Postoperative evaluation of olfactory dysfunction in eosinophilic chronic rhinosinusitis-Comparison of histopathological and clinical findings–

Takashi Kashiwagi[a], Yasuhiro Tsunemi[a], Makoto Akutsu[a], Itsuo Nakajima[a], Shinichi Haruna[a]

[a] Department of Otorhinolaryngology, Dokkyo Medical University
880 Kitakobayashi Mibu-machi Tochigi 321-0293 Japan

Corresponding author: Shinichi Haruna

Tel.: +81-282-87-2164, Fax.: +81-282-86-5928
E-mail: haruna@dokkyomed.ac.jp

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Abstract

Background: Olfactory dysfunction in eosinophilic chronic rhinosinusitis (ECRS) is poorly understood.

Objective: To compare olfactory mucosal injury due to eosinophil infiltration in eosinophilic chronic rhinosinusitis with postoperative olfactory function.

Methods: Seventeen eosinophilic chronic rhinosinusitis patients (ECRS) (ECRS group) and 18 bilateral rhinosinusitis (non-ECRS group) patients were compared. At 3 and 12 months post-ESS, all patients were evaluated for subjective symptoms (nasal obstruction, nasal discharge, olfactory dysfunction), endoscopic nasal findings, CT score and T&T olfactometer recognition threshold test. The eosinophil count, OMP–positive cells and epithelial erosion in olfactory mucosa collected during ESS were compared with the postoperative olfactory function. **Results**: The non-ECRS group showed significant improvement in all clinical findings at 3 and 12 months, but the ECRS group showed worsening of the olfactory dysfunction symptoms and T&T olfactometer recognition threshold at 12 months because of recurrence of sinusitis. The groups differed significantly in the ΔT&T value (i.e., pre-ESS T&T recognition threshold – post-ESS T&T recognition threshold) at both 3 and 12 months, and the degree of olfactory improvement differed. Histologically, the ECRS group showed significantly more eosinophils, fewer OMP-positive cells and greater epithelial erosion than the non-ECRS group. **Conclusion**: Eosinophilic inflammation was thought to cause olfactory mucosal injury/dysfunction.

1. Introduction

Patients with eosinophilic chronic rhinosinusitis (ECRS) frequently complain of olfactory dysfunction [1–3]. CT imaging indicates that ECRS pathological lesions are more likely to be in the ethmoid sinus than the maxillary sinus[4]. For that reason, odor molecules entering through the nostrils are unable to reach the olfactory mucosa distributed in the olfactory cleft. In addition, following endoscopic sinus surgery (ESS) for ECRS, the olfactory cleft is opened, as a result of which odor molecules are readily able to reach the olfactory mucosa and the olfactory dysfunction shows improvement [5]. From that, it was surmised that the olfactory dysfunction of ECRS consists of conductive olfactory dysfunction [6].

Histologically, the respiratory mucosa in ECRS shows infiltration of numerous active eosinophils, epithelial cell injury, secretory cell proliferation and basal cell thickening, i.e., a picture of so-called airway remodeling [7]. A significant correlation was reported between the degree of eosinophil infiltration of the respiratory mucosa and the results of the smell identification test (SIT) [8].

The following reports have been made regarding involvement of olfactory mucosa dysfunction by eosinophil infiltration seen not only in the respiratory mucosa but also the olfactory mucosa.

1）According to histological studies using olfactory mucosa biopsy specimens, the olfactory mucosa was capable of mounting an inflammatory response similar to that seen in the respiratory mucosa of patients with chronic sinusitis. And it was reported that olfactory deficits may be due to cytotoxic proteins arising from infiltration of numerous eosinophils [9].

2）Biopsies of the olfactory mucosa in cases of CRS with olfactory dysfunction showed marked eosinophil infiltration and epithelial cell erosion [10].

