Life Science Journal

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Salivary Epidermal Growth Factor Level in Recurrent Aphthous Ulcer Patients and Smokers: A Comparative Study

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Abstract: Salivary epidermal growth factor (EGF) plays a major role in protection against injuries and helps in maintaining the integrity of oral mucosa by regulating epithelial cell proliferation, growth, and migration. A comparison of EGF level of saliva between smokers, patients who have aphthous ulcer and healthy control group revealed that the mean salivary EGF value in the smoker-group was higher in comparison to the control group. Moreover, the mean salivary EGF value in the recurrent aphthous ulcer (RAU) group was lower than the control group. However, there was no statistically significant difference between the three groups (p=0.222). Nevertheless, salivary epidermal growth factor has an effective impact on the restoration and prevention of recurrent aphthous ulcer. Increased EGF in smokers would be an intermediate circlet for the effect of smoking on the development of RAU.
[Sahar Mahmoud ElRefai, Maha Galal Omar, Ghadeer Ghalab Almutairi, Shahad Saad Alkait, Fatimah Salim Almufarji, Ishrat Rahman. **Salivary Epidermal Growth Factor Level in Recurrent Aphthous Ulcer Patients and Smokers: A Comparative Study.** *Life Sci J* 2022;19(3):11-15]. ISSN 1097-8135 (print); ISSN 2372-613X (online). http://www.lifesciencesite.com. 2. doi:10.7537/marslsj190322.02.

Keywords: Salivary epidermal growth factor; EGF; smokers; aphthous ulcer; RAU.

1. Introduction

The Recurrent aphthous ulcer (RAU) is one of the most common oral mucosal diseases.(1) It is characterized by painful, recurrent ulcerations that affect 5–60% of the general population.(1) It lasts about 1–2 weeks and clinically manifested as round,or oval ulcer with a defined margin, a red halo, and a yellow or grey background.(2) Based on the ulcer size and number RAU can be classified into minor, major, and herpetiform.(3) RAU is a multifactorial illness with unknown etiology. Some factors including: genetics, non-regulation of immune system, malnutrition, stress, local trauma, hormonal disorders and infection have been suggested to cause RAU in specific groups of people. (2)

Tobacco smoking is one of the most common risk factors of oral cancer, oral mucosal lesions, hyper-keratinization and periodontal disease. Thecigarettes chemicals induce biochemical alteration of the epidermal growth factor (EGF). The EGF level is reduced in cigarettes smokers leading to impairment of the buccal EGF receptor functions. This may alter the incidence of recurrent aphthous ulcer as reported by S.L wang et al.(4) Also, some clinical observations show that some smokers experience oral ulcerations upon smoking cessation.

Salivary epidermal growth factor (EGF) consists of 53 amino acids that promote growth and re-epithelization. It plays a major role in protection against injuries and helps in maintaining the integrity of oral mucosa by regulating epithelial cell proliferation, growth and migration.(1) The salivary glands produce salivary EGF, and the primary sourceis the parotid glands. However, the precise control mechanism of EGF secretion into the saliva still needs further studies.(5) It plays a role not only in the oral cavity, but also it has stimulating effects throughout the entire digestive tract. The aim of this study is to compare salivary EGF levels in patients with RAU, smokers, and healthy control group.

2. Material and Methods

The study was conducted in dental clinics at Princess Nourah Bint Abdulrahman University for a period of 3 months. The research was approved by the ethics committee; International Review Board (IRB) at Princess Nourah Bint Abdul Rahman University. A written consent was taken from all the participants who were allowed to withdraw from the study at any time. General examination form was used which included the personal data, medical history, and examination of the salivary glands. After the participants fulfilled the inclusion criteria, the specific examination form was filled. Thirty-three participants were divided into three groups; group I: RAU patients, group II: smoker patients, and group III: healthy control patients. The participants were asked not to drink or eat anything for 2 hours before the saliva collection. They were then instructed to wash their mouth for 2 minutes to decrease bacterial contamination. An amount of about 5ml unstimulated salivary samples were collected from all participants by spitting method. All the samples were stored at □80°C. The samples were analyzed using specific human EGF SimpleStep ELISA kit by Abcam to measure the EGF level (pg/ml). The cross-sectional study data was analyzed using SPSS software (Version 18.0, Inc., Chicago, IL, USA) anddescriptive statistics (mean and standard deviation). ANOVA test was used to compare between the three groups. The difference of mean and standard deviationbetween the 3 groups were taken. If $P < 0.05$ it considered significant.

