivary epidermal growth factor (SEGF) concentration was measured using the sandwich ELISA technique.

Results. Saliva samples were obtained from 36 people, comprised of 21 women and 15 men. The mean of the salivary epidermal growth factor level in the ulcerative stage of RAS patients (1772.05 ± 954.13 pg/mL) was lower than in the remission stage (2020.17 ± 996.94 pg/ml) and the control group (2357.10 ± 1365.96 pg/mL), but the differences were not significant.

Conclusions. Recurrent aphthous stomatitis has no effect on the salivary level of epidermal growth factor (*Dent. Med. Probl.* 2015, 52, 1, 33–38).

Key words: saliva, epidermal growth factor, recurrent aphthous stomatitis.

Słowa kluczowe: ślina, nabłonkowy czynnik wzrostu, afty przewlekle nawrotowe.

Recurrent aphthous stomatitis (RAS) represents a very common oral lesion, affecting 5–60% of the general population [1]. Different mechanisms might be involved in the etiopathogenesis of RAS, but to date, no single definite cause has been confirmed. Pain, ulceration of the oral ep-

ithelium, self-limitation of the condition and recurrence seem to be the ultimate outcomes [2]. The immune pathogenesis for the oral mucosal disease was first proposed by Lehner [3]. There is increasing evidence relating to the local immune dysfunction of RAS [4]. More recent studies have

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focused on a dysfunction of the mucosal cytokine network, including IL-2, IL-6 and IL-10 [5, 6]. An abnormal mucosal cytokine cascade in RAS patients leads to an exaggerated cell-mediated immune response, resulting in localized ulceration of the mucosa [7].

Epidermal growth factor (EGF) was first discovered in the sub-maxillary gland of rats in 1962 [8]. It helps maintain tissue homeostasis by regulating epithelial cell proliferation, growth and migration. It also induces angiogenesis and plays an important role in wound healing and tissue regeneration [9, 10]. Various studies have addressed the alteration and therapeutic effect of EGF in colitis, peptic ulcer, necrotizing enterocolitis, chronic ambulatory peritoneal dialysis (CAPD), mucositis after radiation therapy, recurrent aphthous stomatitis, Behcet disease, wound healing of the tongue, and oral cavity cancer [8–25]. As growth factors are crucial to the healing process, it is rational to hypothesize that EGF level would be diminished in RAS. Therefore, the aim of this study was to investigate and compare salivary EGF levels in 2 stages of RAS and in healthy controls.

Material and Methods

Regarding $\alpha = 0.05$, $\beta = 0.2$, salivary EGF changes of 16, and standard deviation of 7.3, the minimum sample size was estimated to be 17 in each group. With regard to the capacity of each SEGF kit in this case-control study, 18 dental patients with RAS and 18 healthy controls (totally 36 saliva samples) referred to the Department of Oral Medicine, Shahid Beheshti School of Dentistry, Tehran, Iran, from September 2010 to February 2011, were studied. All experiments on the patients were conducted in accordance with the Declaration of Helsinki and informed written consent was obtained from all patients. Meanwhile, the Institutional Review Board of Shahid Beheshti University of Medical Sciences approved the research ethically. Clinical diagnosis of minor RAS lesions (small, painful, recurrent ulcers of diameter no more than 10 mm, on non-keratinized mucosa, of any numbers) was made by a senior resident and then confirmed by an associate professor of oral medicine (the second author). A data form was filled out for each patient including queries about RAS characteristics such as type, number, duration, size and location of RAS, and questions about general health status.

Uncooperative patients and those with a history of diabetes, peptic ulcer, Crohn's disease, ulcerative colitis, Behcet's syndrome, anemia, radiotherapy or chemotherapy, medical treatment

in the previous month, pregnancy, breast feeding, smoking or alcohol consumption, and major or herpetiform aphthous ulcers were excluded from the study. In addition, patients with ulcers of longer duration than 3 days were also excluded. Unstimulated whole saliva samples were collected between 9–11 a.m., to follow the circadian rhythm of EGF secretion [11]. The RAS patients' saliva was collected in 2 phases. The ulcerative phase was in the first 3 days after mucosal ulceration, and the remission phase was 2 weeks after resolution of the ulcers.

