

INFLUENCE OF ALLERGY ON CYTOKINE LEVEL IN NASAL DISCHARGE OF PATIENTS WITH NASAL POLYPS

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Cytokine levels in nasal discharge reflect the inflammatory status of the nasal mucosa. The aim of the paper was to compare the concentrations of cytokines in nasal discharge in allergic and non-allergic patients with nasal polyps (NP). Thirty patients with NP (13 atopic and 17 non-atopic) were included in this study. Samples of nasal discharge were collected from nasal cavities of all 30 subjects. The levels of TNF- α , TNF- β , IL-1 β , IL-2, IL-12, IFN- γ , IL-4, IL-5, IL-6, IL-10, and IL-8 in nasal discharge were measured using commercial flow cytometric kit. We found significantly higher concentrations of IL-4 ($p < 0.01$), IL-5 ($p < 0.05$), IL-6 ($p < 0.05$) and TNF- β ($p < 0.05$) in allergic patients with NP than in non-allergic. Polyps in non-atopic and atopic patients have different immunological patterns. *Acta Medica Medianae* 2010;49(3):40-44.

Key words: allergy, cytokines, nasal polyps

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Introduction

Chronic rhinosinusitis (CRS) is an inflammatory disease of the nose and paranasal sinuses that lasts at least 12 weeks without complete withdrawal of symptoms. Nasal polyposis (NP), which is considered to be a subgroup of CRS, is defined as a chronic inflammatory disease of nasal and paranasal sinuses mucosa followed by formation of benign polyps that spread from sinuses to the nasal cavity. NP is characterized by epithel proliferation, glandular hyperplasia, thickening of the basement membrane, oedema, focal fibrosis and cell infiltration of stroma (1). Polyps most often originate from the anterior ethmoid complex and then they outreach between the middle nasal concha and the lateral nasal wall into the nasal cavity thereby causing symptoms such as nasal obstruction, anosmia, itching, mucous secretion and sneezing (2). NP is a multifactorial disease. Chronic persistent inflammation is a major factor in the development of polyps and among the inflammation starters bacterial, fungal and viral infection, allergy, and environmental

pollution are mentioned (2). Polyp tissue includes various inflammatory cells, among which eosinophils are dominant. They have the primary role in the maintenance of chronic inflammation (1,2).

It is assumed that a weakened Th1-mediated immune response is associated with an increased Th2-mediated immune response, and in conjunction with a chronic infection as well as with an increased presence of eosinophils it leads to polyp formation (3). It is further assumed that the weakened Th1-mediated immune response in these patients is connected with suppression of the specific toll-like receptors involved in the congenital immune response (3).

Nasal discharge represents the first line of defense, in which the leukocytes probably act as part of the defense mechanism along with the mucociliary transport and biochemical properties of the mucus (4). Products of cellular secretion should be detected in order to explain inflammatory changes of the upper respiratory mucosa (5). Nasal discharge contains little amounts of cytokines, which are potent biological factors involved in the regulation of inflammation and immune defense, as well as other mediators of inflammation that are expressed in various epithelial and non-epithelial cells (6). As cytokines play a dominant role in the pathophysiology of respiratory diseases, determining of cytokine profile in nasal discharge may help us understand the mechanisms included in the development of NP.

The prevalence of NP in Europe is around 2,7% (7). On the other hand, allergic rhinitis is a high prevalence disease in developed countries, affecting about 10-20% of the general population (7). However, the relationship between NP and

allergic rhinitis has not been completely defined. The connection between NP and allergic rhinitis has been mainly studied in order to determine whether NP in allergic individuals are different from those in non-allergic ones. The incidence of allergy in patients with NP has been found to range from 10% to 63.2% (8, 9).

The aim of this prospective study is to compare the levels of proinflammatory cytokines, Th1 cytokines, Th2 cytokines as well as one chemokine in nasal discharge of allergic and non-allergic patients with NP.

