

Original Paper

Allergogenic Profile in Patients with Different Subtypes of Chronic Rhinosinusitis with Nasal Polyps

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Key Words

Chronic rhinosinusitis with nasal polyps · Eosinophilic nasal polyps · Noneosinophilic nasal polyps · Eosinophils · Allergy-specific immunoglobulin E

Abstract

Aims: The aim of this study was to analyze the relationship between allergy and different subtypes of chronic rhinosinusitis with nasal polyps (CRSwNP). **Methods:** A total of 83 patients with CRSwNP and 20 patients with nasal septum deviation (NSD) as controls were enrolled in our study. Nasal tissue was obtained from all subjects during surgery. Hematoxylin and eosin staining was performed and the CRSwNP cases were classified into eosinophilic nasal polyps (ENP) and noneosinophilic nasal polyps (nENP) cases according to the percent of eosinophils. The Allergy Screen test was used to detect total and specific immunoglobulin E (IgE) against 5 kinds of common inhalant allergens. **Results:** There were 28 (33.73%) patients with ENP and 55 with nENP. Total IgE levels were significantly increased in ENP compared with nENP patients. The IgE level was significantly correlated with the eosinophil count. According to the 5 kinds of common inhalant allergens, only ENP patients exhibited higher sensitivity to dust mites. **Conclusion:** Patients with ENP showed significantly higher IgE levels when compared with patients with nENP based on allergy.

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Introduction

Chronic rhinosinusitis (CRS) is one of the most common chronic heterogeneous diseases in adults, which results from local inflammation in the upper airways and sinuses [1–4]. It can be classified into 2 types based on the presence of nasal polyps (NPs), including CRS with

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nasal polyps (CRSwNP) and CRS without nasal polyps [5]. Patients with NPs always showed extensive bilateral mucosal thickening and chronic inflammation of the nasal mucosa [6]. Besides, intense eosinophilic infiltration was found in most NP patients, and it was a major pathological hallmark for CRSwNP [7–9]. However, not all NPs are eosinophilic infiltration; more than 50% of CRSwNP patients showed noneosinophilic inflammation [10]. Previous studies reported that there were obvious differences in clinical manifestations between eosinophilic and noneosinophilic inflammation [10–12]. Therefore, different managements based on the phenotyping of CRSwNP [eosinophilic nasal polyps (ENP) and noneosinophilic nasal polyps (nENP)] are necessary to ultimately improve the outcome [7, 13].

It is considered that several factors are involved in the etiology of CRS, such as allergy, bacterial and fungal infections, and structural abnormalities. Allergy was considered to play a key role in the pathogenesis of rhinosinusitis [14–16]. A previous study indicated that there was a 25–75% concordance between allergy and rhinosinusitis [17]. Patients with chronic rhinosinusitis showed increasing prevalence of allergy, and higher levels of eosinophils were exhibited in the sinus aspirates of allergic individuals [18]. Previous evidence showed that allergy might be a predisposing cause of chronic sinusitis and that it increased the severity of CRSwNP [19]. After endoscopic sinus surgery, the allergic patients with CRSwNP are always related with more severe symptoms and high rates of recurrence compared with nonallergic patients [20, 21]. However, the effects of the allergic reaction in different subtypes of CRSwNP are still unclear.

In this study, we analyzed the allergic reactions in ENP and nENP CRSwNP cases. The allergen-specific immunoglobulin E (IgE) of 5 common inhalant allergens was detected, including tree (humulus, pinus, salix), grass (hung ling wood, *Urtica dioica*, *Artemisia argyi*, ragweed), fungi (*Penicillium notatum*, *Aspergillus fumigatus*, branching neurospora), dog and cat epithelium. The purpose of this work was to explore the relationship between allergy and different subtypes of CRSwNP. We expected that our findings could provide a new perspective for CRSwNP treatment.

Methods

Subjects

This study was approved by the Ethics Committee of the Affiliated Hospital of the Medical School of Ningbo University, Ningbo, China. All subjects or their parents provided informed consent before enrollment in the study.

