Flowcytometric Analysis of Lymphocytes and Nonlymphoid Cells in Nasal Lavage of Patients with Nasal Polyposis

NAZAL POLİPLİ HASTALARIN NAZAL LAVAJLARINDA LENFOSİT VE NON-LENFOID HÜCRELERİN FLOVSİTOMETRİK ANALİZİ

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Summary

Local immunologic and inflammatory processes play a prominent role in the development of nasal polyps. T and B lymphocytes, HLA - DR+ epithelial cells are regulatory and effector cells in the complex process of inflammatory responses.

This study was carried on twenty-five patients with nasal polyposis and twenty-five healthy subjects used as control group. Lymphocytes and non -lymphoid cells obtained from the nasal lavage of patients were detected by means of flow cytometric analysis and the results were compared with those from analysis of nasal lavages of healthy persons.

It was found that the ratios of T helper cells / T suppressor cells. T cell / Natural killer cell, T cell / active T cell in patients were significantly lower than those in healthy persons (p«).05). There was no significant difference in T cell / B cell ratio.

Key Words: Nasal lavage. Nasal polyp. Flow cytometry, Lymphocytes, Nonlymphoid cell

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Özet

Lokal immünolojik ve inflamatuvar olavlar nuzal polip gelişiminde önemli rol oynar. T ve B lenfositleri ve HLA-DR+ epitelyal hücreler inflamatuvar cevabın oluşmasında regülatör ve efektör hücrelerdir. Bu çalışmaya nuzal polipli 25 hasta dahil edildi. Kontrol grubu olarak da sağlıklı 25 kişi alındı. Nazal polip ve sağlıklı kişilerden elde edilen nuzal lavajlordaki lenfosit ve non-lenfoid hücreler flovsitoınetrik o/arak analiz edilerek karşılaştırıldı.

Sonuç olarak T helper/T süpresör oranı, T lücre/Natürel killer hücre oranı, T hücre/Aktif T hücre oranı önemli derecede sağlıklı bireylerinkinden düşük bulundu. T hücre/B hücre oranları arasında ise önemli bir fark bulunamadı.

Anahtar Kelimeler: Nazal lavaj, Nazal polip, Flovsitometri, Lenfositler, Non-lenfoid hücreler

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Inflammation is stated to be the predominant mechanism involved in the development of nasal polyposis (1). Human respiratory mucosa may be associated with T-lymphocyte dependent disturbances in peripheral blood (2,3). T and B lymphocytes, which arc regulatory and effector cells in the complex process of inflammatory responses are found in the nasal mucosa (4-6). Nonlymphoid cells, such as HLA-DR+ cell have vital importance in the regulation of immune responses (7). HLA-DR+ epithelial cells which arc also present in the nasal mucosa, may activate T lymphocytes (8).

Nasal lavage has been shown to be a useful tool in the study of inflammatory cells and their mediators in diseases affecting the nose (9-14). Several research groups use nasal lavage to investigate the role of cells and mediators in allergic and nonallcrgic airway diseases. Different methods have been proposed to perform nasal lavage and a detailed morphologic identification of obtained cells has been described (9,10,15-18).

Although mucosal infiltration by T-cell has been demonstrated in upper airways of patients with rhinitis, these cells were not detected in nasal lavage (19,20). In this study, lymphocyte and non-

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lymphoid cells obtained from nasal lavage of patients with nasal polyposis were detected with flow cytometric analysis and the results were compared with those from nasal lavage of healthy persons.

Material and Methods

Patients

Twenty-five patients with nasal polyps (aged between 18 and 65 years with a mean age of 34 years) were included in this study. From these 25 patients, polyp tissue was evaluated by using transnasal endoscope. None of the patients had taken medication (inhaled glucocorticoids, antihistaminics, nasal decongestant agents) during the 2 weeks interval before the nasal lavage examination.

Control groups

The control group was composed of 25 healthy subjects (7 women and 18 men ranging in age from 18 to 34 years with a mean age of 24 years). These subjects did not have any upper respiratory diseases such as nasal allergy, nasal polyps, chronic sinusitis and common cold.

Nasal lavage technigue

A nasal lavage technique described in detail by Naclcrio et al. (11) was employed. With their heads bent back, the patients kept 5 ml of isotonic saline solution at room temperature in each nasal cavity for 10 seconds. The lavage fluid was then returned into a plastic cup, immediately chilled and centrifuged at 4°C at 500 g for 10 min. The sol phase was sepcrated from cellular phase and the supernatant was aliquoted for subsequent analysis.

Flowcytometric analysis

Total cell counts were obtained with flowcytometric analysis as described below:

1. Supernatant was taken out; then 20 1 of CD_3/CD_{19}, CD_4^+/CD_8^+, CD_3/HLA - DR+, $CD/CD_{Uvt\text{-}56}$, CD_{45}/CD_{14} receptors and 200 1 of nasal secretion were added into each tube.

