Original Article

Role of Salivary Tumor Necrosis Factor-alpha and Immunoglobulin-A in Recurrent Aphthous Stomatitis

Batool H Al-Ghurabei* **B.H., M.Sc. PhD**
Mustafa M. Saliah** **MM., BDS**

Summary:

Background: Until today, the etiology of recurrent aphthous stomatitis (RAS) remains unknown, although hints of its etiologic basis lay on genetic susceptibility, infectious agents and alterations in immune mechanics.

Objectives: The aim of this study was to investigate the possible alterations in salivary tumor necrosis factor-alpha (TNF-α) and Immunoglobulin-A (IgA) level in patients with RAS and its relation with clinical types of disease.

Subjects and Methods: Salivary TNF-α levels were investigated in 50 RAS patients and 25 healthy controls by enzyme-linked immunosorbent assay (ELISA). Single radial immune diffusion method was used for estimation of IgA level in two studied groups.

Results: Salivary level of TNF-α was significantly higher in RAS patients than in healthy controls ($p<0.001$), Moreover, the level of TNF-α was significantly increased in minor type of disease than in major and herptiform types ($p<0.05$). On the other hand, the level of IgA showed no significant differences between patients and healthy controls and neither among clinical types of disease ($p>0.05$).

Conclusion: These findings suggest that saliva provides an ideal medium for the detection of pro-inflammatory markers of the oral cavity, moreover; salivary TNF-α may play an important role in pathogenesis of this disease and it may also have an important role in the search of new treatments for this disease. As well as these results indicated to a possible role of mucosal immune system in pathogenesis of RAS.

Key words: Recurrent aphthous stomatitis, salivary TNF-α, salivary Immunoglobulin-A

Fac Med Baghdad 2011; Vol. 53, No. 2
Received Mar. 2011
Accepted May 2011

Introduction:

The precise etiology of recurrent aphthous stomatitis (RAS) remains unknown, although genetic susceptibility, infections and alteration in immune system has been considered as etiologic factors (1). The disease usually has three clinical forms, based on the aspect and size of the ulcerations: minor, major and herpetiform. Minor RAS affects 80% of the patients with RAS, and it is characterized by a shallow painful, oval or round-shaped ulcer, smaller than 5mm in diameter. Major RAS is the most severe form, aphtha diameter can get bigger than 1cm. The third form of the disease, and the less common one is Herpetiform RAS, characterized by multiple and painful 1 to 3mm ulcers, which may coalesce, forming larger and irregular lesions (2). The ulcerative process in RAS is initiated by an unknown antigenic stimulation of the mucosal keratinocytes which leads to T-lymphocyte stimulation, the liberation of cytokines such as TNF-α and different interleukins, and the migration of other lymphocytes, neutrophils and Langerhans cells. Some cytokines like TNF-α could stimulate the expression of MHC class I and II antigens in epithelial basal cells. These cells are recognized by T-lymphocytes triggering a cytotoxic response and causing the ulceration of the mucosa (3). Recently, TNF-α is considered to be one of the most important cytokines implied in the development of new lesions in RAS patients (4). Several studies have reported an increase in salivary and serological TNF-α, especially during the active phase of the disease (3 &5). The possible relevance of TNF-α to the pathogenesis of RAS stemmed from observations that thalidomide, which reduces the activity of TNF-α by accelerating the degradation of its messenger RNA (6), and pentoxifylline, which inhibits TNF-α production (7). The most important humoral mediator for mucosal immunity is secretory IgA, due to cooperating with a number of protection mechanisms (8 & 9), for presenting greater resistance to the proteolytic degradation caused by other immuneglobulins and for being especially located in the digestive and respiratory tracts, which are in close contact with the environment8, preventing the uptake of a large amount of antigens and precluding an overload in the immune system (10). This study was designed to evaluate the role of TNF-α and sIgA level in patients with RAS and its relation with clinical types of disease.