Between January 1, 2010 and June 30, 2013, patients with CRSwNP and controls were recruited from the Affiliated Hospital of the Medical school of Ningbo University. All the patients were examined by nasal speculum. CRSwNP in patients was diagnosed according to the criteria of a European position paper on rhinosinusitis and nasal polyps from the European Academy of Allergology, Clinical Immunology, and US guidelines [22–24]. The inclusion criteria for CRSwNP patients contained the presentation of ≥ 2 symptoms including congestion, anterior/posterior drip, facial pain/pressure, reduction or loss of smell for at least 3 months, mucopurulent discharge from middle meatus, edema/mucosal obstruction primarily in the middle meatus, and mucosal changes within the ostiomeatal complex and/or sinuses. Patients who suffered antrochoanal polyps, cystic fibrosis, fungal sinusitis (mycetoma, allergic fungal sinusitis), and primary ciliary dyskinesia were excluded from this study.

Finally, a total of 83 CRSwNP patients including 49 males and 34 females were included in this study, and 20 cases with nasal septum deviation (NSD) were included as controls. The mean age of the CRSwNP patients was 34.9 years, ranging from 14 to 65 years. Among all CRSwNP patients, 28 (33.73%) were classified into the ENP group and 55 (66.26%) belonged to the nENP group according to the percent of eosinophils in tissues.

The atopic status of all subjects was evaluated by serum allergen-specific IgE detection. Oral glucocorticoid and intranasal steroid sprays were discontinued at least 3 months and 1 month before the test, respectively. All subjects failed to respond to medical therapy and underwent endoscopic sinus surgery. The demographic characteristics and test outcomes of the patients are displayed in table 1.

Table 1. Patient classification and testing data in the serum allergen-specific IgE detection

	ENPs (n = 28)		nENPs (n = 55)		NSDs (n = 20)	
	serum	local	serum	local	serum	local
Total IgE, IU/ml	239.79±65.13* [#]	310.82±90.68* [#]	141.87±47.87 [#]	190.04±81.16	74.55±34.68	109.50±52.48
Eosinophil count, 10 ⁹ /l	0.74±0.23* [#]		0.24±0.10 [#]		0.16±0.07	
Special IgE, IU/ml						
Dust mites	0.54±0.10* [#]		0.29±0.16		0.19±0.11	
Tree	0.01±0.01		0.01±0.01		0.02±0.01	
Grass	0.01±0.01		0.01±0.01		0.02±0.01	
Dog and cat epithelium	0.03±0.01		0.02±0.01		0.01±0.01	
Fungi	0.01±0.01		0.01±0.01		0.01±0.01	

* p < 0.05 vs. nENPs; [#] p < 0.05 vs. NSDs.

Hematoxylin and Eosin Staining

Nasal tissue was obtained from all the subjects during surgery. NP tissues (about 0.5 g) were collected from the apex region of polyps in CRSwNP patients, and the nonpolyp tissues (about 0.5 g) were isolated from inferior turbinates in controls. Part of each specimen was fixed in 10% formalin and embedded in paraffin. Serial sections (4 µm) were cut and dried at 60°C overnight. After dewaxing by dimethylbenzene, the sections were stained with hematoxylin and eosin (HE). The number of eosinophils was counted under microscope (×400 magnification) in 5 fields of the subepithelial area. All CRSwNP cases were classified into ENP and nENP cases according to the percent of eosinophils in tissues [7]; 10% were calculated as the cutoff value.

Detection of Total and Allergen-Specific IgE

The Allergy Screen test (Mediawiss Analytic GmbH, Moers, Germany) and solid-phase immunoassay (Pharmacia CAP system) were used for detecting the total and specific IgE against 5 kinds of common inhalant allergens (ALK Abello, Horsholm, Denmark), including tree (humulus, pinus, salix), grass (hung ling wood, *U. dioica*, *A. argyi*, ragweed), fungi (*P. notatum*, *A. fumigatu*, branching neurospora), dog and cat epithelium according to the manufacturer's instruction.

The sera and tissue samples were collected from all patients included in this paper. The freshly obtained tissue specimens were immersed in 0.9% NaCl solution and were then homogenized with a mechanical homogenizer at 1,000 rpm for 5 min on ice [25, 26]. The supernatants were separated by centrifugation at 4,000 rpm for 10 min at 4°C.

Statistical Analysis

Data were summarized and displayed as means ± standard deviations (SD). All statistical analyses were performed using Microsoft Office Excel 2007 and SPSS 16.0. When the data exhibited regular distribution and equal dispersion in each group, significance in more than 2 variables was analyzed by one-way ANOVA with repeated measures and Tukey's honestly significant difference test. Otherwise, the Kruskal-Wallis and Steel-Dwass tests were used. Spearman's rank correlation coefficient was applied to evaluate the relationship between IgE (serum and local) level and eosinophil count. p < 0.05 was considered to be significantly different.

