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Source / Izvornik: **Journal of Oral Pathology & Medicine, 2006, 35, 241 - 243**

Journal article, Published version

Rad u časopisu, Objavljena verzija rada (izdavačev PDF)

<https://doi.org/10.1111/j.1600-0714.2006.00404.x>

Permanent link / Trajna poveznica: <https://urn.nsk.hr/urn:nbn:hr:127:716793>

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Download date / Datum preuzimanja: **2025-03-20**



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Salivary interleukin-6 and tumor necrosis factor- α in patients with recurrent aphthous ulceration

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BACKGROUND: Recurrent aphthous ulceration (RAU) is a well known oral disease which seems to be mediated principally by the immune system. However, it is still a matter of debate which part of the immune system is implicated in its pathogenesis as a reaction to the still unknown antigen. The aim of this study was to evaluate salivary cytokines, interleukin (IL)-6, and tumor necrosis factor (TNF)- α .

METHODS: In 26 patients with minor RAU, age range of 23–49 years (mean 27.3 years), during both the acute phase and remission and in 26 healthy controls, age range of 22–64 years (mean 30.1 years), salivary IL-6 and TNF- α levels were determined by use of enzyme-linked immunosorbent assay (ELISA). Statistical analysis was performed by use of descriptive statistics.

RESULTS: Significant differences in salivary TNF- α between healthy controls and patients with acute RAU and during the remission period were found ($P < 0.001$) as well as between patients with acute RAU and those during the remission period ($P < 0.001$). No differences in salivary IL-6 between all three groups could be found.

CONCLUSIONS: We might conclude that elevated salivary TNF- α levels during acute RAU and especially during the remission period are of importance in RAU, whereas salivary IL-6 levels seem not to play a role in the RAU disease.

J Oral Pathol Med (2006) 35: 241–3

Keywords: recurrent aphthous; salivary cytokines; ulceration

Introduction

Recurrent aphthous ulceration (RAU) is the most common oral disease affecting 5–20% of the general population (1). Despite extensive investigations, its

pathogenesis still remains poorly understood. The process seen in RAU is probably initiated through an as yet unidentified exogenous or endogenous antigenic stimulation of the keratinocytes, which stimulates secretion of T-cell activation cytokines [i.e. interleukin (IL) and tumor necrosis factor (TNF)- α , and leukoattractant chemokines by the keratinocytes]. This immune response by the activated cytotoxic T lymphocytes induces epithelial damage in the form of cytotoxicity of the oral epithelial cells that leads to the loss of epithelium by direct keratinocyte lysis. Phagocytic mononuclear cells and neutrophilic leukocytes are involved in this immunologic destructive process (2). Changes seen in RAU based on the histologic studies show that active cell-mediated immunity and local release of cytokines is characteristic. Various specimens including tissue samples, serum and even genes in patients with RAU have been investigated in order to complete cytokine profiles in these patients such as IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12, IL-13, interferon (IFN)- γ , TNF- α , TNF- β , transforming growth factor (TGF)- β , and granulocyte-macrophage colony-stimulating factor (GM-CSF; 3–9). However, so far it seems that IL-10, IL-6, TNF- α , and IFN- γ might have an important role in the RAU pathogenesis. Therefore, the aim of this study was to measure levels of salivary IL-6 and TNF- α in RAU patients which might reflect local production of cytokines at the sight of the disease. Additional information was sought as a possible result of the differences in examined cytokines regarding two distinct phases of the RAU disease itself.

Materials and methods

All the patients with RAU and controls were recruited from the School of Dental Medicine, University of Zagreb after informed consent, according to the Helsinki II Agreement was obtained from each participant. There were 26 patients with minor RAU, age range of 23–49 years (mean 27.3 years), during acute phase and remission period (only 13 patients were available). All patients had minor RAU and were selected according to

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Accepted for publication November 24, 2005

Lehner's criteria (10). None of the RAU patients had periodontal disease. The control group consisted of 26 healthy participants, age range of 22–64 years (mean 30.1 years) who were all healthy, did not have history of RAU, were free of periodontal disease, and did not take any medication for 1 month prior to this investigation. None of the participants were smokers.

Whole unstimulated saliva was collected according to Wu-Wang et al. (11) into calibrated tubes (0.1 ml) for 5 min, between 8 and 11 AM. Salivary flow rate was determined as well and all the patients had normal salivary flow rate. Saliva samples were then divided into two smaller tubes and frozen at -70°C until further analysis. For determination of salivary IL-6 and TNF- α , enzyme-linked immunosorbent assay (ELISA) was performed using commercially available kits (Quantikine, R&D systems, Inc., Minneapolis, MN, USA).

Statistical analysis was performed by use of descriptive statistics: ANOVA and values <0.001 ($P < 0.001$) were considered as significant. Additionally *post hoc* Scheffe's test was performed in order to obtain more precise results between tested groups.