Subjects and Methods:

The present study included 5o patients with RAS (31females and 19 males), mean age of (23.1) years, ranged between (16-40years).They were attending the college of dentistry\teaching dental hospital, compared

*Clinical Immunology, College of Dentistry/ University of Baghdad.
** Dept. of Basic Science/College of Dentistry/ University of Baghdad

with 25 apparently healthy individuals considered as a controls. Patient's group was divided in to three groups according to the clinical types of disease (36 patients with minor RAS, 13 with major RAS and 1with herpetiform type). Three ml of saliva were collected from each subject for estimating levels of TNF-α by using commercially available Enzyme-Linked Immunosorbent Assay (ELISA) and performed as recommended in leaflet with kit. [Human TNF-α kit/ DRG International, Inc., USA]. Quantitative estimation of IgA has been done by single radial immunodiffusion assay.

Statistical analysis: It was assessed using P (Mann-Whitney-test), P (Bonferroni-test) and (Kruskal-Wallis-test). P values of P<0.001and P<0.05 were considered significant (11).

Results:

The current study revealed a significant elevation in median salivary level of TNF-α among RAS patients (0.15pg/ml) in comparison to that of healthy control (0.131 pg/ml), (p<0.001), according to table (1). Moreover, the level of TNF-α was significantly increased in minor type of disease than in major and herptiform types (p<0.05), as shown in table (2). As shown in figure (2) and table (3), the area under ROC curve for salivary TNF-α was significantly higher (0.71) from 0.5 value of an equivocal test (P<0.001). The level of salivary IgA showed no significant differences between patients and healthy controls (3.8 ± 0.004 mg/dl vs. 3.7 ±0.029 mg/dl) and neither among clinical types of disease (p>0.05).as clearly shown in table (4 & 5). The area under ROC curve for salivary IgA was slightly higher (0.65) from 0.5 value of an equivocal test, but not significant (p>0.05), table (3).

Table 1: Case-control difference in median salivary TNF-α (pg/ml) concentration.

	Controls	Cases RAS	P (Mann-Whitney)
salivary TNF-α			**0.009**
Range	(0.116 - 0.213)	(0.12 - 0.201)	
Median	0.131	0.15*	
Inter-quartile range	(0.125 - 0.142)	(0.135 - 0.153)	
N	25	50	

*** Significant differences p<0.001**

Table 2: The difference in levels of salivary TNF-α (pg/ml) according to the clinical types of disease.

Type of RAS	No.	Median of salivary TNF-α
1- Minor	36	0.150*
2- Major	13	0.128
3- Herptiform	1	0.136
P (Kruskall-Wallis)	--	P=0.008

*** Significant differences p<0.05**

Table3: ROC area for salivary TNF-α (pg/ml) and salivary IgA (mg/dl) when used as a test to predict cases with RAS differentiating them from controls.

	Area	P
salivary TNF-α	0.71	0.009*
Salivary IgA	0.65	0.07[NS]

*** Significant differences p<0.001**

Table 4: Case-control difference in mean salivary IgA (mg/dl) concentration.

	Controls	Cases (Recurrent Aphthus Ulceration)	P (Mann-Whitney)
Salivary IgA			**0.07[NS]**
Range	(3.4 - 4)	(3.5 - 4)	
Mean ±SD	3.7±0.029	3.8 ± 0.004	
Inter-quartile range	(3.8 - 3.9)	(3.5 - 3.9)	
N	25	50	

NS: no significant.

Table 5: The difference in levels of salivary IgA (mg/dl) according to the clinical types of disease.

Type of RAS	No.	Mean ± SE IgA
1- Minor	36	3.27 ± 0.25
2- Major	13	3.63 ± 0.06
3- Herptiform	1	3.80 ± 0.00
P (student t-test)	--	2.359 ns

NS: no significant.