Inclusion criteria

For recurrent aphthous ulcer patients, they should have history of RAU with at least 3 attacks in the past one year at the different focal sites and currently having RAU (1-7 days) either in active or healing stage. For smokers they should have smoked at least 6-10 cigarettes/ day for at least 6 months.[2] Healthy control group should be medically fit, not taking any medications and have no history of RAU or smoking.

Exclusion criteria

Generally, pregnant, lactating mothers, and patients who have systemic or topical drug therapy in past six months or have spontaneous bleeding are excluded.

Patients with a history of any systemic disease or syndromes resulting in ulcers or any concurrent mucosal lesions, history of smoking. Finally, Smokers who smoke less than the amount determined in the study (<6-10 cigarettes/day), or having present RAU or using smokeless tobacco are also not included in the study.

3. Results

In this study, One-way analysis of variance (ANOVA) test showed the highest mean salivary EGF value was among smokers' group (440.13 ± 150.95). Whereas the aphthous ulcer group exhibited the lowest mean salivary EGF value (344.73± 146.62). The mean value of the healthy control group was 346.18 ± 133.96 (Table 1). However, there was no statistically significant difference between the three groups (p=0.222). (Table 2)

Table 1: descriptive statistics of the study groups

	N	Mean	Std. Dev	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Control	11	346.18	133.96	40.39	256.19	436.18	218.55	648.26
Aphthous ulcer	11	344.73	146.62	44.20	246.22	443.23	179.99	666.59
Smoker	11	440.13	150.95	45.51	338.72	541.54	259.10	745.52
Total	33	377.01	146.63	25.52	325.02	429.01	179.99	745.52

Table 2: shows the one-way analysis of variance (ANOVA) output.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	65739.27	2	32869.63	1.58	0.222
Within Groups	622312.77	30	20743.75		
Total	688052.04	32			

4. Discussions

Recurrent aphthous ulcer (RAU) is one of the most common oral lesions, resulting in periodic pain and ulceration of the mucosa.[1] The main etiological cause of RAU still needs to be illustrated, but several precipitating factors have been identified, including smoking cessation, stress, and hormone imbalance. Recurrent aphthous ulcers provoke an immunological response in which T lymphocytes, cytokines, and tumour necrosis factor lead to epithelial cell necrosis and ulcer formation.[2] Physiological healing of RAU results from a complicated interaction of various types of cells and their ability to produce a series of growth factors and responses.

Salivary epidermal growth factor (EGF) has important cytoprotective factor for injuries healing in the oral cavity. It promotes growth and re-epithelization that helps in maintaining the integrity of oral mucosa by regulating epithelial cell proliferation, growth, and migration. [1] In addition, it has a role not only in the oral cavity, but also it has stimulatingeffects throughout the entire digestive tract. Due to the importance of EGF for the healing process, it is reasonable to hypothesize that EGF level would be decreased in RAU.[1] [5]

One of the most common risk factors for oral cancer, oral mucosal lesions, hyper-keratinization, and periodontal disease is tobacco smoking. Different studies have shown that cigarette chemicals induce biochemical alteration of the EGF as reported by S.L wang et al.[4] Some patients who quit smoking reported acceleration of aphthous ulcer. According to Rezaei et al, this could be explained by the effect of tobacco in increasing mucosal keratinization, which causes the oral mucosa to be less likely to develop ulcers. [2]

The aforementioned findings were encouraging to assess EGF level among subjects with recurrent aphthous ulcer compared to smokers and control group.