3）Tissue biopsies of the superior concha of ECRS patients revealed that the eosinophilic marker CLC (Charcot-Leyden crystal) protein, IL5, eotaxin-3 and cationic protein were elevated and correlated with olfactory loss.

These findings support the hypothesis that olfactory dysfunction in ECRS correlates with local eosinophil influx into the olfactory cleft [11].

Clinically, as well, it was reported that olfactory dysfunction was not always improved even when ESS was performed for ECRS. It was surmised that the olfactory dysfunction associated with ECRS may involve not only conductive olfactory dysfunction but also sensorineural olfactory dysfunction [12].

However, those various reports cited above leave unclear whether the tissue injury due to eosinophil infiltration of the olfactory mucosa in ECRS is clinically related to olfactory dysfunction [9].

In this study, we biopsied the olfactory mucosa at the time of ESS for ECRS and compared the extent of eosinophil infiltration of the olfactory mucosa and the degree of tissue injury with the postoperative olfactory function.

2. Methods

2.1. Subjects

The study subjects consisted of 17 patients with ECRS and asthma who complained of olfactory dysfunction (11 males and 6 females; 19-39 years old (mean 34.1); ECRS group) and 18 patients who complained of olfactory dysfunction with bilateral chronic sinusitis but had no ECRS (11 males and 7 females; 18-40 years old (mean 37.1); non-ECRS group) (Table 1).

As described in the JSREC study, ECRS was diagnosed on the basis of a score of ≥11 points and a tissue eosinophil count of 70 cells/HPR [13].

The patients' age range was 18 to 40 years; patients older than 40 were excluded to eliminate age-related changes in the olfactory mucosa [14].

2.2. Endoscopic sinus surgery (ESS)

Under observation with an endoscope, anterior and posterior ethmoidectomy and sphenoidectomy were performed via the middle meatus. Then, in order to achieve sufficient communication with the maxillary sinus, the fontanel was opened as wide as possible. Ventilation and draining of the sinuses were facilitated to improve the diseased mucosa. For treatment of

the olfactory clefts, the superior meatus was completely opened to remove obstructing polyps and edematous mucosa, and to consequently enable greater intranasal airflow. Septoplasty and conchotomy of the inferior turbinate were additionally performed on 13 patients in the ECRS group and 14 patients in the non-ECRS group. All the patients underwent first-time ESS performed by the same surgeon.

2.3. Postoperative treatment

2.3.1 Postoperative treatment for ECRS

To promote epithelialization of the wound site after surgery, the patients were administered a macrolide antibiotic (clarithromycin) at 2 tablets/day for 2 weeks, followed by 1 tablet/day for about 3 months (macrolide therapy) [15]. At the same time, 1 Celestamine Combination Tablet®/day, which contains betamethasone (0.25 mg) and D-chlorpheniramine maleate (2 mg) (Merck & Co., Inc.; Tokyo, Japan), was ingested daily for one month and then discontinued. A topical steroid and a leukotriene receptor antagonist (LTRA) were used once per day, nasal irrigation was performed with saline solution, and the course was observed. If the once-improved olfactory dysfunction recurred due to a cold, etc., an antibiotic was prescribed for a maximum of one week and an oral steroid (1 Celestamine Combination Tablet®/day) for a maximum of 2 weeks.

2.3.2 Postoperative treatment for non-ECRS

The patients were administered a macrolide antibiotic (clarithromycin) at 2 tablets/day for 2 weeks, followed by 1 tablet/day for about 3 months (macrolide therapy) [15]. Nasal irrigation was performed with saline solution, and the course was observed. If the patient had a cold, an antibiotic was prescribed for a maximum of one week.

2.4. Clinical evaluation

The clinical efficacy of ESS for the two types of CRS was compared by evaluating the subjective nasal symptoms, endoscopic nasal findings, CT score and T&T olfactometer recognition threshold at 3 and 12 months after surgery.

