CYTOKINE PROFILES IN NASAL FLUID IN PATIENTS WITH NASAL POLYPS: A FLOW CYTOMETRIC STUDY

PROFILI CITOKINA U NOSNOM SEKRETU KOD PACIJENATA S NOSNIM POLIPIMA: ISPITIVANJE PROTOČNOM CITOMETRIJOM

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Summary: Biological markers in nasal fluid provide valuable information on nasal pathophysiology. The aims of this study were to compare the cytokine profiles of nasal fluid in subjects with nasal polyps (NP) and co-morbid asthma and NP patients without asthma and to determine the role of these cytokines in the development of NP. Thirty patients with NP (15 asthmatic and 15 nonasthmatic) were included in this prospective study. Nasal secretion samples were collected from nasal cavities of all 30 subjects. The levels of eleven cytokines (TNF-α, TNF-β, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, and IFN-γ) were measured using flow cytometry. The concentrations of Th2 cytokines IL-5, IL-6 and IL-10 were significantly higher in patients with NP and asthma compared with subjects with NP without asthma. We also found significantly higher levels of IFN-α, IL-4, IL-6 and IL-10 in allergic patients with NP and asthma compared with those without asthma. In nonallergic patients with NP and asthma, the concentrations of TNF-α, IL-5 and IL-6 were significantly higher than in nonallergic patients with NP without asthma. Our results show that the presence of Th2 cytokines, especially IL-5 and IL-6 in patients with NP and asthma is a more prominent feature than in those without asthma that relates to the increased eosinophilic inflammation. We have also found a significant influence of allergy on the cytokine profiles both in asthmatic and nonasthmatic patients.

Keywords: nasal polyps, asthma, cytokines, nasal fluid, allergy

Kratak sadržaj: Biološki markeri u nosnom sekretu mogu pružiti dragocete podatke o patofiziologiji bolesti nosa i paranasalnih sinusa. Cilj studije je bio da se uporede profili citokina u nosnom sekretu kod obolelih od nosne polipske (NP) bolesti sa astmom i pacijenata obolelih od NP bez astme i ispita uloga tih citokina u razvoju NP. Trideset pacijenata sa NP (15 sa astmom i 15 bez astme) isklopljeno je u ovu prospektivnu studiju. Uzorci nosnog sekreta su uzeti iz nosne {upljine svakom od 30 pacijenata. Koncentracije jedanaest citokina (TNF-α, TNF-β, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, i IFN-γ) su merene flow-citometrijom. Koncentracije Th2-citokina IL-5, IL-6 i IL-10 bile su statistički značajno više kod pacijenata sa NP i astmom u poređenju sa obolelima od NP bez astme. Takođe smo našli značajno više vrednosti IFN-α, IL-4, IL-6 i IL-10 kod alergičnih pacijenata sa NP i astmom u poređenju sa onima bez astme. Kon nealergičnih pacijenata sa NP i astmom, koncentracije TNF-α, IL-5 i IL-6 bile su značajno više nego kod nealergičnih pacijenata sa NP bez astme. Rezultati su pokazali da je koncentracija Th2-citokina, na- ročito IL-5 i IL-6, kod pacijenata sa NP i astmom izražena karakteristika nego kod onih bez astme, povezana sa poja- čanom eozinofilnom inflamacijom. Takođe je otkriven zna- čajan uticaj alergije na profile citokina i kod astmatičnih i kod neastmatičnih pacijenata.