Results

Descriptive statistical analysis (ANOVA) for salivary IL-6 values between three groups (acute RAU, remission RAU, healthy controls) did not show any significant differences (Table 1).

Additional *post hoc* Scheffe's testing was not performed as no significant differences were found between the three groups. The numbers represent units of measured salivary IL-6 and TNF- α in pg/ml.

Descriptive statistical analysis (ANOVA) for salivary TNF- α values showed significant differences between the three studied groups (acute RAU, remission RAU, healthy controls; Table 2).

There were significant differences between healthy controls and patients with acute RAU ($P < 0.001$). Significant differences were also found between patients

Table 3 *Post hoc* Scheffe's test showing significant differences between healthy controls, patients with acute RAU and during remission period in salivary TNF- α values

(I) groups	(J) groups	Mean difference (I - J)	SE	P-value
Scheffe's test				
Healthy	Acute RAU	-20.12*	7.76	0.04
Healthy	Remission RAU	-46.42*	9.50	<0.001
Acute RAU	Remission RAU	-26.31*	9.50	0.03

* $P < 0.001$.

with acute RAU and those in remission ($P < 0.001$; Table 3).

Discussion

It is postulated that RAU develops as a result of unknown antigenic stimulation, most probably local one and that cell-mediated immunity is activated expressing its activity in different levels of various cytokines. However, which type of immune response and the precise role of investigated cytokines is still unknown.

Circulating leukocytes from patients with RAU have been shown to secrete higher levels of TNF than leukocytes from controls (12) and high levels of TNF- α , have been detected in biopsies of ulcer tissue (4, 13). Taylor et al. (12) reported that TNF- α might play a role in the pathogenesis of RAU because elevated levels in serum of RAU patients were found during the acute phase whereas no differences between patients during remission period and controls could be found. On the contrary, Natah et al. (13) reported no TNF- α immunoreactivity in the epithelial cells of patients with RAU, suggesting that either the concentration of TNF- α is too low or RAU is not a major producer of TNF- α . The same authors (13) conclude that it seems that the major source of TNF- α that acts on RAU vascular endothelium is the adjacent mononuclear inflammatory cells. Yamamoto et al. (14) reported that TNF- α was hardly

Table 1 Analysis of variance for salivary IL-6 values in healthy controls, patients with acute RAU or during the remission period

	N	Mean	SD	SE	95% confidence interval for mean			
					Lower bound	Upper bound	Minimum	Maximum
Healthy	26	9.38	9.23	1.81	5.66	13.11	0	33
Acute RAU	26	12.5	17.51	3.43	5.43	19.57	0	70
Remission RAU	13	8.31	6.07	1.68	4.64	11.98	3	22
Total	65	10.42	12.77	1.58	7.25	13.58	0	70

Table 2 Analysis of variance for salivary TNF- α values in healthy controls, patients with acute RAU or during the remission period

	N	Mean	SD	SE	95% confidence interval for mean			
					Lower bound	Upper bound	Minimum	Maximum
Healthy	26	7.88	8.45	1.66	4.47	11.30	0	26
Acute RAU	26	28	26.19	5.14	17.42	38.58	0	120
Remission RAU	13	54.31	49.63	13.77	24.31	84.30	5	185
Total	65	25.22	32.48	4.03	17.17	33.26	0	185

detected in the sera of patients with RAU, but on the contrary, serum IL-6 levels were increased in patients with acute RAU. Decreased IL-6 levels were found during the remission period. It is interesting to note that we found significant differences between healthy controls and patients with acute RAU as well as between patients with acute RAU and during the remission period regarding salivary TNF- α values. The highest levels of salivary TNF- α were seen in patients with RAU during the remission period, indicating a possible role for TNF- α during healing of RAU, a finding which has not been reported so far. We might conclude that TNF- α plays more important role during healing period in comparison with the previously thought more important role in lysis and cytotoxic destruction of epithelial cells during the active phase of the RAU disease (2). It has been shown that TNF- α has a synergistic effect with IFN- γ as well as IL-10 and maybe their relationship changes during the acute phase and remission period, resulting in the ulcer formation and healing process. In our study we were unable to confirm the previously hypothesized synergistic interaction of TNF- α and IL-6 suggested by Sugerman et al. (15) who reported that TNF- α often exerts its biologic activities together with other cytokines especially with IL-6.

Furthermore, another hypothesis could be suggested, based on the different cytokine profiles reported in the published literature for RAU together with the possibility that RAU maybe a single clinical presentation of a spectrum of diseases with different etiologies rather than one single disease with multifactorial etiology (2). Then we might speculate that different antigens activate different types of cytokines.

To our knowledge this is the first report upon salivary cytokines in RAU and results are difficult to compare with other studies particularly because the majority of other studies did not divide RAU patients into acute phase and those during remission. Because no differences between salivary IL-6 levels were found between patients with RAU and controls, it seems unlikely that IL-6 has a role in RAU disease.

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Acknowledgment

The authors wish to thank Mrs Jennie Chaston for language editing of the manuscript.