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RESEARCH ARTICLE

Levels of Th1 and Th2 Cytokines in Patients with Nasal Polyps

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ABSTRACT

Objective: We aimed to investigate Th1 / Th2 (T helper) cell balance by examining Interleukin-4 (IL-4), Interleukin-5 (IL-5), Interleukin-10 (IL-10), Interleukin-17 (IL-17) and Interferon gamma (IFN γ) levels in serum in patients with nasal polyps (NPs).

Patients and Methods: This study included 45 patients (mean age: 34.8 years) with NPs and a control group (mean age: 37.9) consisting of 45 healthy volunteers.

Results: The mean IL-17 level in the NPs group (4.096 pg/ml) was significantly higher than the control group (2.219 pg/ml) ($p<0.001$). IL-5 levels were significantly higher in the control group. NPs group had significantly lower IL-5 level compared with the control group (1.981 pg/ml vs. 2.720 pg/ml, respectively: $p<0.001$).

The mean IFN γ concentration was significantly lower in the NPs group (0.379 pg/ml) compared to the control group (0.439 pg/ml) ($p=0.011$). No significant differences were found in the IL-4 (3.25 pg/ml vs. 3.38 pg/ml) and IL-10 (11.249 pg/ml vs. 12.156 pg/ml) concentrations between NPs and the control groups ($p=0.799$ and $p=0.255$, respectively).

Conclusions: Increased IL-17 levels were found in NPs patients. There was no significant differences in IL-4 and IL-10 values of the Th1 cytokines among the groups, whereas IL-5 was found to be low in the NPs group. IFN gamma values of Th2 cytokines were found to be low in the NPs group. According to these results, low IFN gamma levels and regulatory cytokine IL-7 activation may be effective in the etiopathogenesis of NPs.

Key words: Nasal Polyps, Interleukin-4, Interleukin-5, Interleukin-10, Interleukin-17, Interferon Gamma

INTRODUCTION

Nasal polyps (NPs) are benign protrusions into the nasal cavity, characterized by chronic inflammation of the nose and paranasal sinuses. The prevalence of NPs in general population is 1-4%. Nasal polyps are two times more common in males than females [1]. Although several theories have been suggested, etiopathogenesis of NPs remains unclear. Factors thought to play a role in the formation of NPs include allergy, bronchial asthma, aspirin allergy genetic factors, anatomic disorders, epithelial rupture, chronic local infections, mucosal contact, Bernoulli's phenomenon, connective tissue disorders, immunological and biochemical factors [2].

The underlying pathology leading to the development of NPs is nasal mucosal edema. Submucosal edema and inflammation may result in epithelial damage, necrosis and rupture from where the edematous mucosa prolapses to develop NPs [3]. Many inflammatory cells, cytokines, growth factors are responsible for the development of edema and inflammation. Cells mainly responsible for inflammation in NPs include eosinophils, leukocytes, mast cells and lymphocytes [4].

Mediators released from eosinophils, neuropeptides and histamine lead to edema and polyp formation by changing ion balance in the medium and promoting collagen synthesis. T cells are specialized cells that have a role in pathogen inactivation by destroying infected cells. T-cells both enhance B and T lymphocyte response by releasing cytokines and limit tissue

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damage by regulating immune responses. T helper cells (Th) consist of at least 5 groups of cells including Th1, Th2, Th17, regulatory T-cells (Treg), and Th9 [5].

Th1 cells mainly secrete IFN γ and IL-2. These cytokines particularly activate macrophages and NK cells to enable them to destroy intracellular pathogens. Th2 cells mainly secrete IL-4 IL-5, IL-10 and IL-13. The cytokines released by the Th2 cells act in hypersensitivity reactions and immunity against extracellular pathogens such as parasites. They also promote B-cell activation and antibody production [6]. Th1 cells are typically predominant cells in NPs. Th2 cells and eosinophils are found at higher rates in polyp tissues from atopic patients, eosinophilic infiltration in NPs has been reported to be associated with Th2 cells [7]. The degree of eosinophilic infiltration in NPs and the presence of cytokines regulating them may be clinically important for treatment decision-making [8]. Interleukin-4 (IL-4) is a major molecular regulator of IgE synthesis. IL-4 is released by Th2 and mast cells. cells. Exposure to an allergen in patients with allergic rhinitis increased both nasal mucosa and the number of peripheral blood Th2 cells [9].