Results

Eosinophil Observation

As shown in figure 1, almost all of the infiltrating cells in the ENP group are eosinophils, whereas most of the infiltrating cells in the nENP group and controls are lymphocytes. The eosinophil level in nasal tissue was $0.74 \pm 0.23 \times 10^9$, $0.24 \pm 0.10 \times 10^9$ and $0.16 \pm 0.07 \times 10^9$, in the ENP, nENP and NSD groups, respectively.

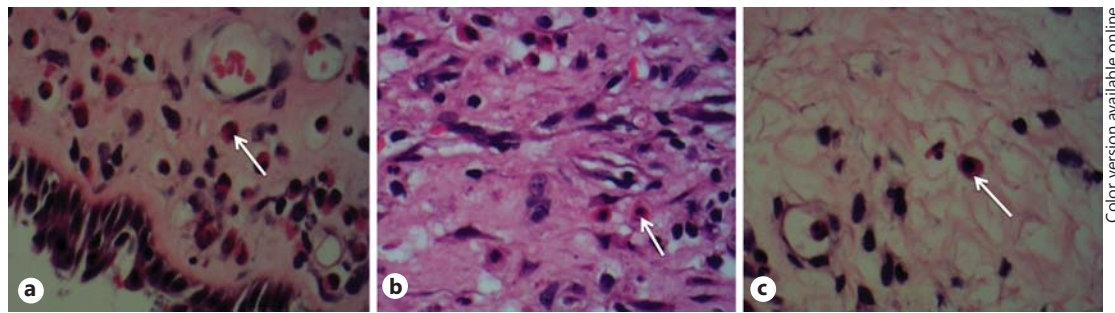


Fig. 1. HE staining of NP sections (×400). **a** Chronic rhinosinusitis with ENP. **b** Chronic rhinosinusitis with nENP. **c** NSD. Arrows represent eosinophilia.

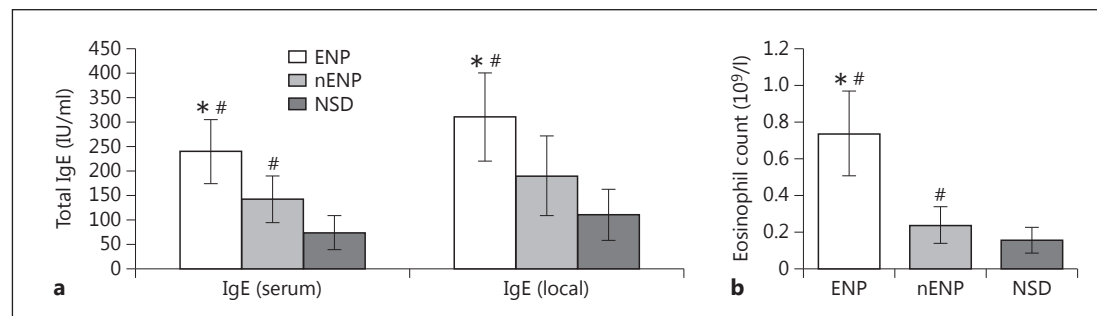


Fig. 2. Comparisons of IgE and peripheral eosinophil count among chronic rhinosinusitis with ENP, chronic rhinosinusitis with nENP and NSD. **a** Total IgE (IU/ml). **b** Eosinophil count (10⁹/l). * $p < 0.05$ vs. nENPs; # $p < 0.05$ vs. NSDs.

Total and Allergen-Specific IgE Levels

Total IgE levels in both the serum and tissue were significantly increased in ENP patients (239.79 ± 123.13 ; 310.82 ± 120.68 IU/ml) compared with nENP (141.87 ± 127.87 ; 190.04 ± 141.16 IU/ml) and NSD cases (control; 74.55 ± 94.68 ; 109.50 ± 112.48 IU/ml). A significant difference was observed in the serum IgE level between the nENP (141.87 ± 127.87 IU/ml) and the control group (74.55 ± 94.68 IU/ml) ($p < 0.05$). However, there was no significant difference in the total IgE level of tissues in nENP patients (190.04 ± 141.16 IU/ml) compared with NSD cases (controls; 109.50 ± 112.48 IU/ml). Besides, the IgE level was significantly correlated with the eosinophil count in ENP ($r = 0.655$) and nENP subjects ($r = 0.833$) (fig. 2). According to the 5 kinds of common inhalant allergens, the ENP patients exhibited higher sensitivity to dust mites and fungi compared with the nENP and NSD (control) groups. There was no significant difference among the other indicators (table 1).