Kljucne reči: nosni polipi, astma, citokini, nosni sekret, alergija

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Introduction

Bronchial asthma is a chronic disease characterized by inflammatory changes and intermittent obstruction of the airways and bronchial hyperresponsiveness. As opposed to »intrinsic« or nonallergic asthma, »extrinsic« (allergic, atopic) asthma is characterized by elevated serum IgE concentrations and association with other allergic manifestations (1). Se-
ven percent of asthma patients have nasal polyps (2). Nasal polyposis (NP), a chronic inflammatory disease of the nasal and paranasal sinus mucosa, is characterized by proliferation of the epithelial layer, glandular hyperplasia, thickening of the basement membrane, edema, focal fibrosis, and cellular infiltration of the stromal layer (3). Polyps originate from the paranasal sinuses, most often from the anterior ethmoid complex and from there they can descend between the middle turbinate and the lateral nasal wall into the nasal cavity causing symptoms such as nasal obstruction, anosmia, sneezing, rhinorrhea, and itching (2). NP is a multifactorial disease with several etiologic factors. Chronic persistent inflammation is a major factor in the development of NP (3). Polyp tissue includes mixed inflammatory cells, of which eosinophils are the most dominant. They have the primary role in the perpetuation of chronic inflammation (2, 3). However, polyp tissue eosinophilia is an entity independent of atopy (3). In non-atopic asthma, polyps are diagnosed more frequently, in about 10–15% patients (2). Asthma and NP are characterized mainly by eosinophilic airway inflammation which is more severe and refractory to conventional medical treatment strategies (4).

It has been suggested that an ineffective local Th1-based immune response in these patients is associated with increased Th2-cytokine-based activity, which contributes to a chronic infection as well as to an increased presence of eosinophils, which then lead to further polyp formation (5). It has been further proposed that the weakened Th1 response in these patients may be secondary to the down-regulation of some specific toll-like receptors involved in the innate immune response (5).

Nasal fluid represents a first line defense medium, in which the leukocyte compartment probably acts as an efficient part of the defense mechanism along with the mucociliary transport system and the biochemical properties of the mucus (6). To characterize inflammatory changes of the upper respiratory mucosa, cellular secretory products in nasal secretions may be determined (7). Nasal secretions contain minute amounts of cytokines, potent biologic factors involved in the regulation of inflammation and immune defense, and other inflammatory mediators expressed by various epithelial and nonepithelial cells (8). As cytokines play a dominant role in the pathophysiology of airway disease, the cytokine profile in nasal secretions may help to recognize mechanisms underlying NP associated with bronchial asthma.

The aim of this cross-sectional study was to investigate the levels of these cellular secretory products in the nasal secretions of asthmatic and non-asthmatic patients with nasal polyps. The attempt was made to identify the possible characteristics of nasal cytokine profiles between these groups.

Materials and Methods

Human subjects

Thirty patients with NP were included in this prospective-analytic 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 and on the results of nasal endoscopy and computed tomography. Fifteen patients were diagnosed with mild persistent bronchial asthma. Diagnosis of asthma was done at the time of inclusion in the study according to the Global Initiative on Asthma (GINA) (9). The assessment of the severity of asthma was done by a pulmologist based on the patient’s medical history, clinical data and pulmonary function testing, including forced expiratory volume in 1 second (FEV1) and methacholine provocation test (Mch PD20). Only patients with polyps associated with mild bronchial asthma, without aspirin sensitivity, were included in the study. The diagnosis of aspirin-induced asthma was done by a positive bronchial aspirin-provocation test. The other exclusion criteria were the presence of antrochoanal polyps, cystic fibrosis, and primary ciliary dyskinesia. None of the subjects included in this investigation had bronchial or respiratory tract infection and none of the subjects had been treated with oral and topical corticosteroids, antibiotics and antihistamines for at least three weeks before the enrollment. Skin prick tests were performed on all patients for sensitivity to 18 common allergens. A test result was considered positive when at least one of the induration diameters was 3 mm higher than that in the negative control. Subjects were considered allergic if they had a serum IgE level > 100 IU/mL.

Clinical score

Only subjects with nasal symptoms whose duration was two years and less were included in this study. The presence of nasal symptoms associated with NP (obstruction, anosmia, sneezing, rhinorrhea, and itching) on the day of enrollment in the study was scored according to Tsicopoulos et al. (10) from 0 to 3: 0 for no symptoms, 1 for mild symptoms, 2 for moderate symptoms, and 3 for severe symptoms, so that the maximal global nasal symptom score was 15.