Subjects and methods

Patients:

Thirty (30) patients with NP (13 allergic and 17 non-allergic) were included in this prospective study. Written informed consent was obtained from all subjects. The study was approved by the Ethics Committee of the Military Medical Academy, Belgrade, Serbia. The diagnosis of NP was based on each patient's medical history as well as on the results of nasal endoscopy and computed tomography (CT) of paranasal sinuses. Only subjects with nasal symptoms that lasted for maximum two years were included in this study. The exclusion criteria were: asthma, aspirin hypersensitivity, antrochoanal and sphenchoanal polyps, cystic fibrosis, and primary ciliary dyskinesia. None of the subjects had any of the acute respiratory infections. None of them was treated with oral or topical corticosteroids, antibiotics and antihistaminics for at least three weeks before being included into the investigation. Skin prick-test was performed on all patients with the aim of determining hypersensitivity on common inhalant allergens: *Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, *Artemisia*, *Alternaria alternata*, cat and dog epithelium, mixture of grass pollen, feathers, birch pollen, mixture of flower pollen. The result was considered positive in the case when at least one of the induration diameters was greater than 3 mm compared to the negative control. Subjects were considered allergic if they had serum IgE level higher than 160 IU/ml.

Nasal discharge sampling and cytokine concentration measuring

Samples of nasal discharge were collected from the nasal cavities of all 30 subjects (17 patients with NP, and 13 patients with NP and allergy) using absorption technique with cotton wool sticks which were inserted into the nasal cavity behind the mucocutaneous junction for 60 sec, as previously described (10,11). Every sample was placed in a 2 ml Eppendorf tube containing 1 ml of transfer medium (phosphate-buffered saline with 50 µg/ml of gentamycin, 340 U/ml of penicillin G and 500 µg/ml of fungisone)

for 30 min with the aim of cytokines diffusion into transfer medium and was then stored at 4°C for 2h maximum. Nasal discharge was then centrifuged at 1000 g for 10 min in order to separate the cellular components. After centrifugation, supernatant was transferred and stored at -70°C until cytokine detection. Concentrations of eleven cytokines (proinflammatory cytokines TNF-α, TNF-β, IL-1β, Th1 cytokines IL-2, IL-12 and IFN-γ, Th2 cytokines IL-4, IL-5, IL-6 and IL-10, as well as chemokine IL-8) were measured in all of the 30 samples using the commercial flow cytometric kit (Flow cytomic, Bender MedSystems GmbH, Vienna, Austria) on the flow cytofluorimeter, according to the manufacturer's instruction. The detection threshold was as follows: 22 pg/mL for TNF-α; 32 pg/mL for TNF-β; 17 pg/mL for IL-1β; 28 pg/mL for IL-2; 20 pg/mL for IL-4; 30 pg/mL for IL-5, 21 pg/mL for IL-6; 13 pg/mL for IL-8; 20 pg/mL for IL-10; 15.1 pg/mL for IL-12; 8 pg/mL for IFN-γ.

Statistical analysis:

Data are expressed as mean values ± standard deviation (± SD). Comparison between groups was made by using the non-parametric Mann-Whitney U test. A *p* value was considered statistically significant if less than 0.05 (*p*<0.05).

Results

There were 8 male and 5 female patients with NP in the allergy group (mean age 43.85 ± 14.37 years) and 13 male and 4 female patients with NP in the group without allergy (mean age 45.24 ± 14.78 years).

When comparing the two main groups of patients (with and without allergy), we did not find any statistically significant differences in the concentrations of TNF-α, IL-1β, IL-2, IL-8, IL-10, IL-12, and IFN-γ in the nasal discharge (Table 1). The concentrations of IL-4, IL-5, IL-6 and TNF-β in nasal discharge were significantly higher in allergic patients with NP than in non-allergic patients with NP (1757 ± 1150.97 pg/mL vs 651.47 ± 1005.94 pg/mL, *p*<0.01; 761.38 ± 885.32 pg/mL vs 202.35 ± 287.26 pg/mL, *p*<0.05; 311.07 ± 245.28 pg/mL vs 91.47 ± 114.41 pg/mL, *p*<0.05; 281.61 ± 325.94 pg/mL vs 108.70 ± 208.81 pg/mL, *p*<0.05) (Table 2).