Subjects were asked to refrain from eating, drinking, smoking and oral cleansing for at least 90 min before saliva collection [11]. Each patient was instructed to spare his/her saliva in the mouth for 5 min without swallowing, and then spit it into a plastic 50 mL falcon tube [12]. At the end of sampling, a diphenhydramine HCl elixir was prescribed for all patients as a symptomatic treatment. After encoding and transferring to the laboratory, the samples were centrifuged (Hettich, Germany) for 10 min, at 5000 G, at a temperature of 4°C to separate debris and epithelial cells. The volume of each saliva sample was recorded and the samples were stored in micro-tubes at -70°C. The salivary flow rate for each patient was measured by dividing the saliva volume by the sampling time (5 min). SEGF concentration was determined using special kits for the sandwich ELISA technique (Quantikine R&D Systems, Minneapolis, MN, USA). As mentioned by the manufacturer, the special kits had no cross-reactions to other cytokines. At first, the standard samples were prepared in special micro-tubes with determined concentrations. The micro-tubes containing standard and study samples were put in special racks according to their codes so that the laboratory technician was blind to their contents. In order to obtain the dilution rate, one sample was diluted to 1/10, 1/20, 1/50 and 1/100, and the dilution of 1/20 was chosen as the preferred one. The samples were diluted to 1/20 with the special buffer and the 200 μ L of standard, sample, and control solutions were added to each well and incubated at 26°C for 2 h. The plates were washed with the buffer three times. Then 200 μ L of peroxidase conjugated polyclonal antibody were added to the wells and incubated for 2 h. The plates were washed with the buffer 3 times. Finally, 200 μ L of the substrate were added to each well and they were incubated for 20 min in a dark place. Lastly, 50 μ L sample plates were assayed by an ELISA reader (Anthos 2020, Australia) at a wavelength of 450 nm with a reference filter of 570 nm.

The data was analyzed using SPSS 18 software. To describe the data, mean \pm SD and fre-

quency were used. Data analysis was done by using χ^2 , and Mann-Whitney tests. After the normalization of salivary epidermal growth factor (SEGF) in the study groups by means of one-sample Kolmogorov-Smirnov test, the mean concentration levels of SEGF in the two groups were measured using an independent sample *T*-test for separate samples (between the control and RAS group) and paired *T*-test for two dependent groups (between the ulcerative and remission phases of RAS). *P* value ≤ 0.05 was considered significant.

Results

Saliva samples were obtained from 36 people, 18 patients with RAS and 18 healthy controls. Out of those, 21 (58.33%) were women and 15 (41.66%) men. In the RAS group, there were 11 (61.11%) women and 7 (38.88%) men, while in the control group, 10 (55.55%) women and 8 (44.44%) men were studied. There was no significant difference between the two groups in terms of sex ($p = 0.735$), according to χ^2 test.

The mean age of RAS patients and the control group were 29 ± 11.37 and 35 ± 12.84 , respectively. Mann-Whitney test detected no difference between the two groups in this regard ($p = 0.111$).

Table 1 shows the results of salivary flow rate of the contributing people. There was no significant difference between two groups in terms of salivary flow rate ($p = 0.2$).

Table 1. Comparison of salivary flow rate in study groups

	N	SFR (mL/min)	Median	Range
Control	18	1.7 ± 0.7	1.5	0.5–3.5
RAS, ulcerative	18	2.09 ± 0.4	2.5	1.5–2.75
RAS, remission	18	1.97 ± 0.7	2.5	0.7–3.5
P = 0.2				

SFR – salivary flow rate.

Table 2. Comparison of mean Salivary EGF (pg/dL) in study groups

Study groups	Mean \pm SD	Degree of freedom	Test statistics	P value
Control	2357.10 ± 1365.96	–	–	0.608
Control vs. ulcerative	–	34	1.49	0.146
Control vs. remission	–	34	0.85	0.404
RAS, ulcerative	1772.05 ± 954.13	–	–	0.306
Ulcerative vs. remission	–	17	0.78	0.442
RAS, remission	2020.17 ± 996.94	–	–	0.934

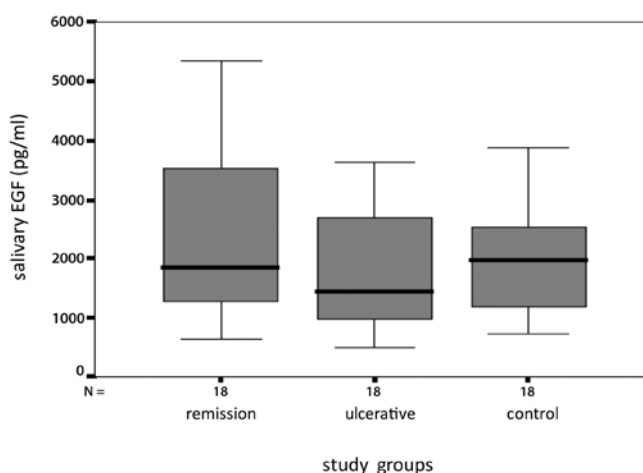


Fig. 1. Salivary EGF (pg/dL) in study groups

Changes of SEGF in the control group, the ulcerative and the remission phases of RAS patients are demonstrated in Table 2. According to one-sample Kolmogorov-Smirnov test, the levels of SEGF in three of normal distribution.

