

The correlation between selected cytokine inhibitors and chronic periodontitis

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Abstract

The aim of this study was to assess occurrence of interleukin-1 receptor antagonist (IL-1Ra) and soluble tumor necrosis factor receptor (sTNF RI) in patients with chronic periodontitis, as well as to establish whether significant relationship exists between the cytokine inhibitors level and clinical parameters of periodontium. Significantly higher IL-1Ra concentration in saliva was observed in the chronic periodontitis group as compared with the control group. The correlation between IL-1Ra saliva concentration and plaque index, bleeding on probing and clinical attachment level has been found in the chronic periodontitis. The results suggest there is a link between saliva level of IL-1Ra and the ongoing inflammatory process in the periodontium.

Key words: chronic periodontitis, cytokines, interleukin-1 receptor antagonist (IL-1Ra), soluble tumor necrosis factor receptor (sTNF RI).

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Introduction

Chronic periodontitis is related in the first place to the presence of bacterial biofilm. Gram-negative bacteria isolated mainly from periodontal pockets constitute a factor initiating the immuno-inflammatory reaction resulting in periodontal tissue destruction [1, 2]. Host's immune response in oral cavity is limited to developing immune mechanisms that lead to antigen elimination. Migration of immune system cells in the inflammatory environment takes place in the presence of cytokines – signal-transducing glycoprotein molecules which bind to corresponding extramembraneous receptors in the target cell. These receptors are specific to only one cytokine [3, 4]. Prior to expression of cytokine-induced receptor proteins, appropriate effector mechanisms of target cells aimed at combating pathogens most often take place. By participating in communication between the immune system and inflammatory reaction, cytokines show mainly local action. Key role of interleukin-1 (IL-1), IL-4, IL-6, IL-8 and tumor necrosis factor α (TNF- α) has been shown in inducing and mod-

ifying effector mechanisms in pathogenesis of periodontitis [5]. Research focused on IL-1 and TNF- α and their inhibitors may be of significant value in diagnosing and assessing progression of periodontal diseases. The aim of this study was to assess the occurrence of interleukin-1 receptor antagonist (IL-1Ra) and soluble tumor necrosis factor receptor (sTNF RI) in patients with chronic periodontitis, as well as to establish whether significant relationship exists between the cytokine inhibitors assessed and parameters of clinical periodontal condition.

Material and methods

The individuals 160 patients, aged 18-55, were participated in the study, including 115 patients with chronic periodontitis, aged 19-55, and 45 persons aged 18-43 with clinically healthy periodontium. Inclusion criteria required allowed for the enrollment of patients without systemic diseases, non-smokers and receiving no medications modifying the microflora and immuno-inflammatory response. Clinical examination included assessment of pocket depth

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(in millimetres, PD), clinical attachment level (in millimetres, CAL), percentage of teeth surfaces with plaque present (%PL) and bleeding on probing (%BOP). Clinical examination was performed according WHO recommendations [6-9]. For the purposes of immunological tests, 2 ml samples of mixed, non-stimulated saliva have been collected. The material was subsequently frozen and stored at -70°C until the test was performed. Concentrations of the selected cytokine inhibitors were measured (pg/ml) by means of ELISA (Quantikine, R&D System). Before the tests were performed, specificity of reaction had been checked.

Statistical analysis

Statistical analysis was performed using the Statsoft Statistica v.7.0 program. To compare the statistical significance of differences in clinical and immunological results between groups T-Student and Mann-Whitney tests were used. To evaluate the correlation between clinical and immunological results the Pearson’s r test was used.

Ethics

The patients and persons from control group gave a consent after details of the protocol were fully explained. The protocol of the study was conforms to the ethical guidelines of the World Medical Association Declaration of Helsinki.

Results of clinical examination

Analysis of results with respect to oral hygiene status, condition of gingivae and periodontium in the group of patients with chronic periodontitis and the control group has shown statistically significant differences ($p < 0.001$) between the groups. Presence of bacterial plaque has been detected in all patients with chronic periodontitis and in the majority of patients from the control group. Mean value of percentage plaque index (%PI \pm SD) amounted to $59.97\% \pm 26.67\%$ in patients with chronic periodontitis and 6.82%

($\pm 4.71\%$) in the control group. Bleeding on probing index (%BOP \pm SD) was $45.50\% \pm 19.72\%$ in the chronic periodontitis group and $0.18\% \pm 0.75\%$ in the control group. Mean value of periodontal pocket depth (PD \pm SD) amounted to $4.27 (\pm 0.75)$ in the chronic periodontitis group and $0.90 (\pm 0.25)$ in the control group. Mean clinical attachment level (CAL \pm SD) was $4.48 (\pm 1.00)$ in patients with chronic periodontitis and $0.81 (\pm 0.24)$ in control group.