Discussion

CRSwNP, known as a mucous membrane inflammation, can be classified into ENP and nENP based on the eosinophil count. CRSwNP is a multifactorial inflammatory disorder that may be caused by allergy, bacterial infection and fungus. However, the definite causes for CRSwNP are poorly understood [27]. Although the allergic cause for NPs has been presumed

since the early 1930s [28], this hypothesis still remains controversial at present. In this study, the relationship of allergy and different subgroups of CRSwNP (ENP and nENP) was investigated.

NP is mainly formed by infiltration in inflammatory cells (especially in eosinophils), which are filled with plasma proteins (especially albumin). It usually exhibits edematous masses in and around the middle nasal meatus or paranasal sinuses [26]. Patients with NPs always show extensive bilateral mucosal thickening and chronic inflammation of the nasal mucosa [6]. NP is a health concern all over the world. In this work, HE staining for nasal tissues showed that almost all of the infiltrating cells in the ENP patients were eosinophils, whereas most of the infiltrating cells in nENP patients and controls were lymphocytes (fig. 1). This was consistent with the formation mechanism of NP described in a previous study [26]. However, not all of the NPs are eosinophil dominated, and the allergy phenomenon is found in 10–96.5% of the NP patients in the Western countries and in about 81.7% of those in the Eastern countries [29]. We speculated that the allergenic profile might be different in ENP and nENP patients.

Allergy is detected by the skin prick test *in vivo* and by serum sIgE detection *in vitro* [30–32]. The skin prick test is inclined to be used in medication guides in clinical practice, such as for antihistamines and glucocorticoids. Serum sIgE detection has a higher specificity and is less affected by drugs and the age of patients [31]. Therefore, we analyzed the IgE level to investigate the allergenic profile in CRSwNP patients. The data of total and allergen-specific IgE levels showed that ENP patients had significantly higher serum and local total IgE levels compared with nENP patients and controls; meanwhile, the peripheral eosinophil count correlated significantly with local and serum total IgE levels in the ENP and nENP groups. However, the local total IgE level was not significantly increased in nENP patients compared with the controls, which suggested that total IgE in the tissues was directly related to the local eosinophil count, regardless of the presence of chronic sinus disease. Similar results were found in previous studies [28, 33, 34]. As reported, the level of total IgE was significantly higher in NP tissue compared with nonpolyp tissue, and total IgE was significantly correlated to the number of eosinophils in NPs [35]. This indicated that the patients with ENP were more likely to be sensitive to eosinophilic inflammation. As outlined in a previous study [36], eosinophils are associated with allergy. The allergen induces an increase in eosinophil cationic protein from eosinophils [37]. Fecal eosinophil cationic protein was reported to be an indicator of food allergy shown as intestinal inflammation in children [38], and eosinophil cationic protein concentration was associated with the allergic disease [39]. Therefore, we speculated that patients with ENP tended to be sensitive to allergy; however, the detailed relationship between ENP and allergy sensitization should be studied further. As outlined in a previous study [40], the high levels of IgE in nasal tissues may result in a greatly enhanced expression of high-affinity IgE receptors (FcεR1) on the surface of mast cells and basophils. The sensitization to various allergens in a substantial proportion of patients may act as a potential trigger for inflammatory mechanisms and the elevated IgE concentrations are related to the eosinophilic inflammation in NP tissues [28, 35]. It is reported that IgE may play a key role in the pathogenesis of NP and the chronic inflammation induced by constant exposure to perennial allergen may be a possible mechanism in the formation of NP [41]. Although the incidence of ENP in CRSwNP was less than 40%, a nonideal treatment effect and a strong tendency for recurrence were always found in ENP compared with nENP patients [42]. Verbruggen et al. [43] reported that the therapy of anti-IgE in combination with a specific immunotherapy may be an effective treatment for allergic rhinitis and NPs.

In conclusion, the patients with ENP showed higher IgE levels and were more likely to be sensitive to allergy. The elevated levels of total IgE and specific IgE in ENPs were closely associated with eosinophilic inflammation in NPs. Thus, the patients with ENP were more likely to be sensitive to eosinophilic inflammation. Treatment based on the features of CRSwNP (ENP and nENP) is necessary to improve the outcomes.

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Disclosure Statement

The authors declare that there are no conflicts of interest.

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