Discussion:

Several publications have recently addressed the potential diagnostic properties of saliva. It has been proposed that saliva could not only be used to help diagnose oral diseases, but as "the body's mirror", it would also have application in the diagnosis of systemic conditions (12 &13). TNF-α is a pro-inflammatory cytokine produced by monocytes, lymphocytes, NK cell and even by some human tumor cells. The TNF-α also acts as a mediator of endotoxic shock and plays an important role in some autoimmune diseases, certain kinds of meningitis, HIV infection and cachexia (14 &15). In line with present findings, high levels of TNF-α, have also been reported by other studies (3, 4 &5) have observed increased levels of TNF-α in saliva and serum of RAS patients. This result underlines the importance of this cytokine in the pathogenesis of RAS. In study conducted by Valle and associates, how studied 20 patients with RAS, they observed that saliva levels of TNF-α was significantly higher in patients with active lesions of RAS compared with controls (4). The exact role that TNF-α plays in the etiology or RAS is not well-known. Its contribution to the pathogenesis of the disease is borne out by two facts: high levels of TNF-α are detected in the first stages of oral ulcerations and some drugs like thalidomide or pentoxifylline with an anti-TNF action, have a high efficacy in the treatment of RAS and other ulcerative diseases such as Adamantiades- Behçet disease (5, 16 &17). The

complex pathogenesis of RAS is probably initiated by an endogenous or exogenous antigenic stimulation of oral mucosal keratinocytes. This causes the production of different cytokines by stimulation of oral lymphocytes. TNF-α is one of these cytokines of a great importance in the beginning of the inflammatory process. This stimulation is the first step of a multistage process which lead to the activation f cytotoxic T-cells and neutrophils, the necrosis of the epithelium and finally the development of an aphthous lesion (17). Guimaraes and colleagues have related the high production of some cytokines, such as TNF-α, IL-1, IL-10 or IL-6 in RAS patients to some genetic polymorphisms. This fact could explain the family history frequently observed in some RAS patients (18). Salivary antibodies which are present in mucosal secretions, particularly IgA, combine with microorganisms to reduce their motility and adhesive properties. Many studies have demonstrated strong correlations between titers of specific salivary IgA antibodies in secretions and resistance to infection. It seems that salivary IgA has an immunoregulatory role and provides protection (1 & 19). Present findings were compatible with the other studies (20 & 21), regarding no differences was found in level of salivary IgA between RAS patients and controls. Furthermore; Bennet and co-workers evaluated the salivary IgA level in normal subjects, tobacco smokers and patients with minor RAS and noticed no significant difference was found (22). In contrast, other reports (1 & 23) mentioned that the level of IgA was increase in saliva of patients with RAS in comparison with healthy controls, in addition they demonstrated a significant increase of IgA level in active lesions in relation to quiescence phase. In 2002 (24) was assessed salivary IgA in oral mucosal disease, their results suggested that IgA and IgG levels increased in RAS,and pointed out to that in remission period of this disease the salivary IgA and IgG levels returned to normal values. On the other hand, current results failed to find any significant differences in mean saliva levels of IgA between clinical types of RAS (minor, major and herptiform), Similarly Pakfetrat and colleagues(23) observed that there was no differences were found in salivary IgA levels between minor and major types. In conclusion these findings suggest that saliva provides an ideal medium for the detection of pro-inflammatory markers of the oral cavity, moreover; salivary TNF-α may play an important role in pathogenesis of this disease and it may also have an important role in the search of new treatments for this disease. As well as these results indicated to a possible role of mucosal immune system in pathogenesis of RAS.

References:

1.Martinez KO, Mendes LL, Alves JB. "Secetory A immunoglobulin, total proteins and salivary flow in recurrent aphthous ulceration". Rev. Bras. Otorrinol. 2007; 73:323-28.

2. Gallo CB, Mimura MA, Sugaya NN. "Recurrent Aphthous Stomatitis".Clinics. 2009; 64 (7):645-8.

3.Natah SS, Häyrinen-Immonen R, Hietanen J, Malmström M, Konttinen YT. "Immunolocalization of tumor necrosis factor-alpha expressing cells in recurrent aphthous ulcer lesions ". J. Oral. Pathol. Med. 2000; 29:19-25.

4. Valle E, Llamosas RC, Vicente JL, Etxebarria AU, Urizar JA. "Salivary levels of Tumour Necrosis Factor-alpha in patients with recurrent aphthous stomatitis". Med. Oral. Patol. Oral. Cir. Bucal. 2011;16 (1): 33-6.