Interleukin-5 (IL-5) is a cytokine that has a critical role in eosinophilic inflammation and is released by Th2. Eosinophils are the only types of white blood cells that have specific IL-5 receptors [6]. IL-5 plays a major role in the activation, differentiation and mobilization of eosinophils Therefore, IL-5 has also an important contribution to the increased eosinophil counts in NPs [7]. Interleukin-10 (IL-10) is an anti-inflammatory cytokine [12]. IL-10 is mainly expressed in Th2 cells, B-lymphocytes and macrophages. The main effect of IL-10 is to preclude cytokine expression in Th1 cells, natural killer (NK) cells and antigen-presenting cells [13]. Interferon gamma (IFN-γ) is produced by Th1 cells and natural killer (NK) cells. Its major functions are to induce somatic cells including macrophages, neutrophils and natural killer cells, to promote cell-mediated immunity (Th2 suppression), to enhance class I MHC production, to increase class II MHC in antigen-presenting cells, to enhance antiviral activity and high endothelial venule formation [13]. This cytokine is also involved in innate immunity and acute inflammation. IFN γ prevents allergic responses of airways and consequently it prevents eosinophilia associated with these responses [13]. Interleukin-17 (IL-17) is a pro-inflammatory and regulatory cytokine which induces a number of mediators of inflammation. IL-17 has been reported to play a role in inflammatory and autoimmune diseases, in the development of cancer and in the regulation of immune system [14,15].

Considering the fact that the etiology of NPs is unknown, this study aimed at clarifying the etiology of NPs , by comparing IL-4, IL-5, IL-10, IL-17 and IFN-γ levels in NPs tissues to the concentrations of these variables in normal, healthy volunteers.

PATIENTS AND METHODS

This study was conducted in the department of Otorhinolaryngology at Medical School of Hitit University, between March 1, 2016 and March 1, 2017. Ethics approval for the study protocol was obtained from the Clinical Trials Ethics Committee of Hitit University, by the decision no. 2016/22. Forty five NPs patients and 45 healthy subjects were included in the study between 18-60 years of age. All Participants provided their written informed consents for participating in the study.

Patients with bilateral NPs were included in the study group. The diagnosis of NPs was made by anterior rhinoscopy and nasal endoscopic examination. Nasal polyps patients with atherosclerosis, malignancies, hypertension, diabetes mellitus, rheumatologic disease, endocrine disease, systemic and metabolic diseases, nose and paranasal surgery patients were excluded from the study. Subjects in the control group did not have any systemic diseases, bronchial asthma or nasal disorders including chronic sinusitis, NPs and allergy. None of the patients or controls was smokers or consumed alcohol. Blood samples were taken before NPs patients began any medical treatment. A 10 ml venous blood sample was drawn into the vacuum test tubes from 8:00 am to 10:00 pm. After waiting for 30-45 minutes, blood samples were centrifuged at 4000 RPM for 10 minutes; serum was separated, and stored in an Eppendorf tube at -80°C until analysis.

Measurements

All samples were stored at -80^{0}C and in the dark to avoid interactions with light. Samples were thawed at room temperature 3 hours before the analysis and centrifuged at 5000 rpm. Test kits included DIA source IL-10 (DIAsource ImmunoAssays SA, Louvain la Neuve, Belgium) INF-γ- EASIA kit (Biosource Europe, Nivelles, Belgium), BMS2017 human IL-17A, BMS225/2 IL-4, BMS278 human IL-5 Platinum Elisa (eBioscience, Vienna, Austria). Kits using ELISA techniques were stored at 2 to 8^{0}C. An auto washer device was used for washing during the assay and different wave lengths were read by an ELX800 ELISA reader and the results were given as pg/ml.

Statistical analysis

To perform statistical analysis, a SPSS statistical software (Version 22.0, SPSS Inc., Chicago, IL, USA, licensed to Hitit University) was used. Kolmogorov-Smirnov and Shapiro-Wilk tests were used to test the normality of the distribution of data. In the descriptive statistics presentation, mean ± standard deviation, median (min-max), and categorical variables were presented as numbers and percentages according to the continuous variables distribution hypotheses. Independent sample t-test was used to compare the mean values of two independent continuous variables, normally distributed. Independent non-dispersive continuous variables were compared with the Mann-Whitney U test. A P

value of 0.05 was considered to indicate a statistically significant difference in all tests.

RESULTS

The mean age of the NPs group (21 male, 24 female) was 34.8 years. The control group (20 males, 25 females) had an average age of 37.9. Groups were similar in terms of age and gender distribution. Intergroup comparisons revealed that IL-17 concentrations were statistically significantly higher in the NPs group (p<0,001) (Table 1) while IFN γ and IL-5 concentrations were significantly higher in the control group (Table 1) (p=0.011, p<0.001, respectively) (Figure 1). No significant intergroup differences were found in IL-4 and IL-10 concentrations (P=0.799, P=0.255, respectively) (Table 1).