Endoscopic physical findings were scored according to Malm (11). The degree of nasal polypos is classified in relation to fixed anatomical landmarks in four steps: 0 = «no polyposis», 1 = «mild polyposis» (small polyps not reaching the upper edge of the inferior turbinate), 2 = »moderate polyposis« (medium sized polyps reaching between the upper and lower edges of the inferior turbinate), 3 = »severe polyposis« (large polyps reaching below the lower edge of the inferior turbinate). The maximal endoscopic score is 6, bilaterally.
Sampling of nasal secretions and flow cytometry

Nasal secretion samples were collected from the nasal cavities of all 30 subjects (15 patients with NP, and 15 patients with NP and asthma) using absorption technique with cotton wool sticks, which were inserted into the nasal cavity for 60 s, as previously described (8, 12, 13). All of the samples were put in a 2 mL eppendorf tube containing 1 mL of transfer media (phosphate-buffered saline with gentamicin 50 μg/mL, penicillin G 340 U/mL, fungizone 500 μg/ml) for 30 min. because of the diffusion of cytokines into the medium and then stored at –40 °C until cytokine determination. The levels of eleven cytokines (TNF-α, TNF-β, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, and IFN-γ) in the nasal secretions. The concentrations of IL-10, IL-6 and IL-5 in nasal fluid were significantly higher in patients with NP and asthma (77.07 ± 67.13 pg/mL; 271.81 ± 223.21 pg/mL; 618.8 ± 585.04 pg/mL) compared with patients with NP without asthma (31.46 ± 52.73 pg/mL; 56.79 ± 87.64 pg/mL; 270.45 ± 723.15 pg/mL) (Table II).

We found significantly higher levels of IFN-γ, IL-6, IL-4 and IL-10 in allergic patients with NP and asthma (61.56 ± 39.05 pg/mL; 325.4 ± 260.92 pg/mL; 1287.34 ± 1717.1 pg/mL; 89.86 ± 59.75 pg/mL) compared with allergic patients with NP without asthma (24.22 ± 40.47 pg/mL; 74.11 ± 86.95 pg/mL; 518.6 ± 1153.39 pg/mL; 26.42 ± 59.08 pg/mL) in non-allergic patients with NP and asthma.

Statistical analyses

Data were expressed as mean ± standard deviation (± SD). Between-group comparisons were made by using the nonparametric Mann-Whitney U test. A p value less than 0.05 was considered statistically significant.

Results

There were 11 male and 4 female patients in the NP group (mean age 42.8 ± 13.71 years, aged from 22 to 65 years) and 10 male and 5 female patients in the NP with asthma group (mean age 46.47 ± 15.25 years, aged from 24 to 65 years). Five patients in the NP group and eight patients in the NP with asthma group were atopic. Only four cytokines (IL-4, IL-5, IL-6 and IL-8) were detected in the nasal fluid in all patients from the NP and asthma group.

The groups did not significantly differ according to the sex, age, and the presence of allergy in the subjects. Comparing the two main groups (NP with asthma and NP without asthma), we did not find any significant difference according to the global nasal symptom score and endoscopic score (Table I). We also did not find significant differences in the levels of TNF-α, TNF-β, IL-1β, IL-2, IL-4, IL-8, IL-12, and IFN-γ in the nasal secretions. The concentrations of IL-10, IL-6 and IL-5 in nasal fluid were significantly higher in patients with NP and asthma (77.07 ± 67.13 pg/mL; 271.81 ± 223.21 pg/mL; 618.8 ± 585.04 pg/mL) compared with patients with NP without asthma (31.46 ± 52.73 pg/mL; 56.79 ± 87.64 pg/mL; 270.45 ± 723.15 pg/mL) (Table II).

Table I Characteristics of the main patient groups.

<table>
<thead>
<tr>
<th></th>
<th>Nasal polyposis</th>
<th>Nasal polyposis + asthma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Age (years)</td>
<td>42.8 ± 13.71</td>
<td>46.47 ± 15.25</td>
</tr>
<tr>
<td>Male/female ratio</td>
<td>11/4</td>
<td>10/5</td>
</tr>
<tr>
<td>FEV1 (L)</td>
<td>102.07 ± 3.32</td>
<td>94.4 ± 5.18</td>
</tr>
<tr>
<td>MchPD20 (μg)</td>
<td>1662.27 ± 59.26</td>
<td>505.8 ± 105.36</td>
</tr>
<tr>
<td>Allergic</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>Nonallergic</td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td>Nasal symptom score</td>
<td>10.6 ± 1.92</td>
<td>11.47 ± 2.26</td>
</tr>
<tr>
<td>Nasal endoscopic score</td>
<td>5.2 ± 1.01</td>
<td>5.07 ± 1.03</td>
</tr>
</tbody>
</table>

All results are expressed as means ± SD

FEV1 = forced expiratory volume in 1 second
Mch PD20 (μg) = amount of metacholine in micrograms

Table II Cytokine levels in nasal secretions.

