

The presence of fungal-specific IgE in serum and sinonasal tissue among patients with sinonasal polyposis

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Abstract Nasal polyposis (NP) is a common inflammatory disorder with different proposed etiologies. There is some evidence that the local production of specific IgE against fungal allergens may contribute to the pathogenesis of this condition. The aim of this study was to compare local and systemic production of fungal-specific IgE among patients with NP. In a prospective cross-sectional study, 141 patients with sinonasal polyposis who were surgical candidates were enrolled. Total IgE and fungal-specific IgE antibodies in serum and tissue homogenates were measured by the ELISA and ImmunoCAP method, respectively. Twelve cases (9.45 %) met the allergic fungal rhinosinusitis (AFRS) criteria while the remaining 129 patients showed histopathological features of chronic hyperplastic rhinosinusitis. Considering a cutoff point of 0.4 KUI/L for specific IgE as a positive reaction against fungal elements, seven and 62 cases showed positive results in serum and homogenate tissue, respectively. All serum positive reactions were seen among patients with AFRS. In conclusion, IgE antibody could be locally produced in the nasal polyp tissue while not detectable in the serum.

Keywords Nasal polyposis · IgE · Fungus · Allergy · Antibody

Introduction

Nasal polyposis (NP) is a chronic inflammatory disease of an uncertain etiology affecting the nasal mucosa and paranasal sinuses. Although the exact prevalence of NP is undetermined, epidemiological studies have suggested a figure of 2–4 % [1]. Patients present with bilateral polyps arising from the paranasal (specifically ethmoidal) sinuses towards the nasal cavity [2]. In spite of the high prevalence, surprisingly the exact etiopathogenesis has remained unknown so far, although eosinophils seem to play an important part. The role of allergy remains unclear and the role of fungal infection in NP is still controversial.

The incidence of atopy in patients with NP varies between 10 and 96.5 % [3–6]. Although patients usually show positive prick tests, the potential role of IgE-mediated hypersensitivity in the development of NP is the subject of debate. Some studies have suggested the occurrence of local allergic reactions due to specific IgE in the mucosa, but not in serum [7].

The local synthesis of multiclonal and specific IgE against *Staphylococcus aureus* enterotoxins sIgE SAE [7–11] in patients with NP is associated with eosinophilic inflammation, yet the role of fungal infection in NP is controversial. Allergic fungal rhinosinusitis (AFRS) was first described as a clinical entity in 1976 [12]. It is defined mainly by the presence of allergic fungal mucin, which is a thick, tenacious, eosinophilic secretion along with diagnostic histological findings [13, 14].

Asero et al. [15] found that patients with NP develop fungal sensitization, particularly to *Candida albicans*, although it was a controversial genus [16]. Ponikau et al. [17], in a study of patients with chronic rhinosinusitis, detected fungi in 96 % of sinusal secretion cultures and identified 40 different fungal species in the paranasal

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sinuses. According to the mentioned authors, the condition is mediated by IgE in response to fungi present in the sinonasal eosinophilic mucin. This theory is supported by the observation of specific IgE in mucus. At present, the almost generally accepted hypothesis excludes the allergic factor and proposes the term “eosinophilic fungal sinusitis” [17, 18], based on the fact that skin testing is negative in many patients presenting CRS with fungal elements. Recently, attempts have been made to explain the importance of local IgE in the absence of systemic IgE [19].

In this study, the local production of antigen-specific IgE within sinus mucosa of AFRS and non-AFRS patients with sinonasal polyposis was evaluated and compared to serum antigen-specific IgE.

Methods and materials

Patients

In a prospective cross-sectional study, from 2007 to 2011, 141 patients with sinonasal polyposis on physical examination and CT scan who were unresponsive to medical treatment and were candidates for surgery were enrolled. Skin prick test (SPT) with a battery of 21 common regional aeroallergens including weeds/grasses, trees, house dust mites cockroach, molds, alternaria and aspergillus extract was performed for all cases.

The study protocol and informed consent form were reviewed and approved by the Research Ethics Committee of Mashhad University of Medical Sciences. An informed consent was obtained from each patient prior to study entrance.

