



Short Communication

Interleukin 6, Tumor Necrosis Factor α and Their Soluble Receptors in the Blood Serum of Patients with Denture Stomatitis and Fungal Infection

JAN KRZYSZTOF PIETRUSKI^{1*}, MAŁGORZATA DOROTA PIETRUSKA², EWA JABŁOŃSKA³, PAWEŁ SACHA⁴,
MARIA ZAREMBA⁴ and WANDA STOKOWSKA²

¹ Department of Prosthodontics, ² Department of Conservative Dentistry, ³ Department of Immunology, ⁴ Department of Microbiology, University Medical School, Białystok, Poland.

Abstract. Determinations of the blood serum levels of interleukin 6 (IL-6), tumor necrosis factor α (TNF- α) and their soluble receptors (sIL-6R, sTNFR) in denture stomatitis patients (DS) were performed. Serum levels of interleukins and their soluble receptors were measured using the ELISA method. In all examined patients mycological diagnostics were conducted using API 20C AUX stripe tests and an automatic ATB machine. Results were compared with those of healthy denture wearers (D), and controls (C). In DS patients, yeasts were isolated in 90.9%, in D in 66.7% of cases. The most often isolated species in both groups was *Candida albicans*. Mean concentrations of IL-6 and TNF- α were statistically significantly higher in DS and D groups compared to controls. Mean concentrations of sIL-6R were similar in all groups; however, concentrations of sTNFR in both DS and D groups were significantly lower compared to controls. There were no correlations found between values of IL-6 and TNF- α nor between examined interleukins and their soluble receptors.

Key words: denture stomatitis; fungal infection; interleukin 6; tumor necrosis factor; soluble receptors.

Introduction

Denture stomatitis (DS), or inflammation of the oral mucosa in denture wearers, is caused by local or general endo- and exogenic agents. Fungal infection, mainly due to yeasts, is an exogenic agent frequently found to coexist with DS (70–100% of cases)^{12, 29}. Fungal antigens, particularly those with glycoprotein structures, affect the immune system cells⁴. They stimulate humoral response, but inhibit cellular response as well as the activity of phagocytizing cells^{8, 9, 11, 15, 19}. In recent years considerable interest has been focused on

the participation of cytokines in fungal infection. Some cytokines are involved in antifungal defense, others can promote infection³². The so-called protective cytokines include interleukin 2 (IL-2), interferon γ (IFN- γ), the granulocyte-macrophage colony stimulating factor (GM-CSF) and tumor necrosis factor^{5, 17, 27}. IL-12 and the transforming growth factor β (TGF- β) play a similar role^{25, 26, 28}.

Microbial infection is associated with an induction of the inflammatory process mediated by a number of soluble and cell-associated factors, with a special role of the so-called “proinflammatory” cytokines IL-1,

* Correspondence to: Dr Jan K. Pietruski, Department of Prosthodontics, University Medical School, M. Skłodowskiej 24A, 15-276 Białystok, Poland, tel.: 48-85-746 83 49.

IL-6, IL-8 and TNF- α ²². Both IL-6 and TNF- α are characterized by multidirectional action which regulates functions of various cell populations responsible for humoral and cellular reactions, phagocytosis and the killing of microorganisms.

Fungal infection and the accompanying inflammatory process affect secretion and release of IL-6 and TNF- α , which in turn condition the development and course of infection. That is why we decided to investigate the concentrations of these cytokines and their soluble receptors in the blood serum of patients with DS and coexisting fungal infection than in the blood serum of healthy denture wearers and people without dentures and to compare our finding in all of these 3 groups.

Materials and Methods

The study population included 37 generally healthy complete denture wearers. Of these, 2 individuals, 18 women and 4 men aged 33–63 years, had symptoms of DS (DS group) and 15 denture wearers, 11 women and 4 men aged 31–67 years, had healthy oral mucosa (D group). Diagnosis of DS was made on the basis of case history and clinical examination according to the classification of Newton as modified by SPIECHOWICZ³⁰. 82% of the examined were included in class II, 18% in classes I and III. The Control group (C) consisted of 15 healthy individuals with preserved dentition, 8 women and 7 men aged 29–57 years, with no yeasts isolated from the oral mucosa. All subjects underwent fungal diagnostics.

Swabs were taken from the palate and alveolar processes, also, from the tooth surfaces in the case of those with preserved dentition and from the mucosal denture surface denture wearers. Culture was started within 1 h in selective Sabouraud medium, where the fungi were incubated at 37°C for 14 days. Species identification was based on the ability to form pseudomycelium, on spore type and capability for hydrocarbon absorption determined by stripe tests API 20C AUX³⁴. Final identification was performed with an automatic ATB machine (BioMerieux). Culture and identification of fungi at the species level were carried out in the Department of Microbiology, Medical University School of Białystok. Blood for the assay of the cytokines and their soluble receptors was collected from the ulnar vein. Concentrations of IL-6 and TNF in serum were determined by the ELISA method using the Quantikine Human IL-6 Immunoassay and the Quantikine TNF- α Immunoassay. The ELISA method was also applied to

determine sIL-6R and sTNFR concentrations in serum using the Quantikine Human IL-6sR Immunoassay and the Quantikine sTNFRp55 Immunoassay, (R&D System, Minneapolis, MN USA).