In this study, unstimulated saliva collection method was used. This method is accurate to evaluate salivary gland status, while stimulated saliva is useful for the study of the functional reserve. Unstimulated saliva has a normal pH range between 6 and 7. Additionally, the stimulated saliva has only a diluted concentration of biomarkers, which makes the biomarkers difficult to detect.[6]

Enzyme-linked immunosorbent assay (ELISA) used in this study is based on antigen-antibody reaction. It provides high specificity, sensitivity, and high efficiency. In addition, the analysis can be accomplished without complicated sample pre-treatment.[7]

The results of the present study showed that the salivary EGF level in patients with RAU was (344.73 ± 146.62) lower than in the healthy control patients (346.18 ± 133.96), but the difference was not statistically significant. Salivary EGF level changes in patients with RAU have been assessed by Gu et al[1], who reported in their study, on saliva samples that the EGF level was lower in the study group than in the control group, with a significant difference between them. The samples from the study group were also taken in the ulcerative and healing stages of RAU.[1] Nevertheless, they had no statistically significant difference between the later stages of RAU in the EGF level being lesser in the ulcerative stage of RAU than in the healing stage, where it approached the concentration level of the control group.[1] It is reasonable to assume that including more patients in our study could have positively impacted the significance of the results.

In addition, increased binding of EGF to its receptors might result in various biological effects, including epithelial proliferation, and angiogenesis. Therefore, it appears that even the transient increase in the salivary levels of EGF is associated with less oral mucosal ulceration [8]

In agreement with this study results,Ramezani et al, revealed that the EGF level was lower in the RAU group than in the control group but with no statistically significant difference.[1] In addition, Adisen et al, showed that the salivary EGF level of RAU patients was lower than of the healthy controls with statistically significant difference.[9]

In contrast, a study by Rezaei, et al revealed no statistically significant difference between the controls and patients with RAU in the salivary EGF.[2] Moreover, salivary EGF concentration was significantly lower in the smokers without aphthous ulcers than the control and case groups. This could be due to the inclusion criteria in their study that involved patients with RAU after improvement of the lesion; a condition that could affect the results. Previous studies have showed that EGF concentration is reduced after the development of stomatitis. In the ulcerative phase of RAU, EGF receptors (EGF-R) are increased in oral mucosa, in order to compensate for the reduction of EGF concentration with subsequent increase in its level in healing stage.[1] [10]

Several studies have shown that smoking lowers the incidence of RAU.[2] [4] [11] In this study, the smokers' salivary EGF level was higher (440.13 ± 150.95) than both the control group (346.18 ± 133.96) and RAU group (344.73 ± 146.62), but the difference was not statistically significant between all the groups. This could be explained by the fact that nicotine induces resistance to epidermal growth factor

receptor tyrosine kinase Inhibitor, this increases the epidermal growth factor receptor expression and leads to elevation of its level in the saliva with less association with tissue injury and ulceration.[4] [5]

Moreover, to our knowledge, not many studies were performed in Saudi Arabia about the EGF level in smokers and the habits that may alter its level such as drinking alcohol. A study published in 2019 by Al-Jerani et al,, showed that the prevalence of alcohol consumption was found to be much less than any other substance in Saudi Arabia. Bernardes et al, revealed in their work that tobacco smoking alongwith alcohol consumption may decrease EGF level in saliva.[12] Most of this study participants were considered heavy smokers with no alcohol drinking habits.[13] This could play a remarkable role in the results of the present study.[14]

The findings of the current study suggest that salivary EGF may affect the potential mucosal tissue healing and that higher levels of EGF are associated with less development of RAU, a finding that could also explain low incidence of RAU among smokers.

5. Conclusion

Salivary epidermal growth factor has an effective impact on the restoration and prevention of recurrent aphthous ulcer. Increased salivary epidermal growth factor in smokers would be an intermediate circlet for the effect of smoking on the development of recurrent aphthous ulcer.

6. Recommendation

Taking into consideration the cytoprotective effect of salivary epidermal growth factor, further studies with larger sample size are needed to improve our understanding to its role in recurrent aphthousulcer incidence and healing.

Acknowledgements:

we would like to thank the administration for facilitating the collection of data in Princess Nourah Bint Abdulrahman University, Riyadh, KSA.

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3/15/2022