Original Article / Çalışma - Araştırma

Cytokine levels in nasal secretions in asthmatic and nonasthmatic patients with nasal polyposis

Astımlı ve astımlı olmayan nazal polipozisli hastaların nazal sekresyonlarındaki sitokin seviyeleri

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Objectives: The aim of this study was to compare the cytokine levels of subjects with nasal polyps (NP) and comorbid asthma and NP patients without asthma.

Patients and Methods: Thirty patients with NP (15 asthmatic and 15 nonasthmatic) were included in this prospective study. Nasal secretion samples were collected from the nasal cavities of all 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 cytometric method.

Results: 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. IFN-γ, IL-4, IL-6 and IL-10 levels were found significantly higher 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.

Conclusion: Our results showed that the presence of Th2 cytokines, especially IL-5 and IL-6 in patients with NP and asthma is a prominent feature that relates to increased eosinophilic inflammation. We also found significant influence of asthma and allergy on the cytokine profiles in nasal secretions of patients with NP.

Key Words: Allergy; asthma; cytokines; nasal polyps; nasal secretions.

Amaç: Bu çalışmada nazal polip (NP)'leri ve hastalığa eşlik eden astımı olan ve olmayan NP'li hastaların sitokin seviyeleri karşılaştırıldı.

Hastalar ve Yöntemler: Bu ileriye yönelik çalışmaya, nazal polipleri olan 30 hasta (15 astımlı ve 15 astımlı olmayan) dahil edildi. Tüm olguların nazal kavitelerinden nazal sekresyon örnekleri biriktirildi. On bir sitokinin (TNF-α, TNF-β, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12 ve IFN-γ) seviyeleri akım sitometri yöntemi ile ölçüldü.

Bulgular: Th2 sitokinlerinin IL-5, IL-6 ve IL-10 konsantrasyonları astımı olan NP'li hastalarda, astımı olmayan NP'li hastalara göre anlamlı derecede yüksek bulundu. Nazal polipli ve astımı olan alerjik hastalarda, IFN-γ, IL-4, IL-6 ve IL-10 seviyeleri, astımı olmayanlara göre anlamlı derecede yüksek bulundu. Alerjik olmayan, nazal polipleri ve astımı olan hastalarda TNF-α, IL-5 ve IL-6 konsantrasyonları, alerjisi ve astımı olmayan NP'li hastalara göre oldukça yüksek bulundu.

Sonuç: Bizim sonuçlarımıza göre, Th2 sitokinlerinin, özellikle de IL-5 ve IL-6'nın varlığı, NP ve astımı olan hastalarda eozinofilik inflamasyonun artışıyla ilişkili belirgin bir özelliktir. Biz aynı zamanda astım ve alerjinin, NP'li hastaların nazal sekresyonlarındaki sitokin profiline önemli etkisinin olduğunu saptadık.

Anahtar Sözcükler: Alerji; astım, sitokinler; nazal polipler; nazal sekresyonlar.

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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.[1] Polyps originate from the paranasal sinuses, most often from the anterior ethmoid complex from where 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.[1] Nasal polyposis is a multifactorial disease with several etiologic factors. Chronic persistent inflammation is a major factor in the development of NP.[2] Polyp tissue includes mixed inflammatory cells, of which eosinophils are the most dominant. They play a primary role in the perpetuation of chronic inflammation.[1,2]

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.[3] 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.[3]

Bronchial asthma is a chronic disease characterized by intermittent obstruction and inflammatory changes of the airways, and bronchial hyperresponsiveness.[4] Seven percent of asthma patients have nasal polyps.[1]

Nasal secretions represent a first line defense medium, in which the leucocyte compartment probably acts as an efficient part of the defense mechanism, along with the mucociliary transport system and the biochemical properties of the mucus.[5] To characterize inflammatory changes of the upper respiratory mucosa, cellular secretory products in nasal secretions may be determined.[6] 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.[7] As cytokines play a dominant role in the pathophysiology of airway disease, the cytokine profile of nasal secretions may help recognize mechanisms underlying NP associated with bronchial asthma.

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

PATIENTS AND METHODS

Human subjects

Thirty patients with NP were included in this prospective-analytic study. There were 15 patients in the NP group (11 males, 4 females; mean age 42.8±13.7 years) and 15 patients in the NP with asthma group (10 males, 5 females; mean age 46.5±15.3 years).

Writen 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 (CT). Fifteen patients had diagnosis of 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).[8] The severity of asthma was assessed by a pulmologist based on the patient's medical history, clinical data and on pulmonary function testing, including forced expiratory volume in 1 second (FEV1) and metacholine 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 made by a positive bronchial aspirin-provocation-test. The other exclusion criteria were the presence of antrochoanal polyps, cystic fibrosis, and primary ciliary dyskinesia. All subjects included in this investigation did not have bronchial or respiratory tract infection and none of the subjects were treated with oral and topical corticosteroids, antibiotics and antihistamines for at least three weeks before 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 of the negative control. Subjects were considered allergic if they had a serum IgE level >100 IU/ml.

