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Delayed type of allergic skin reaction to *Candida albicans* in eosinophilic rhinosinusitis cases

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ABSTRACT

Objective: Eosinophilic chronic rhinosinusitis (ECRS) is frequently complicated by asthma, and recognized as refractory and persistent rhinosinusitis. However, the detailed pathophysiology of ECRS has not been elucidated yet. In this study, we investigated the association between recurrent ECRS and intradermal testing to multi-antigens including *Candida albicans*.
Methods: The subjects were 49 cases of bilateral chronic rhinosinusitis including 24 ECRS cases. They underwent endoscopic sinus surgery and submitted to pathological examination. Prior to surgery, peripheral blood eosinophil count, total and antigen-specific IgE levels (11 categories), and intradermal tests (5 categories) were carried out in all patients. These patients were followed-up for longer than 3 months. We compared the results of preoperative and postoperative clinical examination data between ECRS and non-ECRS (NECRS) cases.
Results: Positive reaction of the delayed type of intradermal testing to *C. albicans* was significantly more often observed in ECRS than NECRS cases. ($P < 0.01$) Additionally, these positive reaction cases exhibited significantly higher recurrence of nasal polyps and symptoms of ECRS ($P < 0.05$).
Conclusion: These results suggest the involvement of (Coombs) type IV allergic reaction to *C. albicans* in the pathophysiology of ECRS.

1. Introduction

Chronic rhinosinusitis with nasal polyps (CRS wNP) [1,2] and the marked infiltration of eosinophils in the paranasal mucosa is termed eosinophilic chronic rhinosinusitis (ECRS) [3–8]. It is frequently complicated by asthma, and recognized as refractory rhinosinusitis that may recur. Recently, diagnostic criteria were proposed through the JESREC study [4], but the detailed mechanism remains to be clarified. Currently, only the systemic administration of oral steroids is effective. For treatment, endoscopic sinus surgery (ESS) and postoperative oral steroid therapy are performed.

Nonatopic eosinophilic inflammation of the airway may be involved in the pathogenesis of ECRS [5,7]. Concerning the detailed mechanism, the involvement of arachidonic acid metabolism disorder [8], Staphylococcus aureus enterotoxin (SAE) superantigen [9], and fungus [10–13] have been reported. These factors may influence the innate/acquired immune systems, causing eosinophilic inflammation and refractory recurrent conditions.

Asthma, which is a frequent complication, has been reported to be related to *Candida albicans* [14–16]. It has been reported that *C. albicans* stimulates the production of IL-5 by helper T-cells (Th-cells) of asthmatic patients, inducing eosinophilic inflammation [17], and that eosinophilic inflammation and allergic airway inflammation are exacerbated by the proliferation of fungi in the intestinal flora, particularly, *C. albicans*

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[18]. The proliferation of *C.* albicans in the intestinal flora may exacerbate allergy through an increase in the blood prostaglandin E2 level, and increase the level of macrophage migration-inhibiting factor (MIF), which induces delayed allergic responses, through the activation of macrophages (an increase in the number of M2 macrophages) [18–24].

In this study, we investigated the association between recurrent ECRS diagnosed based on the diagnostic criteria established in the JESREC study and (immediate, delayed) intradermal test responses to *C. albicans.* We performed this study to consider delayed hypersensitivity reaction-enhancing effects of *C. albicans* in patients with ECRS.

2. Subjects and methods

Of patients who underwent ESS in the Department of Otorhinolaryngology, Nippon Medical School Musashi Kosugi Hospital between August 2012 and July 2015, the subjects were 49 with bilateral chronic rhinosinusitis, excluding those with unilateral rhinosinusitis, inflammation of the maxillary sinus alone, such as odontogenic maxillary sinusitis, a single paranasal lesion, cystic diseases, choanal polyps, or fungal sinusitis and those who had received steroids before surgery.

Allergic fungal rhinosinusitis (AFRS) cases were not include in this study [25].