1） Subjective nasal symptoms (i.e., nasal obstruction, nasal discharge and olfactory dysfunction) were evaluated using a VAS (visual analog scale). The VAS was a self-administered test using a linear scale, and the patients evaluated the severity of their subjective symptoms from 1 (none) on the far left to 7 (severe) on the far right.

2） Endoscopic nasal findings. The intranasal polyp score was evaluated using the following scale: 0 (no polyps), 1 (small polyps localized in the middle nasal meatus), 2 (polyps extending from the middle nasal meatus to the nasal cavity, or olfactory cleft polyps), and 3(polyps filling the nasal cavity).

3） CT score. The CT image of each sinus, i.e., the anterior and posterior ethmoid sinus, frontal sinus, sphenoid sinus and maxillary sinus, was assigned a score of 0–2 [16].

4） Evaluation of olfaction (T&T olfactometer recognition threshold test）: The T&T test consists of five odorants: (A) b-phenyl ethyl alcohol, which smells like a rose; (B) methyl cyclopentenolone, which smells like burning; (C) iso-valeric acid, which smells like sweat; (D) g-undecalactone, which smells like fruit; and (E) skatole, which smells like garbage (Takasago Industry, Tokyo, Japan). Examinations were performed by a single clinical laboratory technician to limit examiner bias. Both detection (○) and recognition (X) thresholds for each odorant were obtained and averaged. Olfactory severity was categorized into five classes according to the mean T&T recognition threshold. Patients were diagnosed as having normal olfactory acuity (normosmia), mild, moderate and severe disorder (hyposmia), and olfactory anesthesia (anosmia), when the mean T&T recognition threshold was 1.0 or less (<1.0), between 1.1 and 2.5, between 2.6 and 4.0, between 4.1 and 5.5, and 5.6 or greater (5.6<), respectively. The postoperative olfactory change was evaluated (ΔT&T= preoperative T&T recognition threshold– postoperative T&T recognition test). The patients were classified into four levels of improvement: “cure” when the mean postoperative T&T recognition threshold was 2.0 or less (＜＝2.0), “remission” when ΔT&T was 1.0 or more, but not as good as

cure, “exacerbation” when ΔT&T was -1.0 or less, and “no change” when the finding was other than those described above [5].

2.5. Comparison of histopathological findings for respiratory and olfactory mucosae

The ECRS and non-ECRS groups were compared in regard to the eosinophil count and status of epithelial cell injury in the respiratory and olfactory mucosal specimens collected during the ESS.

2.5.1. Tissue preparation and staining

Two weeks prior to ESS, in the absence of treatment with steroidal and antibacterial agents, samples of the respiratory mucosa were taken from the middle meatus. Then, during the ESS, samples of the olfactory mucosa were taken as 1- to 2-mm^3 biopsy specimens from along the upper superior turbinate.

The samples were subjected to standard tissue processing, i.e., fixation for 24 hours in 10% buffered neutral formalin solution, followed by dehydration in graded alcohol solutions. They were then embedded in paraffin blocks, serially cut into 3-μm-thick sections, and mounted on glass slides. The sections were stained with hematoxylin-eosin.

Olfactory mucosa was identified by the following:

1) The presence of nerve bundles in the lamina propria and a stratified epithelium,
2) The absence of goblet cells and a thick basement membrane, and
3) Positive staining for olfactory marker protein (OMP).

2.5.2. Immunohistochemistry

Immunohistochemistry was carried out using a three-step indirect streptavidin-biotin complex method (LSAB2 Kit, Dako Corp.; Carpenteria, CA). After deparaffinization of the sections with xylene and rehydration

with graded concentrations of ethanol and Tris-buffered saline, the manufacturer's staining protocol was followed.