Clinical score

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.[9] 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 is 15.

Endoscopic physical findings were scored according to Lildholdt et al.[10] The degree of nasal polyps 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.

Findings on CT scans were graded according to the Lund-Mackay score.[11] The mucosal abnormalities were graded as 0= no abnormality, 1= partial opacification, or 2= total opacification of the frontal, maxillary, anterior ethmoid, posterior ethmoid and sphenoid sinus, bilaterally. The ostiomeatal complexes were scored bilaterally as 0= not occluded or 2= occluded. The maximal CT grading score is 24.

Sampling of nasal secretions and cytokine determination

Nasal secretions samples were collected from 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 seconds, as previously described.[7,12,13] All of the samples were put in a 2 mL Eppendorf tube containing 1 mL of transfer media (phosphate-buffered saline with gentamycin 50 μg/ml, penicillin G 340 U/ml, fungizone 500 μg/ml) for 30 minutes to allow diffusion of cytokines into the medium. The samples were 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) were measured in all of the 30 samples using a commercial flow cytometric kit (Flow cytomix, Bender MedSystems GmbH, Vienna, Austria) on the flow cytofluorimeter.

Statistical analyses

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

RESULTS

Five patients in the NP group and eight patients in the NP with asthma group were atopic. This finding showed a higher percentage of subjects with allergy in the NP with asthma group than in NP group ($p<0.01$; Chi square-test). Only four cytokines (IL-4, IL-5, IL-6 and IL-8) were detected in nasal fluid in all patients from NP and asthma group. The groups did not significantly differ according to the sex and age. Comparing two main groups (NP with asthma and NP without asthma), we did not find any significant difference according to the global nasal symptom score, endoscopic score and Lund-Mackay score (Table 1). 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 to patients with NP without asthma (31.46±52.73 pg/mL; 56.79±87.64 pg/mL; 270.45±723.15 pg/mL), (Table 2).

We also 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 to 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, 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 non-allergic patients with NP without asthma (16.53±23.2 pg/mL; 48.13±91.31 pg/mL; 54.81±119.15 pg/mL), (Table 3).

DISCUSSION

According to our results, the presence of asthma does not modify the symptoms, endoscopic and radiographic characteristics of nasal polyposis. We, however, found significant influence of asthma on the cytokine profiles in nasal fluid. 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

Table 1. Characteristics of the main patient groups

	Nasal polyposis		Nasal polyposis+asthma	
	n	Mean±SD	n	Mean±SD
Patients	15		15	
Age		42.8±13.71		46.47±15.25
Sex				
Male	11		10	
Female	4		5	
FEV1		102.07±3.32		94.4±5.18
MchPD20(μg)		1662.27±59.26		503.8±103.36
Allergic	5		8	
Nonallergic	10		7	
Nasal symptom score		10.6±1.92		11.47±2.26
Nasal endoscopic score		5.2±1.01		5.07±1.03
Lund-Mackay score		17.13±2.59		18.4±2.53

All results are expressed as mean ± SD; FEV1= Forced expiratory volume in 1 second; Mch PD20(μg)= amount of metacholine in micrograms.

the antigen provocation, by direct action of histamine, and by reflex action of metacholine.

Nasal polyposis 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 the 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 found in 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 basement membrane, accumulation of extracellular matrix, proliferation of myofibroblasts, 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 concommitant asthma when compared with nonasthmatic NP patients.[16] The initiating factor that underlies persistent inflammation and microbial colonization in NP is 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.[3] It has been shown that Th2 cytokines down-regulate toll-like receptors (TLR) expression and inhibit TLR signaling function in Th1 lymphocytes membrane.

Table 2. Cytokine levels in nasal secretions

Patients with nasal polyps											
	IL-12	IFN-γ	IL-2	IL-10	IL-8	IL-6	IL-4	IL-5	IL-1β	TNF-α	TNF-β
$\bar{x}$	6.28	37.69	229.26	31.46	169.82	56.79	577.7	270.45	28.24	25.42	166.36
SD	19.49	37.08	167.3	52.73	278.25	87.64	984.05	723.15	47.34	24.59	223.31
Patients with nasal polyps and asthma											
	IL-12	IFN-γ	IL-2	IL-10	IL-8	IL-6	IL-4	IL-5	IL-1β	TNF-α	TNF-β
$\bar{x}$	26.6	69.6	353.7	77.07	133.7	271.81	1020.1	618.8	42.46	46.45	200.83
SD	43.87	68.74	288.63	67.13	100.27	223.27	1458.8	585.04	65.13	42.83	325.68

$\bar{x}$: Mean; SD: Standard deviation; IL: Interleukin; IFN: İnterferon; TNF: Tumor necrosis factor.

