Original Article

An Investigation on the Levels of Vascular Endothelial Growth Factor (VEGF) in the Unstimulated Whole Saliva of Patients with Recurrent Aphthous Stomatitis

F. Agha-Hosseini[1,2], H. Kaviani[3], K. Bamdad[4]

[1] Associate Professor, Department of Oral Medicine, Faculty of Dentistry, Tehran University of Medical Sciences, Tehran, Iran
[2] Associate Professor, Dental Research Center, Tehran University of Medical Sciences, Tehran, Iran
[3] Dentist, Private practice
[3] Lecturer, Department of Immunology, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran

Abstract:

Statement of Problem: Recurrent aphthous stomatitis (RAS) is one of the most common inflammatory diseases encountered in dental practice, but the precise etiology and pathogenesis of the disease is not fully understood. Vascular endothelial growth factor (VEGF) is a multifunctional angiogenic cytokine involved in angiogenesis and wound healing. There is evidence that VEGF could play an important role in recruiting inflammatory infiltrates like those in RAS.

Purpose: The aim of this study was to investigate salivary levels of VEGF in various stages of RAS.

Materials and Methods: In a case/crossover study, salivary VEGF levels were determined in 31 patients with RAS. Their saliva was collected by the spitting method in specially prepared tubes in two stages; the active phase (first week) and the remission phase. Salivary levels were then determined using the Sandwich ELISA technique and the data were analyzed by the Wilcoxon test.

Results: Patients in the remission period had increased VEGF values, 571.774 (347.5499) pq/ml, as compared to the acute stage, 424.758 (235.1474), and the difference was significant ($P< 0.05$).

Conclusion: Salivary VEGF levels seem to be associated with ulcer development in RAS, supporting the concept of a potential association between RAS and VEGF.

Key Words: VEGF; Saliva; Recurrent Aphtous Stomatitis (RAS)

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Corresponding author:
F. Agh-Hosseinii, Department of Oral Medicine, Faculty of Dentistry, Tehran University of Medical Sciences, Keshavarz Bulv., Gods St., Post Code: 14147, Tehran, Iran.
aghahose@sina.tums.ac.ir

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INTRODUCTION

Recurrent Aphthous Stomatitis (RAS) is the single most common oral mucosal lesion and is encountered frequently by dentists in practice [1,2]. The ulcers are painful, can be debilitating, and are often the source of much concern and anxiety for the patient [3]. The pathogenesis of RAS is unknown. Current evidence supports a role for immune dysfunction, although the specific defect has not been identified [3].

There is evidence that vascular endothelium could play an important role in recruiting inflammatory infiltrates like those in RAS [4]. VEGF is a multifunctional angiogenic cytokine involved in angiogenesis and wound healing [4]. It is known that organ development and cell growth is dependent on adequate vascular supply. Adequate angiogenesis is a fundamental requirement for

normal functioning of the organism [5]. VEGF is a secreted dimeric polypeptid that until recently has been believed to be a specific mitogen for endothelial cells subserving angiogenesis and permeability, in development and after injury [6].

Pammer et al [7] demonstrated that VEGF mRNA and protein are constantly expressed in normal salivary glands, and that VEGF is secreted in saliva in concentrations sufficient to be angiogenic. In addition to its protective effects to oral mucosa, saliva directly contributes to oral and extra-oral wound healing [8], and the role of VEGF in this context is poorly understood.

As VEGF may be involved in the maintenance of mucosal homeostasis, as well as in the development and progression of oral mucosal diseases, the aim of this study was to investigate salivary levels of VEGF in two stages of RAS; the acute stage and the remission period.

MATERIALS AND METHODS

In a case/crossover study, 31 patients with RAS, who had been referred to the Oral Medicine Department, Tehran University of Medical Sciences, between September 2003 and June 2004 were examined in order to determine salivary VEGF. Prior to any procedure, informed written consents were obtained from all subjects. Afterwards, a questionnaire was filled out for each patient, recording the following information: type of RAS according to Lehner [9], and the number and duration of RAS (occurring on the first or second week of the disease). Smokers and patients with systemic diseases such as Crohn's disease, ulcerative colitis, Behcet's syndrome and anemia (all confirmed by laboratory tests) and patients undergoing medical treatment were excluded from the study. All subjects were evaluated during two clinical stages of RAS; acute stage (first week) and remission period (clinical healthy appearance of mucosa).

