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SHORT PAPER

IL-25 and IL-33 Serum Levels are not Associated with the Type of Allergen Causing Allergic Rhinitis

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ABSTRACT

Background: Fungal aeroallergens might sensitize the airway which in turn produces a specific cytokine profile. **Objective:** To evaluate the IL-25 and IL-33 profile in patients with fungal allergic rhinitis. **Methods:** The present study examined patients who were evaluated due to allergic rhinitis (AR) at Emam Reza Hospital of Shiraz, Iran. The allergic patients were categorized based on the skin prick test. Blood samples were collected and allergen-specific IgE and cytokine profiles were analyzed. **Results:** 184 patients were enrolled in the study and in 35 of whom fungal rhinitis was confirmed. The levels of specific IgE in patients with fungal allergy were statistically significant compared to those in the control group ($p<0.000$). However, there were no significant differences in IL-25 and IL-33 levels between fungal and none-fungal AR patients. **Conclusion:** Chronic fungal challenge might regulate innate system cytokines in severe persistent AR.

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INTRODUCTION

Allergic rhinitis (AR) is a common symptomatic disorder among the upper airway allergic diseases (1). It is an IgE-mediated chronic inflammatory disease clinically characterized by sneezing, rhinorrhea, nasal itching and nasal obstruction (2). AR diagnosis is based on clinical manifestations, positive skin prick test (SPT), specific serum IgE level and its severity, which is categorized into mild, moderate, and severe grades (3). Several cells, chemokines and cytokines are involved in the immunopathology of upper airway diseases. Among the cytokines, the importance of the T helper cell (Th) 2 cytokines is well established (4). Through producing IL-10 and transforming growth factor-β (TGF-β), regulatory T cells (Tregs) have restrictive effects on Th, while their absence causes IgE synthesis along with the development of allergic inflammation (5). IL-17 is another cytokine which contributes to the induction of allergen-specific Th2 cell activation, eosinophil accumulation, and serum IgE production. IL-17 is a family including IL-17A, B, C, D, E and IL-17F (5,6). IL-17E differs from other members in its function and structure, hence also known as IL-25. This cytokine is able to induce NF-κB activation and stimulate IL-8 production (7). Interleukin-33 (IL-33) has recently been considered as an emerging key factor in the development of allergic diseases. As an 'alarmin', IL-33 is released from a variety of cells following exposure to allergens (8). Although IL-33 has been identified as a member of the IL-1 family, it acts as an intracellular nuclear factor to dampen pro-inflammatory signaling through reducing nuclear factor kappa B (NF-κB) (9). IL-33 exerts its inflammatory function extracellularly through binding to a heterodimer receptor complex (10). Studies conducted throughout the world have shown that fungal sensitivity, particularly to Alternaria species, is common among asthmatic subjects (11). Fungal allergen exposures are generally considered to take place in outdoor environments; however, many species are capable of invading homes through open cracks or windows. Certain species such as *Penicillium* and *Aspergillus* are often recovered in greater amounts from inside the buildings compared with the outside air (12). The objective of this study was to investigate the role of innate cytokines such as IL-25 and IL-33 that may form a target for novel treatment approaches. Although there are studies in the literature evaluating allergic rhinitis cytokines in nasal lavage, we preferred to evaluate serum cytokine levels, especially in patients with allergic fungal rhinitis.

MATERIALS AND METHODS

Patients. This cross-sectional study was performed in the Allergy and Clinical Immunology Departments of Emam Reza Hospital of Shiraz, Iran, between May 2017 and April 2018. The total studied sample consisted of 70 participants divided into fungal allergic patients and non-fungal allergic volunteers based on their skin prick test. The Ethics Committee of the Shiraz University of Medical Sciences approved the study which adhered to the Declaration of Helsinki (2013) guidelines (IR.SUMS.MED.REC.1397.128). All subjects with a minimum one year moderate/severe persistent rhinitis signed a written informed consent form after the study protocol was fully explained to them. A detailed clinical history and a complete physical examination were performed for each patient. Each subject in the non-fungal

allergic group was selected among 184 AR patients attending the allergy outpatient clinic according to a negative skin prick test. The subjects in the fungal allergic group were selected randomly from the mentioned AR patients based on the following criteria: history of persistent rhinitis and positive skin prick test to fungal allergens (>3 mm). In both groups, the exclusion criteria were acute/chronic infections, inflammatory diseases (Systemic Lupus Erytmatose, Rheumatoid Arthritis), systemic immunological disorders, anatomical abnormalities of the upper respiratory tract such as polyps and septal deviation, malignancies and chronic treatment with systemic steroids. Allergy drugs of the patients were voluntarily discontinued two weeks prior to sampling and prick tests.