Preparation of serum and tissue homogenates

Five milliliters of venous blood was taken from all patients at study entrance and sera were separated by centrifugation (3,000 rpm for 20 min). Sera were stored in -20°C before analysis. Nasal polyp tissue samples were taken during polypectomy and after weighing were stored at -70°C . On the day of analysis, tissue samples were thawed and homogenized by a mechanical homogenizer (1,500 rpm for 5 min on ice) in the presence of phosphate buffered saline (1 mL per every 100 mg of tissue, pH 7.4); the supernatant was then collected. For both the polyp supernatant and serum, total IgE and specific IgE in a mixture of six different molds including *penicillium chrysogenum*, *Cladosporium herbarum*, *Aspergillus fumigatus*, *C. albicans*, *Alternaria alternata*, *Setomelanomma rostrata* were measured by commercial Elisa kit (Radim, Italy) and ImmunoCap (Pharmacia, Sweden), respectively. To adjust the level of IgE between serum and polyp tissue, albumin level

was measured in both tissue and serum by the nephelometric method. Smear and culture for fungal elements was performed only in those who were suspected of having AFRS.

Results

The mean age of patients was 37.4 ± 11.6 years and the male to female ratio was 76/65. Twelve cases (9.45 %) met the AFRS criteria while the remaining 129 patients showed histopathological features of chronic hyperplastic rhinosinusitis.

Skin prick test was positive for at least one allergen in 32 cases (22.7 %) (Table 1). Total IgE level and anti-fungal-specific IgE in serum and tissue are shown in Table 2.

Considering a cutoff point of 0.4 KUI/L for specific IgE as a positive reaction against fungal elements, 7 and 62 cases showed positive results in serum and homogenate tissue, respectively. All serum positive reactions were seen among patients with AFRS.

Smear and culture studies for fungal elements among AFRS patients resulted in seven and four cases having *Aspergillus* and *Alternaria* species, respectively, whereas one case had a negative culture.

Discussion

Nasal polyposis is defined as a chronic mucosal inflammatory process of the nasal cavity and paranasal sinuses. Since the etiology remains unclear, eosinophils appear to be an important factor. The role of fungal agents in the

Table 1 The most common allergens among patients with nasal polyposis

	Allergens	Number	Percent
1	<i>Dermatophogoides farinae</i>	7	21.88
2	<i>Dermatophogoides pteronyssinus</i>	5	15.63
3	<i>Alternaria Alternata</i>	6	18.75
4	<i>Aspergillus</i> mix	5	15.63
5	Molds yeasts mix	4	12.50
6	Cockroach	6	18.75
7	Rough pigweed (<i>Amaranthus retroflexus</i>)	9	28.13
8	Bermuda grass (<i>Cynodon dactylon</i>)	6	18.75
9	Fat Hen (<i>Chenopodium album</i>)	12	37.50
10	Sorrel (<i>Rumex acetosa</i>)	4	12.50
11	<i>Kochia scoparia</i>	10	31.25
12	Russian thistle (<i>Salsola kali</i>)	11	34.38
13	Ash (<i>Fraxinus excelsior</i>)	2	6.25

Table 2 Specific anti-fungal IgE level in serum and tissue of AFS compared to non-AFS sinonasal polyposis patients

	No (%)	Positive prick test	Total IgE (KUI/mL)	Anti-fungal-specific IgE		<i>P</i> value
				Serum (KUI/L)	Tissue (KUI/L)	
AFS	12 (8.51 %)	10 (83.33 %)	520.14	0.33	0.92	<0.05
None AFS	129 (91.49 %)	22 (17.05 %)	207.45	0.1	0.6	<0.05
<i>P</i> value		<0.05	<0.05	<0.05	<0.05	

AFS allergic fungal rhinosinusitis

etiology of NP is still controversial. The aim of this study was to determine whether a correlation exists between the presence of fungus-specific IgE antibodies and the development of NP.

Twelve (8.51 %) patients were categorized in the AFRS group according to Bent and Kuhn diagnostic criteria [14] whereas 129 (91.49 %) cases formed the chronic hyperplastic sinusitis group. In the available literature the prevalence of AFRS in the CRS population ranges from 5 to 51 % [20, 21]. Allergic fungal sinusitis (AFS) is a type I hypersensitivity reaction to fungal antigens in which patients usually present with nasal polyps [22]. IgE antibodies are a key factor in this hypersensitivity disease [23] and their detection by skin tests or by in vitro experiments reveals atopic sensitization.

The prevalence of allergic sensitization in patients with NP varies between 10 and 96.5 % in various studies and is determined by skin tests or specific IgE levels. In our recent study on 127 patients with NP, the prevalence of AFS was reported to be 9.45 % in the north east of Iran [24].