Saliva was collected between 9:00 and 11:00 A.M to avoid circadian variation. Participants were asked not to drink or eat two hours prior to saliva collection. All patients were asked to retain mixed saliva in their mouth for 1 to 3 minutes without swallowing and then to expectorate into clean plastic containers. The samples were immediately stored at -20 °C until analysis. Patients were followed until they had no ulcer, or exhibited clinically healthy appearance of the oral mucosa. Then the same procedure was repeated.

Salivary concentrations of VEGF were determined by the Human VEGF ELISA kit (cat No: 17171) and Sandwich ELISA. Results were expressed as mean (standard deviation) in pg of VEGF/ml saliva. Statistical analysis was performed using the Wilcoxon test (paired non- parametric test) and significance was set at $P< 0.05$.

RESULTS

31 patients were involved in this study, conducted between September 2003 and June 2004. Of these patients, 19 (61.3%) were female with a mean age of 32.16 (15.04) and 12 (38.7%) were male with a mean age of 32.61(13.69). Six patients (19.4%) demonstrated major RAS, and 25 (80.6%) had minor RAS.

The average level of salivary VEGF (ALSV) in the acute and remission period were 424.758 (235.147) and 571.774 (347.550) respectively and the ALSV in acute stage was significantly lower than that in the remission period ($P<0.05$). In minor RAS the ALSV was significantly lower in the acute stage as compared to those in the remission period ($P<0.05$), but the difference of ASLV in the acute stage and remission period was not significant in major RAS ($P<0.05$) (Table I).

DISCUSSION

Recurrent aphthous stomatitis is one of the

Table I: Salivary levels of VEGF in patients with RAS in the acute and remission stages in major and minor RAS.

Type	VEGF levels (pq/ml)	Mean	SD	P-Value
Minor	Acute stage	395.500	223.602	0.017*
	Remission stage	602.400	37.462	
Major	Acute stage	534.167	271.964	0.526
	Remission stage	444.167	128.935	

* represent significant different.

most common inflammatory diseases affecting the oral mucosa. While the clinical characteristics of RAS are well defined, our knowledge of the precise etiology and pathogenesis of the disease is at best incomplete. However, various factors such as local trauma, smoking [10] nutrition and vitamin deficiency [11,12], viruses like human herpes virus6 (HHV-6), Human cytomegalovirus (HCMV) and varicella zoster virus (VZV) [13], bacteria (streptococus sanguis), stress [14], genetics [15], drugs [16,17], allergy [18,19] and immunology [20] may contribute to the pathogenesis of this clinical entity.

Brozovic et al [4] have suggested a possible important role for salivary VEGF in the pathogenesis of RAS. They also concluded that the ALSV was significantly lower in the acute stage as compared to the remission period. Our observations confirmed these results; acute stage with the mean value of 424.758 (235.1474) pg/ml, and remission stage 571.774 (347.5499)pg/ ml (P-value <0.05), however the amounts of ALSV were different in two study (424 compared to 700). A possible explanation could be the use of different laboratory kits and procedures.

It was demonstrated that the ALSV in minor RAS was significantly lower in the acute stage as compared to the remission period (Table II), but no relationship was observed between the ALSV in the acute stage and the remission period in major RAS (P<0.05). These findings are in contrast to the study conducted by Brosovic et al [4]. The smaller sample size employed in our study, may explain the lack of relation that was encountered in major RAS.