Skin Prick Test (SPT). Skin prick test was performed by the same experienced personnel at Emam Reza Hospital of Shiraz. According to a previously validated protocol, they applied one drop of each allergen extract (Allergy Laboratories Inc., Oklahoma City, USA) from a panel containing *Aspergillus*, *Alternaria*, *Penicillium*, grass, mugwort, ragweed, birch, cypress, house dust mite, cat and dog dander and food allergens at least 3 cm apart (6). Histamine was used as a positive control and the diluents of each allergen (Allergy Laboratories Inc., Oklahoma City, USA) were employed as a negative control. The sensitivity of the skin test was determined by the size of the wheal. The largest diameter of the wheal was specified as the size of the wheal after 20 minutes. A wheal diameter of 3 mm or higher, accompanied by erythema, was considered as a positive reaction.

Detection of Cytokines. Peripheral blood samples were drawn from an antecubital vein to assess the serum cytokine levels. The samples were collected into tubes containing heparin. Immediately after sampling, tubes were centrifuged at 1000 ×*g* for 10 min and aliquots of plasma were stored at -20ºC. The serum IL-25 and IL-33 levels were measured by ELISA (Bioassay Technology Laboratory, USA) according to the manufacturer's protocols. The minimal detectable concentrations were 20 ng/L for IL-25 and 5 ng/L for IL-33. All samples were detected in duplicate.

Measurement of Allergen Specific IgE. Specific IgE antibodies to *Aspergilluse fumigatus, Alternaria alternate* and *Penicillium* were measured using a flouroimmunoassay (CAP system; Pharmacia, Uppsala). Sera were primarily assayed undiluted, and when necessary, assays were repeated with the diluted sera to enable the quantitative measurement of specific IgE antibodies. Once specific antibodies were detected for each tested fungal allergen, the sera belonging to patients were tested for IgE level.

Nasal and Ophthalmic Symptom Scores. The nasal symptom scores of atopic patients were recorded in both groups. The severity of each participant's nasal symptom score was totally assessed by calculating the value of each symptom including nasal rhinorrhea, sneezing, itching and congestion. The severity of ophthalmic symptom score was completely assessed in both atopic patients through evaluating symptoms such as lacrimation and eye itching.

Statistical Analysis. In this study, SPSS software version 16 (SPSS Inc., Chicago, IL, USA) was used for data analysis. Cytokine levels were tested for the normality of distribution; moreover, using the ANOVA, the differences between the two mentioned groups were compared regarding plasma levels of IgE and cytokines. Mann-Whitney Test, as a nonparametric test, was performed to compare dependent and other variables. All statistical tests employed a significance level of P-value<0.05.

RESULTS and DISCUSSION

Thirty-five non-fungal allergic volunteers as controls (ages 30.43 ± 10.00 years) and 35 participants with fungal allergic rhinitis (FAR) as patients (ages 32.37 + 10.47 years) completed the study. The demographic and clinical characteristics of patients and control subjects are shown in Table 1. Based on our findings, there was no significant difference between the two groups regarding nasal rhinorrhea, itching, sneezing and congestion. Moreover, the total nasal symptom score (TNSS) in non-fungal allergic volunteers was similar to that of patients with FAR.

Table 1. Demographical data of patients with fungal (case) and none-fungal (control) allergic rhinitis.

Variables	Control (n=35)	Case (n=35)	p-value	Significance
Gender (female/ male)	18/17	25/10	0.086	NS
Age (years)	30.43 ± 10.004	32.37± 10.474	0.430	NS
Smoking(Y/N)	2/33	3/32	0643	NS
Nasal rhinorrhea (Y/N)	32/3	35/0	0.077	NS
Itching (Y/N)	14/21	21/14	0.094	NS
Sneezing (Y/N)	20/15	27/8	0.075	NS
Congestion (Y/N)	24/1	24/1	1.000	NS
Lacrimation (Y/N)	9/26	19/16	0.015	S
Dyspnea (Y/N)	11/24	14/21	0.454	NS
SPT positivity (≥ 3 mm)	0	35	0.000	S
IgE	0.35 ± 0.00	2.62 ± 0.60	0.000	S

SPT: Skin prick test, NS: Not statistically significant.