The results obtained were subjected to mathematical and statistical analysis. After the data distribution ascertainment, the Mann-Whitney *U*-test was used to compare the mean values. Statistical differences were considered significant when $p < 0.05$.

Results

Yeasts were isolated from the mucosa of 20 DS patients (90.9%), *Candida albicans* being the prevailing species in the DS group (14 cases – 63.6%). In the D group, yeasts were found in 10 cases (66.7%) and *C. albicans* in 8 (53.3%). The incidence of the respective yeast species is presented in Fig. 1. IL-6 was observed in the serum of all the 22 DS cases. It was not found in 1 case of D and in 3 cases of the C group. The mean IL-6 concentration in the DS group was 29.68 pg/ml and in the group of 14 people with *C. albicans* 32.86 pg/ml. In the D group mean values of IL-6 were 24.36 pg/ml and 27.5 pg/ml, respectively. The mean value of the control group was 5.09 pg/ml. The mean values in the D and DS groups were statistically significantly different from the mean value in control group. All the examined of the DS, D and C groups had sIL-6R detected in serum. The mean concentrations in the DS group (44.15 pg/ml), in D group (44.17 pg/ml) and in the C group (39.9 pg/ml) showed no statistically significant differences. No correlation was revealed between the concentrations of IL-6 and sIL-6R. Serum of only 19 DS patients was examined for the presence of TNF- α . TNF- α was detected in the serum in all of these 19 DS cases and in 15 D cases. It was not found in one control case. The mean TNF concentration in the DS group was 62.43 pg/ml, in the D group 40.87 pg/ml

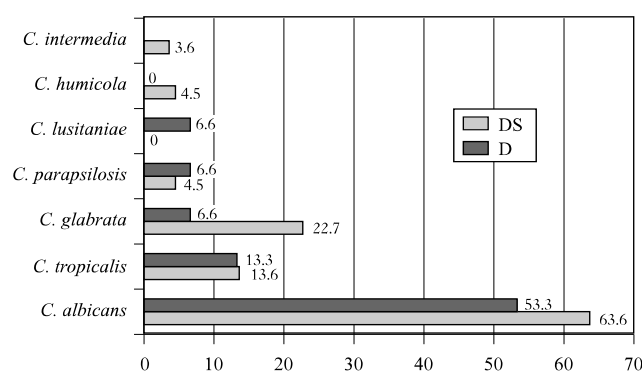


Fig. 1. Occurrence of respective yeast species in DS (denture stomatitis) and D (denture wearers) groups

Table 1. Mean concentrations of IL-6, sIL-R6, TNF- α and sTNFR 55 in blood serum denture stomatitis (DS), denture wearers (D) and control (C) patients (min.-max. values in brackets)

Group	IL-6 (pg/ml)	sIL-6R (pg/ml)	TNF- α (pg/ml)	sTNFRp55 (pg/ml)
DS	29.68 (± 20.35)* (0–73) p = 0.000390	44.15 (± 9.2) (21.2–62)	62.43* (± 31.3) (5–95) p = 0.006289	0.37* (± 0.13) (0.8–0.81) p = 0.000069
DS <i>C. albicans</i> +	32.86* (± 21.97) (7–73) p = 0.000390	46.29 (± 10.37) (21.2–62)	78.0* (± 22.7) (47–95) p = 0.006289	0.37* (± 0.16) (0.18–0.81) p = 0.000069
D	24.36* (± 14.63) (5–45) p = 0.000173	44.17 (± 8.7) (21.2–57.6)	40.87* (± 19.42) (5–65) p = 0.003369	0.50* (± 0.18) (0.24–0.84) p = 0.012832
D. <i>C. albicans</i> +	27.5* (± 16.2) (11–45) p = 0.000173	45.1 (± 7.24) (37.2–57.6)	41.09* (± 25.7) (5–53) p = 0.003369	0.50* (± 0.2) (0.24–0.84) p = 0.012832
C	5.09 (± 4.4) (0–10)	39.9 (± 5.95) (29.6–48)	11.42 (± 7.04) (0–19.3)	0.69 (± 0.13) (0.51–0.86)

* Statistically significant difference from C group.

and in the C group 11.42 pg/ml. The mean TNF- α values found in the DS and D groups differed significantly from the mean value of the control group. In 12 DS cases with *C. albicans*, the mean TNF value was the highest, 78.0 pg/ml, while in 8 D cases it was 41.09 pg/ml. The mean concentration of sTNFRp55 in the DS group was 0.37 pg/ml and in the D group 0.50 pg/ml, being significantly lower compared with the mean control value (0.69 pg/ml). Significant differences were not found in the mean values of IL-6, sIL-R6, TNF- α and sTNFRp55 when comparing the DS and D groups. No correlation was observed between the concentrations of TNF- α and sTNFRp55. There was also no correlation between the concentrations of IL-6 and TNF- α . The results are presented in Table 1.