Results of immunological tests

The mean concentration (\pm SD) of IL-1Ra in saliva of patients with chronic periodontitis amounted to $29491.75 \text{ pg/ml} (\pm 7853.62)$ and was significantly higher as compared to results in the control group: $20088.22 \text{ pg/ml} (\pm 7639.59)$. No significant differences have been found between sTNF RI levels (\pm SD) in patients with chronic periodontitis ($622.19 \text{ pg/ml} \pm 358.53$) and with clinically healthy periodontium ($689.53 \text{ pg/ml} \pm 414.71$). Statistical analysis performed in the chronic periodontitis group has shown a positive correlations between mean IL-1Ra saliva concentration and mean values of plaque index, bleeding on probing and clinical attachment level (Figs. 1, 2, 3). No relationship has been shown to exist between mean IL-1Ra saliva level and periodontal pocket depth and between mean IL-1Ra saliva level and clinical parameters assessed in the control group. Statistical analysis has also shown no relationship between mean sTNF RI saliva concentration and parameters of clinical periodontal condition in patients from both the chronic periodontitis as well as the control group.

Discussion

Assessment of clinical parameters is of special importance in determining the activity of a periodontal disease [6, 7]. In the presented studies, statistically significant differences have been observed in mean values of parameters for assessment of oral hygiene and condition of gingivae

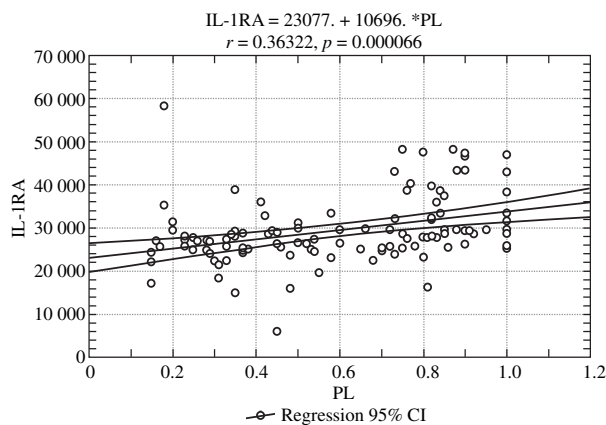


Fig. 1. Correlation between the saliva levels of IL-1Ra and plaque index (PL) in chronic periodontitis ($p < 0.05$)

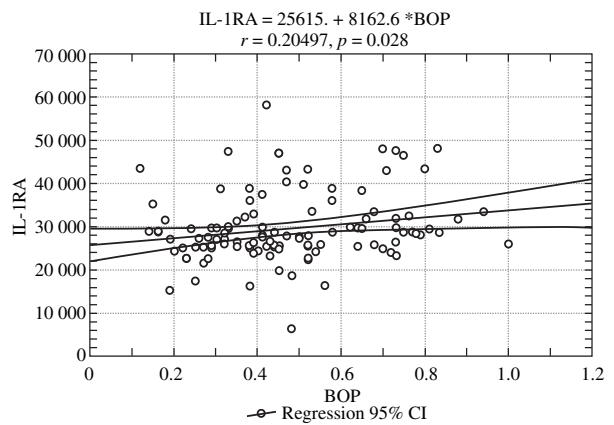


Fig. 2. Correlation between the saliva levels of IL-1Ra and bleeding on probing (BOP) in chronic periodontitis ($p < 0.05$)

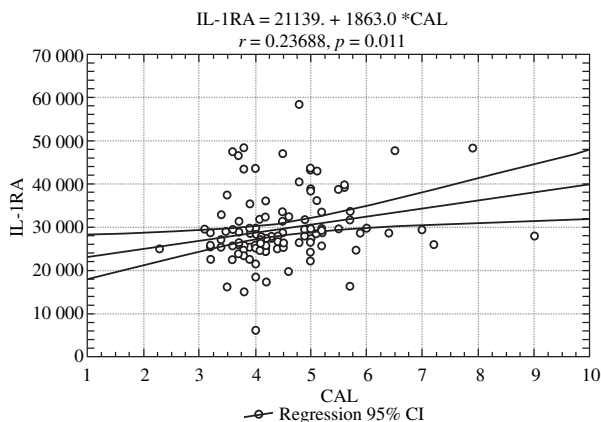


Fig. 3. Correlation between the saliva levels of IL-1Ra and clinical attachment level (CAL) in chronic periodontitis ($p < 0.05$)