The sections were incubated in bovine serum albumin to reduce nonspecific staining, followed by incubation for 1 hour at room temperature with the primary antibody (anti-OMP), which is a polyclonal goat antiserum generated against OMP whole protein (Wako; Richmond VA) and diluted 1:200 in PBS. A color reaction was developed using 3,3'-diaminobenzidine (DAB) as the chromogen, resulting in a brown-colored precipitate at the antigen site. Counterstaining was performed with Mayer's hematoxylin, and the specimen was mounted and cover-slipped with an aqueous-based mounting medium (Dako Glycergel Mounting Medium, Code No. C563).

2.5.3. Measurements

Eosinophils in the lamina propria and OMP–positive cells in the epithelium were counted in 10 randomly selected fields. All cells in the lamina propria that stained red with hematoxylin-eosin were considered to be eosinophils, while all cells in the epithelium that stained brown with OMP antibody were considered to be OMP-positive cells. The positive cell counts were expressed as mean counts per high-power field. Also, the height of the stratified epithelium of the olfactory mucosa in both ECRS and non-ECRS was measured in 10 different fields and expressed as the mean height in each group. All cell counting and measurements of the epithelial height were performed in a blinded manner by two investigators.

2.6. Statistical analysis

Data are presented as the mean value ± standard deviation (SD). Statistical significance was assessed with the nonparametric Mann-Whitey *U* test and Spearman's rank correlation using Prism (ver 5.0; GraphPad Software, Inc.; San Diego, CA, USA). A p-value of <0.05 was considered significant.

Ethics Committee

This study was performed as a retrospective, case-controlled study after being approved by the Institutional Ethics Committee of Dokkyo Medical Hospital (No. 1917).

3. Results

For both the ECRS and non-ECRS groups, the pre-ESS clinical findings showed severe subjective symptoms (nasal obstruction, nasal discharge, olfactory dysfunction), and CT scans showed bilateral pansinusitis. In addition, the polyp scores and T&T recognition threshold values were both high, with no differences between the two groups. However, the ECRS group showed a significantly higher value for eosinophils (%) (Table 1).

3.1. Comparison of histological findings for the respiratory and olfactory mucosae

The eosinophil counts in the respiratory mucosa in the ECRS and non-ECRS groups were 96.9±35.2/HPF, and 39.0±48.0/HPF, respectively, while the respective counts in the olfactory mucosa were 25±2.9/HPF and 5.0±2.0/ HPF. Both of the counts in the ECRS group were significantly higher (Table 2). In the ECRS group, the eosinophil counts in the respiratory and olfactory mucosae were significantly correlated, and eosinophil infiltration was indicated to be increased in both mucosa. However, the number of eosinophils/ HPF in the olfactory mucosa was much less than that in the respiratory mucosa (Figure 1, left). On the other hand, the number of eosinophils/ HPF in the olfactory mucosa and the eosinophil percent in the blood showed a similar increasing trend but did not show a significant correlation (Figure 1, right). Figure 2 upper pictures presents images of severe eosinophil infiltration, i.e., 129 cells in the respiratory mucosa and 26 cells in the olfactory mucosa. In both images, epithelial erosion—which means deprivation of epithelial cells, or existence of an intercellular space with partial deprivation—is seen more striking in the respiratory mucosa than in the olfactory mucosa. In the ECRS group, respiratory epithelium erosion was observed in 13/17 patients, with striking eosinophil infiltration, while olfactory epithelium erosion was seen in 6/17 patients. At the same time, the number of OMP–positive cells in the olfactory mucosa was 25±11/HPF in the ECRS group and 85±12/HPF in the non–ECRS group, with the former tending to be significantly smaller. The height of the stratified epithelium seemed to be lower in the ECRS group (53±19 μm) than in the non-ECRS group (67±14 μm)(p=0.067) (Table 2, Figure 2 lower pictures).

Therefore, the ECRS group showed severe eosinophil infiltration of both the respiratory mucosa and the olfactory mucosa, and the olfactory mucosal epithelium tended to be more damaged than in the non-ECRS group.