Discussion

We isolated a high percentage of yeasts from denture wearers in the DS and D groups. *C. albicans*, characterized by pathogenicity and the ability to induce host immune reaction, was the most common. The infection progression is known to depend on yeast strain virulence, infection intensity and host resistance. There are clinical reports about the progression of fungal generalized infection in patients with primary or secondary immune defects. Experiments and clinical observations indicate that the infection itself and the presence of fungal antigens also affect immune reactions^{8, 11, 19}. Experiments in mice have demonstrated that fungal antigens, mannose and glycoprotein fractions have an effect on the Th lymphocyte-dependent cellular immunity. Resistance of mice to *Candida* in-

fections is associated with the activity of Th1 lymphocytes, while the progression of the infection with Th2¹⁸. These relationships result from the secretion and release of interleukins by Th1 and Th2 cells, which in different ways regulate functions of various cell populations.

We observed higher concentrations of IL-6 and TNF- α in DS and D cases, compared with controls, particularly in the cases with *C. albicans*. Similar results were obtained by other authors who evaluated IL-6 in denture stomatitis²³. They found a high concentration of IL-2 in serum and enhanced release of IL-3 in cell cultures in DS patients and in denture wearers without denture stomatitis but with yeasts in the oral cavity. Few studies have been concerned with IL-6 concentration in serum in candidiasis. A very high serum concentration of IL-6, 7 times the control values, was observed in patients with chronic pseudomembranous candidiasis (CMC)³³. According to LILIC et al.¹⁶, CMC patients have an impaired cellular response to fungal antigens, which leads to increased IL-6 secretion. The role of IL-6 in candidiasis has been confirmed by the studies of ROMANI et al.²⁴, who found that mice with an IL-6 deficiency are very susceptible to yeast infection.

The elevated TNF- α serum concentration observed in our study may result from its increased release by various cells of the immune system stimulated by fungal antigens. TNF- α plays a special role in antifungal resistance. It stimulates proliferation and cytotoxicity of T lymphocytes, activates phagocytizing cells and regulates secretion of other cytokines¹⁰. Thus, fungi, by stimulating TNF- α secretion, initiate mechanisms of their elimination.

The release of the cytokines examined and, thus,

their concentrations in systemic fluids depend also on their interaction. TNF- α is a strong inducer of IL-6 synthesis and release by neutrophils⁶. On the other hand, IL-6 inhibits TNF- α production and TNF- α -mediated cytotoxicity of mouse monocytes¹. The present study found no correlation between IL-6 and TNF- α concentrations in serum, which suggests that the IL-6 and TNF- α concentrations in blood may be the result of the action of many mediators, including other cytokines responsible for their release. IL-1 and IL-2 enhance secretion of IL-6 and TNF- α ^{6, 22}.

The efficacy of IL-6 and TNF- α is associated with the presence of their soluble receptors, sIL-6R and sTNF. They are able to bind the circulating cytokines and compete in this way with membrane receptors. The sIL-6R acts as an IL-6 antagonist and regulates its biological activity. We did not observe any differences in the mean concentrations of sIL-6R between the DS, D and C groups. No correlation was found between the concentrations of IL-6 and sIL-6R. The results suggests that the presence of yeasts does not have a direct effect on the increase in sIL-6R release. This has been confirmed by the studies of other authors who found *in vitro* no increase in the secretion of sIL-6R by the cells of CMC patients after antigenic stimulation by fungi²². It is likely that this level of the receptor prevents excessive IL-6 activity. The sTNFRp55 and sTNFRp75 take part in the mechanism regulating the action of TNF- α ^{7, 20}. Like TNF- α , they are produced by a number of cells: monocytes, macrophages, T lymphocytes, NK cells, fibroblasts and neutrophilic granulocytes^{3, 7, 13}. TNFR can block binding of the circulation TNF- α , inhibit its activity, block its binding with the cell membrane receptor and thus is a TNF- α antagonist³. High concentrations of sTNFRp55 were detected in the blood of patients suffering from diseases of connective tissue showing features of autoimmunization, in rheumatoid arthritis, visceral lupus and in neoplastic diseases^{2, 14, 21}. It can be assumed that the increase in the secretion of TNF- α receptors in a response to the elevated TNF concentration.

The studies of STEINSHAN et al.³¹ indicate that defense antifungal mechanisms, irrespective of the TNF- α effect, are directly related to the action of the soluble receptors, being the protective factors in *C. albicans* infection. Transgenic mice with sTNFRp55 deficiency are very susceptible to the progression of candidiasis. We found low values of the soluble receptor, which may result from its binding with TNF- α released in excess or may indicate impairment of its secretion due to the immunosuppressive action of fungal antigens on the receptor-releasing cells.

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