Discussion

Content of nasal discharge reflects the inflammatory status, as well as the evolution of mucosal disease. However, mechanisms of cytokines release in nasal discharge have not been investigated yet. The results published by Ohkubo et al. (12) showed that IL-6 is being released into the nasal discharge in allergic rhinitis mainly by the migrating cells and epithelial cells and it is all mediated by direct action of histamine, reflective effect of metacholine as well as after antigen stimulation.

Table 1. Cytokine levels in nasal discharge in atopic and non-atopic patients (in pg/mL)

Atopic NP patients

	IL-12	IFN- γ	IL-2	IL-10	IL-8	IL-6	IL-4	IL-5	IL-1 β	TNF- α	TNF- β
x	17,62	41,77	343,62	65,46	192,07	311,07	1757	761,38	52,77	37,77	281,61
SD	37,54	45,76	252,24	65,34	290,58	245,28	1150,9	885,32	76,03	28,62	325,94

Non-atopic NP patients

	IL-12	IFN- γ	IL-2	IL-10	IL-8	IL-6	IL-4	IL-5	IL-1 β	TNF- α	TNF- β
x	15,53	79,35	251,64	45,65	120,88	91,47	651,47	202,35	22,11	34,47	108,71
SD	34,03	64,38	230,21	62,9	107,31	114,41	1005,9	287,26	31,88	41,59	208,81

Table 2. Comparison between cytokine levels in nasal discharge in allergic and non-allergic patients

Cytokines	With allergy	Without allergy	p value
IL-4	1757 \pm 1150,9	651,47 \pm 1005,9	$p < 0,01$
IL-5	761,38 \pm 885,32	202,35 \pm 287,26	$P < 0,05$
IL-6	311,07 \pm 245,28	91,47 \pm 114,41	$P < 0,05$
TNF- β	281,61 \pm 325,94	108,71 \pm 208,81	$P < 0,05$

The initiating factor that triggers chronic inflammation and microbial colonization in NP is not known yet. Although inflammation mediated by Th2 cells is the main characteristic of the disease, it is not known what triggers the local production of Th2 cytokines and infiltration of eosinophils and lymphocytes (3). It has been shown that Th2 cytokines reduce the toll-like receptors' expression and inhibit their signaling function in lymphocyte membrane. That is how Th2 cytokines inhibit the production of Th1 cytokines, resulting in the reducing of antimicrobial resistance of nasal mucosa (3). The results of our research also showed significantly higher concentrations of Th2 cytokines (IL-4, IL-5 and IL-6) in nasal discharge of patients with NP and allergy than in nasal discharge of non-allergic patients. Previous results showed that IL-5 is one of the key proteins in the pathomechanism of tissue eosinophilia, by enhancing the differentiation, activation, expansion, mobilisation, as well as the survival of eosinophils (13). It is widely accepted that IL-5 plays an important role in the pathogenesis of allergic rhinitis where it induces eosinophil mobilisation, growth and differentiation of the B-cells (13). The main sources of IL-5 are eosinophils, Th2-lymphocytes and mast cells (13). Infiltration and activation of eosinophils is a hallmark of NP tissue (1,13). Preliminary results presented by Fan et al. (1) suggest that T-cell-derived IL-5 as well as autosecretion of IL-5 from activated eosinophils may be the cause of extension and persistence of the inflammation in NP. Activated eosinophils release large amounts of cytotoxic proteins as well as growth factors thereby causing epithelium injury, epithelial basement membrane thickening, stromal fibrosis and angiogenesis, and glandular hyperplasia (1, 14). IL-6 is an important Th2 cytokine that stimulates the production of IgE as well as the proliferation and maturation of mast cells (15).