2. Tubes were incubated for 20 minutes at room temperature.

3. They were analysed on Coulter - Multi - Q - prep device.

4. Then tubes were flowcytometrically analysed on Coulter Epics Elite - ESP device and approximately 10.000 cells were counted per each tube.

Statistical analysis

A11 data were expressed as mean value standard deviation. Mann-Whitney-U test is used for statistical analysis. A p value of less than 0.05 was considered to be statisticaly significant. Lymphocytes and nonlymphoid cells of patients obtained from nasal lavage were analysed by flow cytometric technique and the results were compared with the healthy persons. Statgraf version 5.0 statistical package was used to revaluate the data.

Results

Lymphocyte subsets ratio in nasal lavage of patients with nasal polyps and healthy subjects has been shown in Table 1.

The differences between the ($CD_3/CD_{1()4\text{-}56}$), (CD_4^+/CD_3^+), (CD_3/HLA-DR+) values obtained from the patients and healthy subjects are slatisti-

Table 1. Lymphocyte subsets ratio in lavage of patients with nasal polyps and healthy subjects

	Tccll/Natural killer cell (CD_3/CD_{16+56})	T helper cell / T suppressor cell (CD_4-/CD_k^+)	T cell/B cell ($CD_3/CD|_9$)	T cell/Active T cell (**CD3**/HLA-DR+)
Patients	3.52 ().%	0.92± 0.42	4.07=1=1.12	4.90±1.25
Healthy persons	5.52±1.34	2.05± 0.89	3.75±1 .25	5.85± 1.55
P value of the difference	p<().()5	p<0.()5	p>0.05	p<0.05

cally significant ($p<0.05$). However the difference in the (CD_3/CD_{1y}) ratios of patients and healthy subjects was statistically insignificant ($p>0.05$).

Discussion

Nasal polyps are obstructing tissue formations containing oedema fluid, but has an intact surface epithelium similar to that of the human nasal mucosa (21,22). Histologically, polyps are characterized by an infiltration of inflammatory cells and proliferation of connective tissue in stroma tissue cosinophilia (23). Eosinophils are the most common cells and are found in about 80% of nasal polyps (23). Other inflammatory cells include macrophages, plasma cells, mast cells and lymphocytes (24). Causes and pathogenesis of nasal and paranasal polyposis have still not been clarified. The role of allergy, or TgE-mediatcd hypersensivity is still controversial.

The density of the glands in nasal polyps is significantly less than that in the normal turbinate mucosa and no evidence of true scromucinous gland development as in the inferior and middle turbinates has been demonstrated (6). The density of eosinophilic and basophiloid cells is also markedly greater in the nasal polyp than in the inferior turbinate (25). A11 of these findings suggest that the nasal polyp is an inflammatory growth that is controlled by the local microenvironment (26,27).

Stoop et al.(28) demonstrated that immunologic and inflammatory processes play a prominent role in the development of nasal polyps. The relatively low number of cells in polyps and in the unaffected middle turbinates of the patients in combination with the high number of cells in these tissue could perhaps indirectly result in a less sufficient humoral immune response, but is certainly evidence of an altered T cell-mediated immune defence (29).

It is possible that high number of CD_x could be beneficial because of their suppressive and down-regulating effect on the chronic local inflammatory response, although the precise role of these cells is still a matter of controversy (30,31). Significantly more cells are found in healthy subjects than in patients with nasal polyps (6,29). Also in our study, significantly more cells were found in nasal lavage of healthy subjects than in patients. Besides, T Helper cell/ T Suppressor cell ratio in patients was significantly lower than that in healthy persons ($p<0.05$).

HLA-DR+ cells possibly play a role in uptake and presentation of antigens. HLA-DR+ epithelial cells may also activate cells, as demonstrated by Mayer and Shlien (8). This could suppress the chronic inflammatory reaction in the polyps and in middle turbinates of the patients. In our study T cell to HLA-DR+ ratio in patients was found to be significantly lower than that in healthy persons. Stoop et al.(29) demonstrated that T cell to HLA-DR+ ratio obtained by immunohistochemical technique was much higher in polyps and in middle turbinates than in the inferior turbinates. Our study support the hypothesis that the pathogenesis of nasal polyps is associated with chronic inflammation and T-cell disturbance in specifis sites of the (para) nasal mucosa.

T suppressor cell, active T cell, natural killer cell were found higher in nasal lavage of patients with nasal polyposis than in the healthy subjects. Therefore it can be said that these cells may play an important role in pathogenesis of nasal polyposis and down regulation of chronic inflammatory response.

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