1) Pathological diagnosis, definitive diagnosis of ECRS

A specimen of the ethmoid sinus mucosa was collected during surgery, and submitted for pathological examination. According to the criteria established in the JESREC study, patients with a tissue eosinophil count of ≥70 at a 400-fold magnification were definitively diagnosed with ECRS [4]. Based on the results of pathological examination, ECRS was differentiated from non-ECRS (NECRS). The background of ECRS and NECRS patients (n = 24 and 25, respectively) is shown in Table 1. There were no differences in the age or sex between the two groups.

2) Preoperative examination items

Prior to surgery, the following items were investigated in all patients:

1. Peripheral blood eosinophil count, total IgE level, and antigen-specific IgE level

The method of the IgE test were used an Oriton IgE kit® (Nippon Chemiphar, JAPAN). Examination items of antigen-specific IgE were House dust, *Dermatophagoides pteronyssinus*, Cedar, white cedar, alder, *Ambrosia*, *Dactylis glomerata*, cat and dog skin, *C. albicans*, and *Aspergillus.*

2. Nasal discharge eosinophil count

Nasal discharge samples were taken from the middle nasal meatus by cotton swab. Eosinophil in nasal discharge were checked by smear stained by Wrigh's stain solution and Giemsa's stain solution (Muto Pure Chemicals Co., Ltd. Japan).

3. Intradermal test

The method of the intradermal test is as follows. Examination items were House dust, Cedar, *C. albicans*, *Alternaria*, *Aspergillus* and Control which was negative control (Torii Pharmaceutical Co., Ltd. Japan). The control consisted water solution including 0.9%NaCl and 0.5% phenol. The other allergens diluted 10,000 times. The items were subcutaneously injected 0.02 ml on the anterior surface of the forearm. Immediate reactions were evaluated 15 min after intradermal test, and delayed reactions after 48 h. Patients with a flare diameter of ≥20 mm or an urticarial lesion diameter of ≥9 mm were regarded as showing positive reactions [26].

4. Culture tests

In 19 of the 49 subjects, culture tests of maxillary/ethmoid sinus fluid and mucosa with inflammation were conducted. Culture test was performed more than a week to detect fungi by using these mediums, Pourmedia Sheep Blood Agar M58, Pourmedia Modified DRIGALSKI Aga, Candia GS Agar, Sabouraud Dextrose Agar (Eiken Chemical Co. Ltd. Japan).

For all cases of 49, pathological examination was conducted to detect fungi as well.

3) Postoperative evaluation of recurrence

Regular examinations were conducted at the outpatient clinic of our hospital for more than 3 months after surgery. Refractory rhinosinusitis induces topical mucosal thickening even after ESS, leading to recurrent polyps. In patients with refractory ECRS, polyps may recur after ESS, transiently subsiding through the use of oral/nasal steroids. However, the discontinuation of steroid therapy may result in recurrence, requiring additional steroid therapy or making withdrawal from steroids difficult in some patients. Therefore, patients who received the systemic administration of steroids during postoperative follow-up were regarded as showing an unfavorable course.

4) Comparison and analysis

We compared the results of preoperative examinations and postoperative follow-up between the ECRS and NECRS groups. For statistical analysis, the Mann–Whitney U-test, Fisher's exact probability, and Yates' chi square test were used.

Prior to this study, its protocol was approved by the Ethics Review Board of Nippon Medical School Musashi Kosugi Hospital (Permission number: 252-25-20). Furthermore, UMIN registration was conducted.

Table 1
Classification of ECRS and NECRS based on the results of pathological (N = 49).

	Number of patients	Age	Bronchial asthma	Aspirin-induced asthma	Sex (M:F)
ECRS	24	49.3 years (71-28)	9	1	13:11
NECRS	25	49.5 years (78-18)	2	0	18:7

ECRS = eosinophilic rhinosinusitis, NECRS = non-eosinophilic rhinosinusitis.

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