Table 1. Comparison of nasal polyp and control groups in terms of IL-17, IFN γ, IL-5, IL-4, and IL-10

	Group	N	Mean ± SD	Median (min-max)	*p* value
IL-17	**Polyp**	45	4.09 ± 12.17	2.18 (1.90 - 83.86)	**<0.001**
	Control	45	2.21 ± 0.71	2.01 (1.86 – 5.00)	
IFN γ	**Polyp**	45	0.37 ± 0.12	0.37 (0.25 - 0.90)	**0.011**
	Control	45	0.43 ± 0.19	0.39 (0.34 – 1.68)	
IL-5	**Polyp**	45	1.98 ± 0.69	1.64 (0.58 - 3.64)	**<0.001**
	Control	45	2.72 ± 1.28	2.27 (1.26 – 8.64)	
IL-4	**Polyp**	45	3.25 ± 1.71	2.95 (1.10 - 11.46)	0.799
	Control	45	3.38 ± 1.86	3.10 (1.08 – 11.12)	
IL-10	**Polyp**	45	11.24 ± 5.00	11.66 (3.62 - 30.78)	0.255
	Control	45	12.15 ± 1.74	11.66 (9.65 – 17.89)	

SD: Standard Deviation, Min: Minimum, Max: Maximum
IL-4; Interleukin-4, IL-5; Interleukin-5, IL-10; Interleukin-10, IL-17; Interleukin-17,
IFN γ; Interferon Gamma

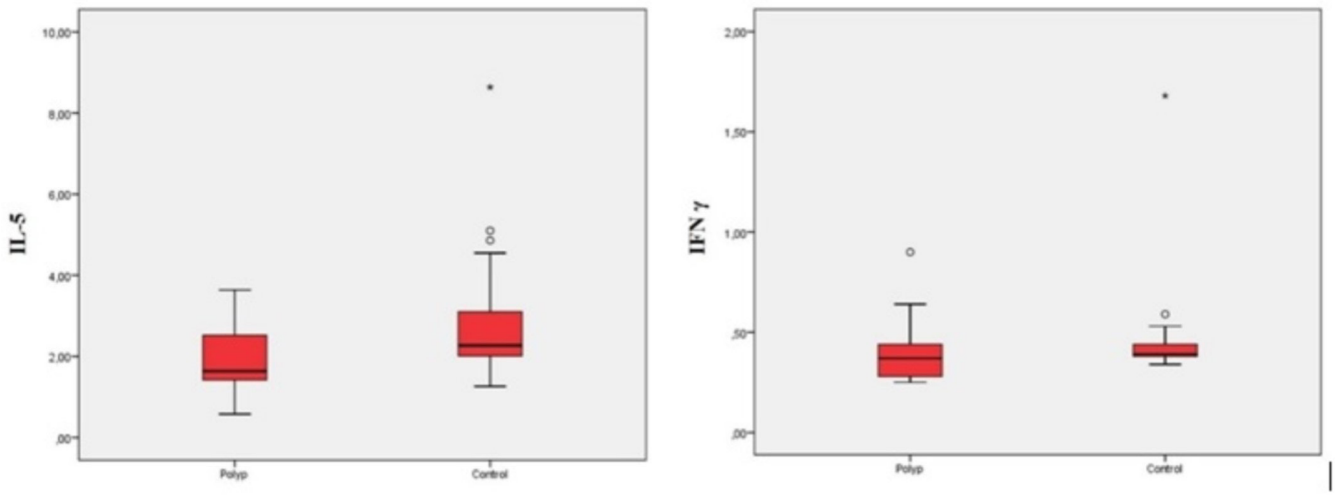

Figure 1. Comparison of nasal polyp and control groups in terms of IL-17, IFN γ, IL-5, IL-4 and IL-10.

DISCUSSION

In our study, we found statistically significant intergroup differences in IL-17, IFN γ and IL-5 concentrations. IL-17 concentrations were statistically significantly higher in the NPs group while IFN γ and IL-5 concentrations were significantly higher in the control groups. No significant intergroup differences were found in IL-4 and IL-10 concentrations.

Our literature search revealed a number of studies investigating concentrations of various cytokines to find out NPs etiology and the results were conflicting. These studies in NPs are usually cytokine studies in the nasal polyp tissue and we have found that there is a limited number of studies on serum levels. Despite a great deal of literature, NPs etiology was unclear therefore we decided to investigateIL-4, IL-5, IL-10, IL-17 and IFN-γ concentrations in patients with NPs.