IL-6 is predominant in nasal mucosa in patients with allergic rhinitis, as well as other Th2 types of cytokines such as IL-4, IL-5 and IL-13 are (15). Immunohistochemical staining and in situ hybridization indicated that Th2-lymphocytes, macrophages, eosinophils, and epithelium cells are the main sources of IL-6 (15). The pathogenesis of NP also includes fibroblasts through the production of IL-6 that modulates the activation of immune responses (plasma cell formation) and stroma synthesis (15). Van Zele et al. (16) showed the increased colonization of NP by *Staphylococcus aureus* culture as well as the presence of specific IgE direct against the *Staphylococcus aureus* exotoxins in the NP tissue. Rates of colonization by this bacterium as well as the presence of IgE are higher in subjects with NP and co-morbid allergic asthma (16). Hellings et al. (17) demonstrated that nasal application of *Staphylococcus aureus* exotoxin B in mice in experimental environment causes the onset of allergic rhinitis and asthma, parallel to the increase of Th2 cytokine level in bronchial secret and in systemic circulation. Xu et al (18) also found the significantly increased levels of IL-6, as well as IL-4 and IFN- γ in *Staphylococcus aureus* exotoxin B-stimulated NP.

It has been shown that IL-4, among other Th2 cytokines, promotes or causes the selective influx of eosinophils in tissue (13, 19). It is assumed that IL-4 may be involved in the induction of vascular cell adhesion molecule-1 (VCAM-1) expression on the microvascular endothelium in NP (13). To infiltrate sites of inflammation, eosinophils leave the bloodstream and pass through the endothelium in four steps, namely rolling, adhesion, transendothelial migration, and chemotaxis (13). Adhesion molecules, such as VCAM-1 play an important role during adhesion of eosinophils to endothelial cells (13). Voegels et al.(19), found that allergic NP patients have

significantly higher levels of IL-4 in the NP tissue than the non-allergic. Cheng et al. (20) found significantly higher percentage of IL-4-producing Th2 cells in the polyps of atopic patients than in the non-atopic.

Our results showed significantly higher concentrations of the strong proinflammatory cytokine TNF- β in nasal discharge of atopic NP patients than in the nonatopic ones. The role of this cytokine is the stimulation of numerous cells growth and differentiation, especially T and B lymphocytes. This finding could be explained by

higher level of inflammatory reaction in allergic patients than in the nonallergic.

Conclusion

Our results showed that the presence of Th2 cytokines IL-4, IL-5 and IL-6 is a more prominent feature in atopic than in non-atopic patients with NP which relates to the increased inflammatory process, mediated by eosinophils. We concluded that polyps in atopic and non-atopic patients have different immunological patterns.

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UTICAJ ALERGIJA NA NIVO CITOKINA U NOSNOM SEKRETU KOD BOLESNIKA SA NOSNIM POLIPIMA

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Koncentracije citokina u nosnom sekretu odražavaju stanje zapaljenja nosne sluzokože.

Cilj rada bio je uporediti koncentracije citokina u nosnom sekretu kod alergičnih i nealergičnih bolesnika sa nosnom polipozom (NP).

Tridesetoro obolelih od NP (13-oro atopičnih i 17-oro neatopičnih) uključeno je u ovu studiju. Uzorci sekreta uzeti su iz nosne duplje kod svih 30-oro bolesnika. Koncentracije TNF- α , TNF- β , IL-1 β , IL-2, IL-12, IFN- γ , IL-4, IL-5, IL-6, IL-10 i IL-8 merene su u nosnom sekretu korišćenjem komercijalnog flow-citometrijskog kita.

Pronađene su statistički značajno više koncentracije IL-4 ($p < 0,01$), IL-5 ($p < 0,05$), IL-6 ($p < 0,05$) i TNF- β ($p < 0,05$) kod alergičnih bolesnika sa NP u odnosu na nealergične.

Neatopični i atopični polipi imaju različit imunološki tok. *Acta Medica Medianae* 2010;49(3):40-44.

Ključne reči: *alergija, citokini, nosni polipi*