<table>
<thead>
<tr>
<th></th>
<th>Patients with nasal polyps</th>
<th>Patients with nasal polyps and asthma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IL-12</td>
<td>IFN-γ</td>
</tr>
<tr>
<td>x</td>
<td>6.28</td>
<td>37.69</td>
</tr>
<tr>
<td>SD</td>
<td>19.49</td>
<td>37.08</td>
</tr>
<tr>
<td></td>
<td>IL-12</td>
<td>IFN-γ</td>
</tr>
<tr>
<td>x</td>
<td>26.6</td>
<td>69.6</td>
</tr>
<tr>
<td>SD</td>
<td>43.87</td>
<td>68.74</td>
</tr>
</tbody>
</table>
Table III  Cytokine levels in nasal fluid in allergic and nonallergic patients.

<table>
<thead>
<tr>
<th></th>
<th>Allergic patients with nasal polyps</th>
<th>Nonallergic patients with nasal polyps</th>
<th>Allergic patients with nasal polyps and asthma</th>
<th>Nonallergic patients with nasal polyps and asthma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IL-12</td>
<td>IFN-γ</td>
<td>IL-2</td>
<td>IL-10</td>
</tr>
<tr>
<td>x</td>
<td>0</td>
<td>24.22</td>
<td>288.82</td>
<td>26.42</td>
</tr>
<tr>
<td>SD</td>
<td>0</td>
<td>40.47</td>
<td>145.46</td>
<td>59.08</td>
</tr>
</tbody>
</table>

asthma, the concentrations of TNF-α, IL-6 and IL-5 (60.25 ± 49.77 pg/mL; 210.57 ± 169.37 pg/mL; 413.38 ± 332.22 pg/mL) were significantly higher than in nonallergic patients with NP without asthma (16.53 ± 23.2 pg/mL; 48.13 ± 91.31 pg/mL; 54.81 ± 119.15 pg/mL) (Table III).

Discussion

It was shown that nasal secretion reflects the inflammatory status of the nasal mucosa and the evolution of mucosal disease. However, the mechanisms of cytokine release in nasal fluid are not well known. Results published by Ohkubo et al. (14) showed that IL-6 was released to the nasal mucosa mainly from the migrating cells and epithelial cells as a result of the antigen provocation, and released to nasal secretion through cholinergic control and from epithelial cells by direct action of histamine.

NP may have a negative impact on lower airway biology, being involved in aggravation of bronchial disease. The mechanisms that connect upper and lower airway dysfunction are: nasal bronchial reflex, mouth breathing caused by nasal obstruction, and pulmonary aspiration of nasal contents (15). It has been shown in a rabbit model of chronic sinusitis that postnasal drainage of inflammatory mediators may affect lower airway responsiveness (15). Therefore, one can hypothesize that a local nasal inflammatory stimulus may induce a systemic effect leading to bronchial eosinophilic inflammation (15).

Similar typical findings can be reached in the microscopic examination of NP when compared with the bronchial mucosa in patients with asthma. In both tissues there is epithelial damage, goblet cell hyperplasia, thickening of the basement membrane, accumulation of extracellular matrix, fibrosis and eosinophil-dominated inflammation (16). The link between these two diseases is further made plausible by the observation that the nasal polyp eosinophilic inflammation is significantly higher in NP patients with concomitant asthma when compared with nonasthmatic NP patients (16). In patients with NP and co-existing allergic rhinitis, the eosinophils seem to be attracted mainly by the release of IL-5 (17). In contrast, in the case of absence of allergy, eosinophils appear to be recruited mainly by the release of granulocyte-macrophage colony-stimulating factor (GM-CFS) (17). The eosinophilic influx is higher in asthmatic patients when compared with nonasthmatic patients (16).