The serum levels of specific IgE in patients with fungal allergic were significantly higher than the control group (p-value<0.000). Although the serum level of IL-25 was higher in fungal allergic patients (1907.28 ± 584.44 pg/mL) compared with the control group in our cytokine measurements (761.78 ± 123.04 pg/mL), the difference was not statistically significant (p-value=0.719) (Table 2).

IL-25 is a cell-derived cytokine exacerbate in allergic inflammation by the production of specific Th2 cytokines (13,17). We observed that allergic patients with positive SPT to fungi produced higher IL-25 concentrations compared with AR control. Although some animal studies have focused on the role of IL-25 in the development of acute allergic inflammation, the relationship between this innate cytokine and the chronic form of AR is yet to be fully fathomed (7). According to our data, the total ophthalmic symptom score was higher in FAR patients compared to the control group (p=0.017). IL-25 indirectly contributed to lung pathology in humans which might be considered it as a cause of ophthalmic symptoms (13). Taken together, this late IL-25 up-regulation in SPT^{+} AR patients might be a compensatory immune mechanism for defense against fungal infection. Moreover, the serum levels of IL-33 in fungal and non-fungal AR groups were 237.55 ± 92.93 pg/mL and 345.89 ± 107.68 pg/mL, respectively (P-value=0.743).

Table 2. IgE and cytokine levels (Mean ± SD) in both patients and control group based on fungal prick test positivity.

Groups	Prick (n)	IL25 (ng/L) p-value	IL33 (ng/L) p-value	IgE (kU/L) p-value
Patients	*Aspergillus* (23)	2365.88 ± 856.84 (1.000)	253.13 ± 119.45 (0.833)	2.97 ± 0.82 (0.001)
	Alternaria (9)	1123.34 ± 531.72 (0.329)	330.86 ± 201.14 (0.948)	2.6 ± 1.05 (0.000)
	Penicillium (5)	543.44 ± 156.93 (0.651)	21.82 ± 3.89 (0.262)	3.28 ± 1.85 (0.000)
Controls	Negative (35)	761.78 ± 123.04	345.89 ± 107.68	0.35 ± 0.00

After exposure, the IL-33 release was upregulated and it exerted its inflammatory function and reduced nuclear factor kappa B (9,10). Some evidence showed that IL-33 and its receptor were expressed in patients with AR, suggesting IL-33/ST2 may play an important role in the pathogenesis of allergic nasal diseases (13,14). According to our data, a serum IL-33 level was reduced in a patient with positive prick test to fungi, which is in line with the Asaka *et al.* study (15). Interestingly, in a study on Japanese cedar pollinosis patients with AR, IL-33 protein was not detected in the serum; however, its level significantly increased in sinus mucosa and correlated with the total nasal symptom score (8,13). Besides, our findings are not consistent with the results of Asaka *et al.* who reported higher IL-33 levels in chronic rhinosinusitis (CRS) patient's serum compared to the controls (8,15). It is to be borne in mind that IL-33 in AR patients increased compared to healthy controls, but we evaluated two groups with clinically diagnosed moderate/severe AR. Moreover, a group of researchers reported that the immunosuppression in patients surviving after sepsis was associated with IL-33 function (9,13). Although serum levels of IL-25 were higher in the FAR patients sensitized with *Aspergillus* and *Alternaria*, this cytokine was lower in *Penicillium* sensitized patients compared to the control group. Evidence suggests that fungal sensitization might be a risk factor for the development of airway allergy and stimulate allergen-presenting cells, generating a specific cytokine profile (9,10). The findings of this study revealed a change in the serum levels of the mentioned cytokines in the patients with FAR compared with non-fungal AR as controls. The immunological patterns of CRS are different based on the presence or absence of nasal polyps. In western countries, CRS with nasal polyps shows type 2 inflammation while in Asia, mixed inflammatory patterns are reported. Such inconsistency might be ascribed to both geographical differences and the nature of fungal allergens (16). Therefore, the reduction in innate cytokine levels in the present study's participants with moderate/severe persistent rhinitis might be assumed as an immune polarization mechanism for the challenged chronicle fungi allergens. Among the limitations of our study, mention can be made of the small sample size, especially regarding some fungal species and a few numbers of male patients. Moreover, other cytokines were not measured, which might be another limitation. The patho-immunology of severe persistent rhinitis patients is still not well understood; however, chronic fungal challenge elicits a persistent AR in susceptible people and triggers the production of

innate system cytokines, polarizing the immune system. Our findings might pave the way for further investigation on the role of these cytokines in fungal allergic rhinitis.

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