Many comparative studies have demonstrated higher IL-5 concentrations in NPs tissues that those in control tissues. Zhao et al [16]. Compared tissue IL-5 levels of nasal polyp and inferior concha. They found that IL-5 expression in the nasal polyp tissue was significantly higher than in the inferior concha. IL-5 high expression may be closely associated with the formation and development of nasal polyps. Kubato et al [17]. In their study of NPs, polyps were divided into two groups as eosinophilic and non- eosinophilic. They compared IL-5 levels in the polyp tissue between the two groups and they found that the IL5 levels in the eosinophilic group were higher than in the non-eosinophilic group.

Bachert et al. [18] reported significant associations between combined increase of IL-5 and eotaxin in NPs tissues and the increased concentration of eosinophilic cationic protein (ECP) which is an indicator of eosinophilic activation. Another study demonstrated an association between IL-5 levels in nasal secretions and NPs recurrences [19]. Allen et al. [20] found that IL-5 levels in polyps' tissue were significantly higher than controls. In the same study, they reported a significant correlation between IL-5 levels and steroid use. In our study, we found that serum IL-5 levels were significantly lower in the NPs group. We did not find a study of serum IL-5 levels in the literature, although there are many studies on polyp tissue IL-5 levels in NPs. We think that the lower level of IL-5 in the nasal polyp group in our study is not a clinical benefit. In our study, lower serum IL-5 levels in the NPs group may be due to the fact that this cytokine is more effective in the peripheral target tissues, when higher IL-5 levels are thought to be present in polyp tissues in previous studies.

IL-10 is an anti-inflammatory cytokine [12]. Moore et al. [21] demonstrated that IL-10 levels were increased in nasal tissues and peripheral blood in patients with allergic rhinitis who had been successfully treated with immunetherapy in long-term. Domdey et al. [22] IL-10 levels were found to be increased in atopic patients while such increases were not detected in patients without atopy. In our study, no significant intergroup differences were found in IL-10 levels.

Özkara Ş et al. [23] Reported IL-10 levels in serum were statistically higher in patients with NPs and allergic rhinitis than control group. However, there was no significant difference between the non-allergic NPs patients and the control group. We found that there was no difference in IL-10 levels between the polyp patients and the control group in our study. We did not divide polyp patients into allergic and nonallergic groups. The reason why there is no difference between the two groups may be that the patient group may be composed of patients with more nonallergic polyps.

Molet SM et al [24]. Compared IL-17 expression in nasal polyp tissue and concha, and they reported that IL-17 levels in the polyp tissue were statistically higher than in the control group. Jiang XD et al [25]. investigated IL-17 levels in nasal polyp patients and control patients. The levels of IL-17 in the polyp tissue were significantly higher than the control group. In the patient group, no difference was found between IL-17 levels between ezinophilic and non ezinophilic polyp tissues. There was no difference in serum IL-17 levels between the Np group and the control group. But, In our study, we found that serum IL-17 levels in nasal polyposis patients were higher than in the control group.

Özkara Ş et al [23], reported IFN-γ, IL-4 and IL-10 levels in serum were statistically higher in patients with NPs and allergic rhinitis than control group. However, there was no significant difference between the non-allergic NPs patients and the control group. Dellacono FR et al [26]. In patients with NPs, IFN-γ levels in polyp's tissue were higher than controls. Increased IFN-γ levels and association with allergy, asthma and topical steroid use are reported. They suggested that IF -γ levels activate lymphocytes and eosinophils in the NP periphery. Similarly, we found that IL-4 and IL-10 levels in serum were higher in patients with nasal polyps than in the control group. but we found that IFN-γ levels were lower in patients with nasal polyps. The reason for the lower serum IFN-γ levels in patients with NPs may be due to the fact that IFN-γ accumulation is predominantly in the polyp and its effects are more effective in the periphery.

The limitations of our study include the absence of comparisons between cytokine levels in polyp tissues and cytokine levels in normal mucosal tissues. In addition, we believe that it might be beneficial to investigate the effects of steroid therapy on cytokine levels. It would also be useful to investigate these cytokine levels in patients with eosinophilic and non eosinophilic NPs.

In conclusion, in our study, intergroup comparisons did not reveal significant differences in Th2 cytokines IL-4 and IL-10 while IL-5 concentration was lower in the NPs group. Among Th1 cytokines, IFN-γ concentrations were found to be lower in NPs group. The levels of IL-17 were found to be higher in the NPs group. According to these results, regulator cytokine IL-17

activation and low IFN gamma may be effective in the etiopathogenesis of NPs. In conclusion, the etiology of nasal polyp is very complex and more comprehensive studies are needed to clarify etiology underlying NPs.

Conflict of Interests: The authors declare that they have no conflict of interest.

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