3.2. JESREC scores and olfactory findings

JESREC scores significantly had the relationship for the T&T recognition threshold values and a tendency of relationship for the eosinophil numbers in olfactory mucosa, but no relationship for a symptom of olfactory dysfunction (Figure 3). JESREC scores might be not suitable to evaluate the olfactory function of ECRS although JESREC study was excellent to diagnose the ECRS.

3.3. Postoperative clinical findings

The clinical symptoms (nasal obstruction, nasal discharge, olfactory dysfunction) were significantly improved in the non-ECRS group at both 3 and 12 months post-ESS. The ECRS group showed similar improvement in nasal obstruction and nasal discharge at both 3 and 12 months post-ESS, and olfactory dysfunction at 3 months post-ESS; however, at 12 months post-ESS olfactory dysfunction was not found to be significantly improved compared with pre-ESS (Figure 4).

In both patient groups, the CT and polyp scores at 3 and 12 months post-ESS were significantly improved compared with pre-ESS. On the other hand, the CT and polyp scores in the ECRS group showed significant differences between 3 and 12 months post-ESS, and both scores became worse with time (Figures 5).

In the ECRS group, the T&T recognition threshold values were 3.14±0.11 at 3 months post-ESS and 4.47±0.12 after 12 months. The value at 3 months was significantly improved compared with the pre-ESS score, but the score at 12 months did not show a statistically significant difference. On the other hand, in the non-ECRS group, the T&T recognition threshold values were 2.74±0.18 at 3 months post-ESS and 2.73±0.12 after 12 months. Even at 12 months post-ESS, the improvement was statistically significant. In addition, although the T&T recognition threshold values did not differ between the two groups at 3 months post-ESS, at 12 months post-ESS the scores were significantly different (Figure 6).

The value of ΔT&T was also compared between the groups. In the ECRS group, the value was 1.62±0.16 at 3 months post-ESS and 0.44±0.17 at 12 months post-ESS. In the non–ECRS group, the respective values were 2.14±0.19 and 2.27±0.21 (Figure 7). In both groups, the difference between the values at 3 and 12 months was significant, and the degree of improvement in olfactory dysfunction differed between the groups.

3.4. Relationship between histological findings for the olfactory mucosa and postoperative olfactory function

In the ECRS group, the tissue injury seemed to occur in the olfactory mucosa obtained during the ESS because more eosinophils and epithelial erosion, few OMP-positive cells and low height of the stratified epithelium were observed. Also, the value of ΔT&T indicated a lesser degree of improvement in olfactory dysfunction, although the T&T recognition threshold value was improved at 3 months. Therefore, it was thought that eosinophilic infiltration causes olfactory dysfunction due to olfactory epithelial cell injury, as well as respiratory epithelial cell injury. On the other hand, the non-ECRS group showed little olfactory epithelial injury, and improvement of olfactory function continued until 12 months post-ESS.

4. Discussion

Patients with ECRS notice their olfactory dysfunction from the early stages of the disease, and their QOL is seriously impacted [17]. When steroid drugs are administered to suppress eosinophilic inflammation, the olfactory dysfunction is ameliorated [6]. However, it recurs when the treatment is discontinued, and steroid dependence readily develops [18].

The rate of improvement in olfactory dysfunction following ESS for CRS is about 70% [19]. The prognosis is considered to be worse for ECRS compared with non–ECRS [5].

The olfactory dysfunction of ECRS was reported to show correlations with the blood eosinophil count and the eosinophil count in the respiratory mucosa tissue [8]. Like the respiratory mucosa, the olfactory mucosa of ECRS patients shows eosinophil infiltration, and injury to the olfactory mucosa epithelium is suspected to occur [9]. However, little has been reported regarding a relationship between the symptom of olfactory

dysfunction and olfactory mucosal injury caused by eosinophilic inflammation.