5. Boras VV, Lukac J, Brailo V, Picek P, Kordić D, Zilić IA. "Salivaryinterleukin-6 and tumor necrosis factor-alpha in patients with recurrentaphthous ulceration". J. Oral.Pathol. Med. 2006;35:241-3 .

6.Moreira AL, Sampaio EP, Zmuidzinas A, Frindt P, Smith KA, Kaplan G. "Thalidomide exerts its inhibitory action on tumor necrosis factor alpha by enhancing mRNA degradation". J. Exp. Med. 1993;177:1675-80,.

7. Zabel P, Schade FU, Schlaak M. "Inhibition of endogenous TNF formation by pentoxifylline". Immunobiology. 1993; 187: 447-63.

8. Vetvik H, Grewal HM, Haugen IL, Ahren C, Haneberg B. "Mucosal antibodies can be measured in air-dried samples of saliva and feces". J. Immunol. Metho. 1998;215(1):163-72.

9. Brandtzaeg P. "Molecular and cellular aspects of the secretory immunoglobulin system". Acta. Pathol. Microbiol. Immunol. Scand. 1995;103(1):1-19

10. Slavkin HC. "Protecting the mouth against microbial infections". J. Am. Dent. Assoc. 1998;129(7):1025-30.

11. Sorlie DE."Medical biostatistics and epidemiology:Examination and broad review". 1st Ed, 74-88; Appleton and Lange Com; Norwalk Connecticut, 1995.

12. Wong DT. Salivary diagnostics powered by nanotechnologies, proteomics and genomics. J Am Dent Assoc. 2006;137:313–321.

13. Hu S, Loo JA, Wong DT. "Human saliva proteome analysis and disease biomarker discovery". Expert. Rev. Proteomics. 2007; 4:531–538.

14.Saini A, Al-Shanti N, Stewart CE. "Waste management - cytokines, growth factors and cachexia". Cytokine Growth Factor Rev. 2006;17:475-86.

15. Brabers NA, Nottet HS. "Role of the pro-inflammatory cytokines TNF-alpha and IL-1beta in HIV-associated dementia". Eur. J. Clin. Invest. 2006;36:447-58.

16. Eguia A, Villarroel M, Martínez-Conde R, Echebarría MA, Aguirre JM. "Adamantiades-Behçet disease: an enigmatic process with oral

manifestations". Med. Oral. Patol. Oral. Cir. Bucal. 2006;11: 6-11.

17. Sun A, Wang JT, Chia JS, Chiang CP. "Levamisole can modulate the serum tumor necrosis factor-alpha level in patients with recurrent aphthous ulcerations". J. Oral. Pathol. Med. 2006;35:111-6.

18. Guimarães AL, Correia-Silva Jde F, Sá AR, Victória JM, Diniz MG, Costa Fde O. " Investigation of functional gene polymorphisms IL-1beta, IL-6, IL-10 and TNF-alpha in individuals with recurrent aphthous stomatitis". Arch. Oral. Biol. 2007;52:268-72.

19. Russmann H, Lissner R, Schmidt H, Karck H. " IgA\IgM and secretory immunity".Sepsis.1999;3:219-24.

20. Malmström M, Salo OP, Fyhrquist F. " Immunogenetic markers and immune response in patients with recurrent oral stomatitis". Int. J. Oral. Surg. 1983; 12: 23-30.

21. Bagg J, Williams BD, Amos N, Dagalis P, Walker DM. "Absence of circulating IgG immune complexes in minor recurrent aphthous ulceration". J. Oral.Pathol.1987;16:53-6.

22. Bennet KR, Read PC. "Salivary IgA in normal subjects, tobacco smokers and patients with minor aphthous ulceration". Oral. Surg. Oral. Med. Oral. Pathol. 1982;53:461-65.

23. Pakfertrat A, Falak F, Sankian M, Abbaszadach H. " Salivary IgA in patients with recurrent aphthous ulceration". J. Appli. Scienc. 2010;10(23):3117-21.

24. Sistig S, Vucicevic B, Lukac J, Kusic Z. " Salivary IgA and IgG subclasses in oral mucosal disease". Oral. Dis. 2002, 8:282-86.