The initiating factors that underlie persistent inflammation and microbial colonization in NP are not well understood. Although Th2 inflammation is a central characteristic of the disease process, what triggers the local production of Th2 cytokines and infiltration of eosinophils and lymphocytes in the first place is unknown (5). It has been shown that Th2 cytokines down-regulate toll-like receptors (TLR) expression and
inhibit TLR signaling function in Th1 lymphocytes membrane. Thus, Th2 cytokines inhibit Th1 cytokines production resulting in the down-regulation of antimicrobial mucosal immunity (5). Hamilos et al. (18) found significantly higher levels of IL-5 in nasal polytissue from asthmatic than in those from nonasthmatic subjects. The results of our research have also shown a significantly higher concentration of Th2 cytokines (IL-5, IL-6 and IL-10) in nasal secretions in patients with NP and asthma than in patients without asthma.

Previous data point to IL-5 as one of the key proteins in the pathomechanism of tissue eosinophilia, enhancing the differentiation, activation, expansion, mobilization, and in situ survival of eosinophils (17). It is widely accepted that IL-5 plays an important role in the pathogenesis of bronchial asthma where it induces eosinophil mobilization, B-cell growth and differentiation (1, 17). The main sources of IL-5 were eosinophils and mast cells (1, 17). IL-6 is an important Th2 type cytokine involved in the induction of IgE synthesis as well as in mast cell proliferation and maturation (14). As well as other Th2 type cytokines such as IL-4, IL-5 and IL-13, IL-6 is predominant in nasal mucosa in patients with allergic rhinitis (14). Immunohistochemical staining and in situ hybridization also indicated that macrophages, eosinophils, and lining epithelium were the main sources of IL-6 (19). The pathogenesis of NP involves nasal polyp fibroblasts through synthetizing IL-6 to modulate the activation of immune responses (plasma cell formation) and synthesis of stroma (19). Van Zele et al. (20) showed increased colonization of NP by Staphylococcus aureus and presence of specific IgE directed against Staphylococcus aureus exotoxins in NP tissue. Rate of colonization and IgE presence in NP tissue was increased in subjects with NP and co-morbid asthma (20). Hellings et al. (21) demonstrated that nasal application of Staphylococcus aureus exotoxin B in mice is capable of aggravating experimental allergic rhinitis and asthma, paralleled with an increase in bronchial and systemic Th2 cytokine levels. IL-10 is an antiinflammatory Th2 cytokine produced by T-lymphocytes, monocytes and macrophages. It impedes macrophage activation and leads to marked immunosuppression (1).

Our results showed higher concentrations of IL-4, IL-6, IL-10 and IFN-γ in nasal fluid in allergic patients with NP and asthma than in non-allergic. Xu et al. (22) also found significantly increased levels of IL-4, IL-6 and IFN-γ in Staphylococcus aureus exotoxin B-stimulated nasal polyps.

IFN-γ is a Th1 cytokine which leads via macrophage activation to extensive inflammatory processes that also enable the killing of intracellular pathogens (1). Dellacono et al. (23) hypothesized that elevated levels of IFN-γ activate lymphocytes and eosinophils within the NP tissue. They found a positive correlation between the increased IFN-γ levels and presence of allergy and asthma in NP patients (23). Among some other Th2 cytokines, IL-4 evidently delivers signals that support or cause selective influx of eosinophils (17). It has been speculated that IL-4 may be involved in the induction of vascular cell adhesion molecule-1 (VCAM-1) expression on microvascular endothelium in NP (17). To infiltrate sites of inflammation, eosinophils leave the bloodstream and pass through the endothelium in four steps, namely rolling, adhesion, transendothelial migration, and chemotaxis (24). Adhesion molecules, such as VCAM-1, play an important role during adhesion to endothelial cells (17, 24, 25). Experiments performed by Ohori et al. (24) demonstrated that TNF-α stimulation induces VCAM-1 protein and mRNA expression in human nasal polyfibrinoblasts.

In conclusion, our results confirmed the previous discovery that the presence of Th2 cytokines, especially IL-5 and IL-6 in NP is a prominent feature that relates to the increased eosinophilic inflammatory process. Our findings also suggest that upregulation of Th2 cytokines is a more significant characteristic of NP in asthmatic than in nonasthmatic subjects. We also found significant influence of allergy on the cytokine profiles both in asthmatic and nonasthmatic patients.

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References


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