In the present study, SPT was positive for at least one allergen in 32 cases (22.7 %); 83.3 % of AFRS patients had a positive prick test while this figure was just 17.0 % among the non-AFRS cases. Ponikau et al. [17] conducted a prospective study on 210 patients with NP and a control group. They performed SPTs with 18 fungal allergens in both groups and found 25 % of the study group to exhibit a positive response to at least one allergen.

Another approach in order to investigate allergy is to detect the IgE antibodies directly in the nasal mucosa. The possibility of local production of allergen-specific IgE antibodies was first brought up in 1975 [25] and confirmed by Fokkens [26]. The difference between total IgE levels in patients with AFRS and the non-AFRS group was significant in the current study which confirms the atopic or allergic nature of AFRS patients. But being atopic or not, could not exclude local IgE production or local reaction to specific allergens as stated by Kim et al. [27]. So the specific IgE quantification was performed and considering the cutoff point of 0.4 KIU/L for specific anti-fungal IgE level as positive, we found that serum-specific anti-fungal IgE level shows insignificant difference among even the

AFRS patients (2 % of all patients, all from the AFRS group), although the difference in IgE level between the AFRS and non-AFRS group was still meaningful.

This finding is compatible with previous studies such as Collins et al. [28] who hypothesized that AFRS is the result of a local, not systemic, hypersensitivity reaction. This study, which was based on finding fungus-specific IgE in the mucin of AFRS as well as non-AFRS patients, suggested evidence for a local type I hypersensitivity response which could be entirely localized to the nose and paranasal sinuses without signs of systemic involvement.

In the study by Chang and colleagues [29], the AFS group showed a high positive serum IgE response to mites and house dust (14/14), but none had a positive serum IgE response to *Aspergillus* (0/14). Manning and Holman [30] reported that 66.6 % of patients in the AFS group were tested positive for *Bipolaris*-specific IgE by RAST and 88.9 % had a positive test for IgG by ELISA. In contrast, Schubert reported 67 cases with AFS, having elevated total serum IgE at diagnosis but with no elevation of serum fungal-specific IgE [31].

In the present study, no other specific antibodies were included. Moreover, the anatomical or histopathological site of local IgE was not classified, in contrast to the study by Ahn et al. [32] which found more fungal and non-fungal-specific IgE in the inferior turbinate of AFRS patients in comparison to controls or chronic rhinosinusitis patients. Wise et al. [33] also detected more fungal-specific and even non-fungal-specific IgE in sinus mucosal subepithelium rather than epithelium of AFRS patients.

In our study 58.3 % of all AFRS patients had a high serum-specific IgE level which accounted for 4.9 % of all patients. The question which rises here is how this could happen. The possible explanation is that the concentration of specific IgE to fungi may be too low to be detected in serum. The reason why we obtained positive tissue sIgE CAP results in 42.7 % of AFRS patients may be due to the higher concentrations. Secondly, we chose to detect only a few kinds of sIgE in our study; it may be possible that we failed to detect other kinds of fungal-specific IgE in the participants.

As previously mentioned, eosinophilic inflammation—which was not assessed in our study—could be the

underlying factor for local reaction in AFRS patients. In a recent study by Matsuwaki et al. [34] eosinophilic cationic protein was used as an indicator of eosinophilic reaction in the tissue and revealed that local total IgE and antigen-specific IgE including fungi in NP are related to the local eosinophilic inflammation in AFRS and CRS patients.

It was noticed that serum IgE tests do not always show a good correlation between AFRS and allergic reactions. In previous studies, type I allergy was defined as either a positive skin test or the detection of specific IgE in the serum [30, 31, 35]. However, Shatkin et al. [36] showed that this definition omitted cases in which IgE is produced locally in the sinonasal mucosa, so remaining undetectable in serum or by skin tests. In order to find a better and more reliable method for confirming the diagnosis of AFS, our study was designed to detect tissue-specific IgE to common fungal agents using the CAP system. By applying this method, we found that 43.97 % of all patients have a significant increase in their fungal-specific IgE on polyp homogenates and among patients with AFRS diagnosis this number reaches 100 %.

IgE antibody could be locally produced in the nasal polyp tissue while not detectable in the serum. It seems that local reaction to fungi is more common than it could be found in the serum of patients. These findings may prove a greater interaction between fungal antigens and the sinonasal tissue and may lead to emerging treatments based on immunologic responses as performed by Dolleman and colleagues [37] regarding immunotherapy in AFRS.

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Conflict of interest None.

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