In this study, olfactory mucosa specimens were collected from ECRS and non-ECRS patients during ESS, and the degree of injury that the olfactory mucosa epithelium had incurred was investigated. Subsequently, the two patient groups were compared in regard to the post-ESS improvement in the olfactory dysfunction.

In the non-ECRS group, significant improvement was seen in terms of the subjective symptoms (nasal obstruction, nasal discharge, olfactory dysfunction), CT scan, polyp score and T&T recognition threshold value even at 12 months post-ESS compared with the baseline, i.e., pre-ESS values. In contrast, in the ECRS group, the above clinical findings showed significant improvement at 3 months post-ESS, but improvement was not seen at 12 months. That is, there was relapse in the paranasal sinuses: edema developed in the paranasal sinus mucosa and olfactory cleft, and conductive olfactory dysfunction occurred. These findings of sinusitis recurrence are in agreement with those of Oka et al [5].

Histological studies revealed significantly larger numbers of eosinophils in both the respiratory mucosa and the olfactory mucosa in the ECRS group compared with the non-ECRS group. Moreover, in the ECRS group, a significant correlation was found between the eosinophil counts in the respiratory and olfactory mucosa. This indicates that there was eosinophil infiltration not only into the respiratory mucosa but also simultaneously into the olfactory mucosa. However, it is unclear why the total counts of eosinophil infiltration differed between the olfactory and respiratory mucosae. There may be a different mechanism of eosinophil infiltration from blood vessel into the tissue.

Also, olfactory mucosa epithelial cell erosion was greater in the ECRS group than in the non-ECRS group, while OMP-positive cells were significantly fewer and the height of the stratified epithelium seemed to be lower in the ECRS group. Robert et al. proposed that the olfactory mucosa was capable of mounting an inflammatory response similar to that seen in the respiratory mucosa [9]. The respiratory mucosa in ECRS was reported to show epithelial cell loss (due to cytotoxic proteins such as MBP, etc.), basement membrane thickening and increased goblet cells, and airway remodeling resembling the pathology of asthma was observed [7].

Accordingly, there is a possibility that, in the ECRS group, the olfactory epithelial injury might have been caused by cytotoxic proteins similar to in the respiratory mucosa due to eosinophilic infiltration, resulting in olfactory mucosa dysfunction (sensorineural olfactory dysfunction). For that reason, the value of ΔT&T at 3 months was significantly lower compared with the non-ECRS group, although the clinical findings had not worsened. Moreover, it was surmised that with additional development of conductive olfactory dysfunction at 12 months post-ESS, the status was mixed olfactory dysfunction.

However, there are several concerns in this study. The biopsied olfactory mucosa specimens were quite small, i.e., 1–2 mm^3, leaving the question of how representative they are of the olfactory mucosa as a whole. In addition, it was reported that olfactory cells are regenerated from residual basal cells or supporting cells, with the turnover taking 30–60 days [20]. It is unclear how epithelial damage due to eosinophilic infiltration affects regeneration of the olfactory mucosa in postoperative ECRS. Moreover, pathological changes, including an influx of lymphocytes, macrophages and neutrophils, were seen in the olfactory mucosa in chronic sinusitis and anosmia [9]. In the future, many samples of olfactory mucosa from ECRS should be collected, and other immune cells and regeneration of the olfactory mucosa of ECRS should be investigated.

Conclusion

In ECRS, eosinophils infiltrated both the respiratory mucosa and the olfactory mucosa, leading to epithelial mucosa injury due to cytotoxic proteins. At 3 months post-ESS, the degree of improvement in olfactory dysfunction in the ECRS group was less than in the non-ECRS group, and it was thought that sensorineural olfactory dysfunction developed due to eosinophil infiltration of the olfactory mucosa. At 12 months post-ESS, ECRS had relapsed, and it was surmised that additional development of conductive olfactory dysfunction resulted in a state of mixed olfactory dysfunction.

Disclosure Statement

There were no conflicts of interest in this study.

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