Table 3. Cytokine levels in nasal fluid in allergic and nonallergic patients

Allergic patients with nasal polyps											
	IL-12	IFN-γ	IL-2	IL-10	IL-8	IL-6	IL-4	IL-5	IL-1β	TNF-α	TNF-β
$\bar{x}$	0	24.22	288.82	26.42	274.56	74.11	518.6	701.73	39.15	43.2	275.01
SD	0	40.47	145.46	59.08	474.1	86.95	1153.4	1203.9	75.83	17.68	302.65
Nonallergic patients with nasal polyps											
	IL-12	IFN-γ	IL-2	IL-10	IL-8	IL-6	IL-4	IL-5	IL-1β	TNF-α	TNF-β
$\bar{x}$	9.42	44.42	199.51	33.98	117.45	48.45	607.25	54.81	22.78	16.53	112.04
SD	23.62	35.5	176.58	52.46	106.74	91.31	955.08	119.15	28.84	23.2	164.41
Allergic patients with nasal polyps and asthma											
	IL-12	IFN-γ	IL-2	IL-10	IL-8	IL-6	IL-4	IL-5	IL-1β	TNF-α	TNF-β
$\bar{x}$	28.58	61.56	377.97	89.86	140.62	325.4	1287.3	798.55	61.22	34.38	285.56
SD	45.23	39.05	305.78	59.75	91.76	260.92	1717.1	714.71	79.92	34.47	360.09
Nonallergic patients with nasal polyps and asthma											
	IL-12	IFN-γ	IL-2	IL-10	IL-8	IL-6	IL-4	IL-5	IL-1β	TNF-α	TNF-β
	24.34	78.78	325.96	62.46	125.79	210.57	714.57	413.38	21.03	60.25	104.0
SD	45.76	95.2	289.17	76.69	116.19	169.37	1149.6	332.22	37.94	49.77	275.16

$\bar{x}$: Mean; SD: Standard deviation; IL: Interleukin; IFN: İnterferon; TNF: Tumor necrosis factor.

Thus, Th2 cytokines inhibit Th1 cytokines production resulting in down-regulation of antimicrobial mucosal immunity.[3] Th2-type cytokines are thought to regulate inflammatory cell recruitment, activation, survival, and the release of inflammatory mediators.[17] Hamilos et al.[18] found significantly higher levels of IL-5 in nasal polyp tissue from asthmatic than from nonasthmatic subjects. The results of our research have shown significantly higher concentrations of Th2 cytokines (IL-5, IL-6 and IL-10) in nasal secretions in patients with NP and asthma than in patients without asthma.

Prevous data point to IL-5 as one of the key proteins in the pathomechanism of tissue eosinophilia, enhancing the differentiation, activation, expansion, mobilisation, 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 mobilisation, B-cell growth and differentiation.[17] The main sources of IL-5 in nasal polyps were eosinophils and mast cells.[17] T-cell-derived IL-5 and autosecretion of IL-5 from activated eosinophils could be the reasons for the persistence and extension of inflammation in NP.[17] Interleukin-6 is an important Th2 type cytokine involved in the induction of IgE synthesis as well as in mast cell and fibroblast proliferation and maturation.[14] Like 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 indicate that macrophages, eosinophils, fibroblasts 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 the presence of specific IgE directed against *Staphylococcus aureus* exotoxins in NP tissue. Rates of colonization and IgE presence in NP tissue was increased in subjects with NP and comorbid asthma.[20] Hellings et al.[21] demonstrated that nasal aplication of *Staphylococcus aureus* exotoxin B in mice is capable of aggravating experimental allergic rhinitis and asthma, parallelled with an increase in bronchial and systemic Th2 cytokine levels. IL-10 is an anti-inflammatory Th2 cytokine produced by T-lymphocytes, monocytes and macrophages. It impedes macrophage activation and leads to marked immunosupression.[4]

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 to extensive inflammatory processes that also enables the killing of intracellular pathogen via macrophage activation.[4] 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] Experiments performed by Ohori et al.[24] demonstrated that TNF-α stimulation induces VCAM-1 protein production and mRNA expression in human nasal polyp fibroblasts. Epithelial and immunocompetent cells, such as eosinophils, macrophages and mast cells produce TNF-α. These findings suggest that TNF-α increases VCAM-1 production in nasal fibroblasts and activates the transmigration of eosinophils, which induce further production of TNF-α and accelerate the accumulation of eosinophils in NP.[24]

In conclusion, our results showed 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 more significant characteristic of NP in asthmatic than in nonasthmatic subjects. We also found significant influence of asthma and allergy on the cytokine profiles in nasal fluid in NP patients. We conclude that nasal polyps have different immunological patterns among asthmatic and nonasthmatic patients.

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