VEGF could be considered as a potent angiogenic agent in the pathogenesis of vasculitis in RAS, and it has been shown that large amounts of VEGF are present in normal saliva [4]. Saliva is a clear slightly acidic mucoserous exocrine secretion, composed of a variety of electrolytes, immuno-globulins, proteins and enzymes. Inaddition to its digestive function and its protective effects on the oral and upper digestive tract mucosa, it plays an important role in the maintenance of oral health, tissue repair and wound healing [21]. Most of the effects of saliva on wound healing have been previously attributed to epidermal growth factor (EGF) and transforming growth factor (TGFα), which promote re-epithelization of superficial wounds [21]. VEGF is considered to be the most important mediator of angiogensis during wound healing, and according to Schroeder's study [22], immune complex vasculitis is essential in the pathogenesis of oral aphthous ulceration. Mitogenic effects of VEGF and its synergy with basic fibroblast growth factor (bFGF) could complement the role of EGF/TGFα, resulting in an efficient formation of new vessels and enhanced would healing [23].

As shown in Table I, patients with minor RAS had lower standard deviation (SD) values in the remission stage as compared to the SD in patients with major RAS in the same stage.

This may be due to the age difference observed between the two groups: sixty four percent of the patients with minor RAS were in the third decade, while 66.6% of the major RAS patients were in fifth decade of life. In addition, other unknown factors may be involved in this phenomenon. As a result further investigation is required in order to gain a better understanding of this common disorder and to elucidate the relation between RAS and VEGF.

CONCLUSION

The results of this study support the theory of a potential relationship between RAS and VEGF, which may be considered as an etiological factor. However, this issue still remains open and needs further investigation and confirmation by other controlled clinical studies.

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بررسی سطح فاکتور رشد اندوتلیالی عروق در بزاق غیر تحریکی بیماران مبتلا به آفت راجعه

ف. آقاحسینی [۱و۲]- ه. کاویانی [۳]- ک. بامداد [۴]

[۱] نویسنده مسؤول؛ دانشیار گروه آموزشی بیماریهای دهان، دانشکده دندانپزشکی، دانشگاه علوم پزشکی تهران. تهران، ایران
[۲] دانشیار مرکز تحقیقات دندانپزشکی، دانشکده دندانپزشکی، دانشگاه علوم پزشکی تهران. تهران، ایران
[۳] دندانپزشک عمومی
[۴] مربی گروه آموزشی ایمونولوژی، دانشکده پزشکی، دانشگاه علوم پزشکی تهران. تهران، ایران

چکیده

بیان مسأله: آفت راجعه دهان یکی از شایعترین بیماریهای التهابی حیطه دندانپزشکی است اما اتیولوژی و پاتوژنز آن بخوبی شناخته نشده است. عامل رشد اندوتلیال عروق (VEGF) یک سیتوکین رگ‌ساز چند کاره است که در روند ساخت عروق و ترمیم زخم‌ها، کارآیی دارد. شواهدی مبنی بر دخالت این عامل در ضایعاتی چون آفت راجعه وجود دارد.

هدف: مطالعه حاضر با هدف تعیین سطح بزاقی VEGF در طی مراحل مختلف آفت راجعه انجام شد.

روش تحقیق: در این مطالعه case crossover، میزان بزاق ۳۱ بیمار مبتلا به آفت راجعه، به روش Spitting در دو مرحله درون لوله‌ای مخصوص جمع‌آوری گردید؛ یک بار در طی مرحله فعال بیماری (هفته اول) و بار دیگر در مرحله بهبودی. سطح سرمی فاکتور با استفاده از تکنیک Sandwich ELISA تعیین شد و نتایج دو گروه با استفاده از آزمون Wilcoxon با هم مقایسه گردید.

یافته‌ها: میزان VEGF بزاقی در طی مرحله بهبود (۳۴۷/۵۵ Pg/ml±۵۷۱/۷۷) به طور معنی‌داری بیش از مرحله حاد بیماری (۲۳۵/۱۵ Pg/ml±۴۲۴/۷۶) بود (P<۰/۰۵).

نتیجه‌گیری: میزان VEGF بزاق با روند تشکیل زخم همبستگی دارد و این همبستگی فرضیه ارتباط اتیولوژیک آفت راجعه دهان و VEGF را تقویت می‌کند.

واژه‌های کلیدی: عامل رشد اندوتلیال عروق؛ بزاق؛ آفت راجعه دهان

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