

Periodontal disease and cytokine inhibitors

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Abstract

The aim of the presented paper was to examine the occurrence in saliva of interleukin – 1 receptor antagonist (IL-1 ra) and soluble receptor type I of tumor necrosis factor (sTNF RI) in patients with periodontitis. The levels of interleukin-1 receptor antagonist and soluble TNF receptor type I (s TNF RI, p55) in saliva were determined with an immunoenzymatic ELISA method using a factory-ready quantitative enzymatic test manufactured by Quantikine, R&D System. The level of occurrence for interleukin-1 receptor antagonist (IL-1 ra) in saliva was statistically significantly higher ($p < 0.05$) in persons with periodontitis as compared with the control group.

Key words: periodontal disease, interleukin – 1 receptor antagonist (IL-1 ra), soluble receptor type I of tumor necrosis factor (sTNF RI)

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Introduction

In the course of infection and simultaneous activation of specific and non-specific anti-bacterial defense mechanisms, the connective periodontal tissue is usually damaged. Depending on the infection size and defense response, the damage to tissues can be reversible or persistent. The key role in the destruction of periodontal tissues plays interleukin-1 and TNF-alpha. It is known that the interleukin-1 level in the blood serum and gingival fluid correlates with the clinical indicators of periodontium state [1, 2]. It has been proved that bacteria *Porphyromonas gingivalis* and *Actinobacillus actinomycetemcomitans* have the ability to stimulate monocytes and macrophages to produce interleukin-1 (IL-1) and TNF – tumor necrosis factor [3, 4]. These cytokines induce the activity of osteoclasts, causing bone resorption. There are numerous macrophages in the periodontal tissues producing considerable amounts of IL-1, which favors inflammatory processes in periodontium, destroys periodontal connective tissue and bones [5]. It is believed that both the IL-1 receptor antagonist and soluble s TNF RI receptor are physiological protections against the potential overproduction of IL-1 and TNF and against undesirable effects of excessive activity of these cytokines [6, 7].

Periodontitis is a disease always accompanied by a bacterial infection. We still do not know everything about immune and inflammatory response to infections in the course of periodontitis. IL-1 ra and s TNF RI are one of the most important anti-inflammatory mediators. The increase in the levels of these cytokines in systemic fluids statistically correlates with the disease activity [8, 9]. The results of latest research on the significance of the above listed cytokines in periodontitis are not univocal and we still need more data concerning this subject. The aim of the presented paper was to examine the occurrence in saliva interleukin – 1 receptor antagonist (IL-1 ra) and soluble receptor type I of tumor necrosis factor (sTNF RI) in patients with periodontitis.

Materials and methods

The tests were performed in the group of 80 persons, aged 18-54, including 45 females and 35 males. The test group consisted of 50 persons suffering from chronic periodontitis, aged 26-54 (30 females, 20 males). The control group consisted of 30 persons with clinically healthy periodontium, aged 18-49 (15 females, 15 males). The samples of mixed non-stimulated saliva were taken 2 hours after breakfast, then refrigerated and stored at -20°C . After defreezing, all samples

were centrifuged 1000xg for 20 minutes. The levels of interleukin-1 receptor antagonist and soluble TNF receptor type I (s TNF RI, p55) in saliva were determined with an immunoenzymatic ELISA method using a factory-ready quantitative enzymatic test manufactured by Quantikine, R&D System Europe Ltd., Abingdon, Oxon, United Kingdom, according to the manufacturer's recommendations. The minimum cytokine level that can be detected with these tests amounts to: 22 pg/ml for IL-1 ra and 3 pg/ml for s TNF RI. Before performing the tests, response specificity was checked. To achieve this, antibodies anti-IL-1 ra (AF-280-NA) and anti-s TNF RI (AF225) made by R&D Company in concentrations recommended by the manufacturer were added to selected samples. The incubation was carried out at room temperature for 1 h. After this period the concentrations of IL-1 ra and s TNF RI in the samples with antibodies and without were checked, with the above mentioned enzymatic tests manufactured by R&D Company.

Statistical analysis

Data were analysed by using Mann-Whitney statistical test. A *p*-value of < 0.05 was considered significant.

Results

Mean concentration values in saliva for interleukin-1 receptor antagonist (IL-1 ra) and soluble TNF receptor type I (s TNF RI) in persons suffering from chronic periodontitis and in control group are presented in table 1. The level of occurrence for interleukin-1 receptor antagonist (IL-1 ra) in saliva was statistically significantly higher (*p*<0.05) in persons with periodontitis as compared with the values for the control group. The levels of soluble TNF receptor type I (s TNF RI) in saliva in persons suffering from periodontitis and in the control group did not differ statistically significantly and their values were similar.

Discussion

The excess of locally produced cytokines is released to the peripheral blood, which results in negative changes to the entire immune response. The tissue macrophages are main source of IL-1 ra. That's why the concentration of IL-1 ra is always higher in the site of inflammation than in peripheral vessels. It is believed that the increase of IL-1 concentration in gingival fluid is one of the markers for assessing the gravity of periodontitis. Kabashima et al. maintain that the presence of IL-1 ra in gingival fluid confirms an inflammatory process in progress because in their investigations they have not proved the occurrence of IL-1 ra in the gingival fluid taken both from the persons with clinically healthy periodontium and from the non-inflammatory sites in persons with periodontitis [10]. Very low concentration of IL-1 ra in gingival fluid of persons suffering from advanced periodontitis presented in some

papers is probably associated with the fact of hydrolyzing the IL-1 ra particles by bacteria of a species: *Porphyromonas gingivalis*. It should be also added that *Actinobacillus actinomycetemcomitans* bacteria do not have this feature. Thus, it can be assumed that the composition of bacterial flora has a significant and regulating influence on the concentration values in systemic fluids, including gingival fluid and saliva. The elevated concentration of IL-1 ra in gingival fluid in persons with chronic periodontitis has been noted many times. In our own investigations statistically significant (*p*<0.05) higher concentration values of IL-1 ra in saliva in patients with periodontitis were noted as compared with the control group. In persons suffering from periodontitis the increase of anti-inflammatory cytokines in gingival fluid is often found. In the case of periodontitis evoked by bacteria of species: *Porphyromonas gingivalis* and/or *Actinobacillus actinomycetemcomitans*, antigens coming from these microorganisms induce the production and secretion of both IL-1 ra, and s TNF RI by the peripheral blood lymphocytes [11, 12]. The chronic overproduction of pro- and anti-inflammatory cytokines leads initially to an excessive inflammatory response and then to a compensatory anti-inflammatory response, which in the case of periodontitis may cause some undesirable complications such as intensification of clinical symptoms or oral cavity mycosis. Any damage to periodontal tissues causes significant changes in the production of pro- and anti-inflammatory cytokines. The balance existing in physiological conditions between pro- and anti-inflammatory immune responses may be disturbed considerably in the course of periodontitis. Our own investigations showed a statistically significant increase in concentration of IL-1 receptor antagonist (IL-1 ra) in persons suffering from chronic periodontitis as compared with persons with clinically healthy periodontium.

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Table 1. Mean concentration values in saliva for IL-1 ra and sTNF RI in persons with chronic periodontitis and in control group

	Chronic periodontitis	Healthy periodontium
IL-1 ra [pg/ml]	26316.55 (SD=4090.70)*	20901.49 (SD=6178.38)*
sTNF RI [pg/ml]	683.71 (SD=398.33)	719.28 (SD=425.13)

* *p*<0.05

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