and periodontium – %PI, %BOP, PD, CAL – between patients with chronic periodontitis and the control group. Many previous studies have shown a link between indices of periodontium state and the advancement of inflammation [8, 9]. The tests performed have also found relationships between the aforementioned clinical parameters and saliva concentration of interleukin-1 receptor antagonist (IL-1Ra): a positive correlation between IL-1Ra level and plaque index in the chronic periodontitis group ($p < 0.05$), positive correlation between IL-1Ra level and bleeding on probing index ($p < 0.05$) and a positive correlation between IL-1Ra level and clinical attachment level ($p < 0.05$). No relationship has been found to exist between mean IL-1Ra saliva concentration and pocket depth or between mean IL-1Ra concentration and clinical parameters in the control group. The results suggest there is a link between saliva level of interleukin-1 receptor antagonist (IL-1Ra) and the ongoing inflammatory process in the periodontium. Significantly higher IL-1Ra concentration has been found in the group of patients with chronic periodontitis as compared with the control group. According to Kabashima *et al.* [10] presence of IL-1Ra in gingival fluid is always evidence of an ongoing inflammatory process, because these authors did not detect IL-1Ra in patients with clinically healthy periodontium. Holmlund *et al.* [11, 12] assessed IL-1Ra concentration in gingival fluid collected from periodontal pockets prior to and following treatment. IL-1Ra level was significantly higher in the material collected from patients with chronic periodontitis than in the control group; a statistically significant decrease in IL-1Ra concentration following treatment was also observed. Studies in mice have shown that IL-1Ra released by cells stimulated by *A. actinomycetemcomitans* inhibits bone resorption [13, 14]. Delima *et al.* used recombinant human IL-1Ra injected into gingival papillae of monkeys, in which periodontitis had been experimentally induced. After treatment, statistically significant inhibition of alveolar bone loss by 91% and significant reduction of clinical attachment level by 51% was

observed [15]. Furthermore, results of studies by Ishihara *et al.* and Rawlinson *et al.* did not confirm the abovementioned results [16, 17]. These authors have shown that IL-1Ra concentration in gingival fluid was lower in patients with chronic periodontitis than in patients with clinically healthy periodontium, which might suggest a protective role of IL-1Ra and indicate that concentration of the produced inhibitor is not sufficient in order to inhibit the inflammatory process. The study by Rawlinson *et al.* [17, 18] shows that there is a reverse relationship between IL-1 α and IL-1Ra concentrations in gingival fluid collected from patients with chronic periodontitis. It should be emphasized that the intracellular form of IL-1Ra can fulfil its function until it is released from necrotic or decaying tissues together with IL-1 β from macrophages and IL-1 α from keratinocytes. Assessing concentration of IL-1Ra and IL-1 β in GCF in experimentally induced periodontitis, Waschul *et al.* [19] did not establish statistically significant differences in concentrations of these cytokines. Authors own study has shown presence of IL-1Ra in saliva of patients with clinically healthy periodontium. The glycoprotein described may not demonstrate the action of a cytokine inhibitor under certain circumstances during an ongoing inflammatory process in the periodontium. Periopathogenic bacteria may affect the cytokine network in periodontal tissues and gingival fluid by means of blocking the activity of inhibitors. It has been shown that anti-inflammatory action of IL-1Ra can be inhibited by *Porphyromonas gingivalis*, which are able to hydrolyse this cytokine [20]. Yoshinari *et al.* [21] have introduced the concept of index of total interleukin-1 activity. Its value is a product of IL-1 α and IL-1 β concentrations and IL-1Ra concentration in GCF. Authors have observed the highest value of this index in patients with biggest alveolar bone loss. They have also shown that there is a positive correlation between the index and clinical state parameters (PD, GI) and a decrease in the index following treatment [21]. The tests performed have shown no significant difference in sTNF RI saliva levels between patients from the chronic periodontitis group and control group. This cytokine is excreted in an early phase of inflammation as a response to newly produced TNF and rapidly regains baseline concentration. No relationship has been shown to exist between sTNF RI concentration and indices of clinical periodontium condition. Soluble forms of receptors fulfil the physiological function as inhibitors of this cytokine, while lack of significant difference in sTNF RI concentration in the group of patients with chronic periodontitis and control group should be interpreted by the chronic nature of the periodontal disease. Soluble tumor necrosis factor receptors are present in peripheral blood serum and urine of healthy people. Increase in their concentration is observed in the course of various bacterial and viral infections, e.g. in patients with acquired immunodeficiency syndrome (AIDS), as well as with neoplastic diseases [22, 23]. Assessing peripheral

blood in patients with chronic periodontitis, McFarlane *et al.* have shown a positive correlation between TNF concentration increase and soluble TNF receptor [24]. In a study on concentration of TNF- α and its receptors p55 and p75 in inflamed periodontal tissues, Tervahartiala *et al.* have observed that an increase in infiltration of the periodontal pocket with macrophages, fibroblasts and endothelial cells was accompanied by increased expression of p55 receptor for TNF- α . The p75 receptor, however, was not detected. Increased expression of p55 receptor was accompanied by high activity of a collagenolytic metalloproteinases [25]. Occurrence of abnormalities in expression of membrane TNF receptors on polymorphonuclear leukocytes in the course of periodontitis may be one of the reasons for lack of significant difference in sTNF RI levels between patients from chronic periodontitis and control group. This issue requires further thorough studies.

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