The difference between the mean concentration of SEGF in the ulcerative and remission stages of RAS patients was not statistically significant using a paired *t*-test ($p = 0.442$). Meanwhile, the result of an independent sample *t*-test showed neither significant difference between the control group and the ulcerative phase of RAS ($p = 0.146$) nor between the control group and the remission phase of RAS ($p = 0.404$) (Table 2).

Figure 1 shows that the mean of SEGF in the ulcerative phase of RAS is lower than in the remission phase and the control group.

Discussion

Recurrent aphthous stomatitis is one of the most common diseases affecting oral mucosa with different etiologies [12, 26]. Local and systemic conditions, genetic, immunological and microbial factors all may play a role in wound healing and re-epithelialization of oral and gastrointestinal mucosa [12, 27, 28]. Salivary secretions have protective ef-

fects on maintaining the health of the mouth [12]. Previously, role of EGF in the pathogenesis of different neoplasms as well as oral lesions has been addressed in several studies [12–14, 17, 29–31]. The present study compares salivary EGF levels in two phases of RAS with healthy controls.

All participants in this study were chosen from dental patients referred to the Oral Medicine Department in order to match the study and control groups more efficiently. There was no significant difference between the groups in terms of age and sex. Unlike Adışen and Guh, we measured the salivary flow rate of patients, which no significant difference was found between the groups [13, 14]. Therefore, SEGf was not affected by salivary flow rate. Patients with a history of systemic disease affecting the SEGf level were excluded from the study, similarly to Wu-Wang and Dumbrigue studies [12, 17].

The diagnosis of RAS was based on Lehner's clinical criteria, 1969, like Wu-Wang [12]. The timing of the first saliva collection was in accordance with Wu-Wang [12] and Guh [14], but due to some limitations of Wu-Wang study, the second saliva collection was performed after two weeks so that during the elapsed time the underlying pathological mechanisms of RAS returned to normal condition. In order to minimize the effect of the circadian rhythms of SEGf secretion, all samples were collected during a special time (9–11 am), similarly to Wu-Wang [12]. All the patients with RAS were provided with a uniform topical prescription in consideration of medical ethics. In this study, the sandwich ELISA technique used to measure the salivary EGF level as a reliable method, similar to the Adışen study [13].

Although not significant, there was a 12.28% increase in SEGf mean in the remission phase compared to the ulcerative phase of RAS, similar

to Wu-Wang and different from Adışen [12, 13]. Meanwhile, the difference between the SEGf level of the ulcerative or remission phases of RAS and the control group were not found to be significant.

In our research, the variation in EGF levels can be rationally related to the clinical stages of RAS. The EGF concentration level in the acute stage of RAS decreases and in the remission stage approaches that of the control group. Previous studies have showed that EGF concentration is reduced after the development of *stomatitis* [12]. In the ulcerative phase of RAS, EGF receptors (EGF-R) are increased in oral mucosa, in order to compensate for the reduction of EGF concentration [8]. On the other hand, in this study, the SEGf level in the remission stage was 14.29% lower than in the healthy control, but the difference was not significant, similar to Adışen [13] and Wu-Wang studies [12].

A few studies suggest that salivary EGF concentration can be affected by renal function [14, 15]. In this study, no data about renal function of the groups were available. Therefore, we were not certain whether the EGF measurement of the groups might be attributed, at least partly, to this co-morbid factor. Moreover, further research should be done to assess if EGF can be influential in RAS healing and improving the quality of life. A strong negative correlation between EGF level and the severity of mucositis was found in the Dumbrigue study [17].

The present study addressed minor *aphthae*, but it is possible that the salivary level of EGF is different in other forms of oral ulcers, including major and herpetic aphthous ulcers; so further research is needed in this regard.

In conclusion, recurrent aphthous stomatitis has no effect on salivary level of epidermal growth factor.

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