Measurement of Japanese Cedar Pollen-Specific IgE in Nasal Secretions

Hiroshi Sakaida¹, Sawako Masuda² and Kazuhiko Takeuchi¹

ABSTRACT
Background: Japanese cedar pollen (JCP) is the most common allergen for seasonal allergic rhinitis in Japan. Little is known about local production of immunoglobulin (Ig)E in people with or without Japanese cedar pollinosis. The aims of this study were to measure levels of JCP-specific IgE in nasal secretions and determine correlations with levels in serum.

Methods: Forty-six subjects were enrolled in this study, comprising 24 symptomatic subjects, 9 asymptomatic subjects sensitized to JCP, and 13 subjects not sensitized to JCP. Nasal secretions were obtained during a period of Japanese cedar dispersal, and levels of JCP-specific IgE were measured with CAP-fluorescent enzyme immunoassay. Serum JCP-specific IgE and total IgE were also measured using the same method.

Results: Among the 46 subjects enrolled, JCP-specific IgE in nasal secretions was measurable in 43 subjects. Irrespective of symptom development, sensitized subjects showed higher levels of JCP-specific IgE in nasal secretions than non-sensitized subjects. A significant moderate correlation was observed between JCP-specific IgE levels in nasal secretions and serum in all 43 subjects. With stratification by subject group, only symptomatic subjects showed a substantial correlation between JCP-specific IgE levels in nasal secretions and serum.

Conclusions: Our results imply a certain association between JCP-specific IgE in nasal secretions and sensitization of Japanese cedar pollinosis. Therefore, levels of allergen-specific IgE in nasal secretions can be used as an alternative diagnostic marker for allergic rhinitis patients.

KEY WORDS
allergen-specific IgE, allergic rhinitis, diagnostic marker, Japanese cedar pollinosis, nasal secretion

INTRODUCTION
Allergic rhinitis (AR) is an immunoglobulin (Ig)E-mediated inflammatory disease of the nasal mucosa resulting from exposure to an inhalant allergen. IgE plays an important role in the development of AR. One of the ways to determine the presence of sensitization to allergens is to measure allergen-specific IgE levels in serum, and this approach is used widely in daily practice. Many studies have detected IgE not only in serum, but also in nasal secretions in patients with AR,¹² showing local production of IgE in the nasal mucosa. Recent research has shown local production of IgE in patients who are negative for serum IgE, leading to the concept of local AR.³⁸ Local IgE production is thought to play a more important role than IgE in the serum in allergic inflammation.

The origin of allergen-specific IgE in nasal secretions has been addressed by many studies. In patients with AR, allergen-specific IgE is detected in nasal mucosa⁹ in addition to nasal secretions.² Moreover, somatic hypermutation and class switching to IgE have been observed in the nasal mucosa in patients with AR,¹⁰ suggesting that locally detected IgE is produced in nasal mucosa rather than a mere transudate from serum. Until recently, the major sources and sites of allergen-specific IgE production have not been fully identified. However, a recent study showed that the majority of allergen-specific IgE in peripheral blood is not derived from IgE-secreting cells in the blood, suggesting local IgE production in tissues as a major source of allergen-specific IgE.¹¹ Given such
findings, allergen-specific IgE in both nasal secretions and serum may be derived from common sites of production.

Allergens vary from one region to another. In Japan, Japanese cedar pollen (JCP) from Cryptomeria japonica represents the most common allergen for seasonal AR, affecting more than 30% of the population as Japanese celllar pollinosis, and the morbidity rate has been rising. Despite many clinical and basic investigations into Japanese celllar pollinosis, little is known about the production of IgE in individuals with or without Japanese cedar pollinosis. No standardized method for quantitative measurement of IgE in nasal secretions is clinically available. Furthermore, the correlation between concentrations of IgE in serum and in nasal secretions has not been elucidated. The aims of this study were both to measure JCP-specific IgE in nasal secretions using a clinically available method and to evaluate correlations with its serum counterpart in subjects with or without Japanese cedar pollinosis.

METHODS

STUDY SUBJECTS

A total of 46 subjects (age range, 1-78 years) with or without Japanese cedar pollinosis were recruited in the present study. These included healthy subjects as well as patients who were under treatment for various diseases in the outpatient clinic of our department. Patients with chronic rhinosinusitis were excluded from this study.

STUDY DESIGN

The study was performed from January to March 2012, during a period of JCP dispersal in Japan. Symptoms associated with allergic rhinitis and levels of JCP-specific IgE in both serum and nasal secretions were evaluated. Medication was not restricted for subjects in this study. During the study period, subjects took medications as usual for their underlying medical conditions, including Japanese cedar pollinosis.

Subjects were classified into the following 3 groups according to sensitization to JCP and presence of symptoms of Japanese cedar pollinosis: Group 1, which included symptomatic subjects who were sensitized to JCP; Group 2, which included asymptomatic subjects who were sensitized to JCP; and Group 3, which included asymptomatic subjects who were not sensitized to JCP. The presence of Japanese cedar pollinosis was defined as development of typical symptoms affecting the nose and eyes during JCP dispersal and eosinophilia in nasal secretions. Sensitization was defined as serum JCP-specific IgE levels ≥0.70 kUA/mL (IgE ImmunoCAP class ≥2).

This study was approved by the ethics committee of Mie University. Written informed consent was obtained from all subjects and parents or guardians of subjects under 20 years old.

PREPARATION OF NASAL SECRETIONS FOR SPECIFIC IgE MEASUREMENT

Nasal secretions were obtained using an ATOMS tap® (Lumenis, Tokyo, Japan). The ATOMS tap® is a device originally designed for collecting middle ear effusion for the diagnosis of otitis media. The instrument is equipped with a suction tip about 2 mm in diameter and 60 mm in length, and a bottle into which the aspirated sample is collected. The tip was inserted into the nasal cavity of subjects to aspirate nasal secretions. The obtained nasal secretions were frozen at -20°C until analysis. After thawing, the nasal secretions were diluted with 0.05 M phosphate saline buffer. Samples were centrifuged for 5 min, and supernatants were collected for measurement of IgE.

MEASUREMENT OF TOTAL AND SPECIFIC IgE TITERS OF SERUM AND NASAL SECRETIONS

Levels of JCP-specific IgE in nasal secretions and serum were measured with CAP-fluorescent enzyme immunoassay (FEIA) (Phadia, part of Thermo Fisher Scientific, Tokyo, Japan) in the same manner used in daily practice. Total IgE in serum was also measured this way.

SPIKE AND RECOVERY TESTS

Nasal secretion contains many substances including IgA and mucin, which may interfere with the assay. To validate and assess the accuracy of the assay, spike and recovery tests were performed as follows. Among the set of the samples of nasal secretions preserved, 4 samples whose concentrations of JCP-specific IgE had been determined below 0.1 U/mL were selected and thawed. The concentrations of IgE of the samples were measured and found to be identical to those measured before preservation. A human serum containing high concentration of IgE was used as a spiking solution. The spiking solution was added to each sample at 1:10 dilution. The spiking solution was also added to the standard diluent (0.05 M phosphate saline) at the same dilution. The standard dilute has been proven not to interfere with the assay. The concentrations of IgE in each spiked sample and the spiked standard diluent were determined. The recovery rate was calculated as the ratio of the value of each spiked sample to that of the spiked standard dilute.

LINEARITY OF DILUTION TESTS

Among the set of the samples of nasal secretions, a sample with high concentration of JCP specific IgE was selected. The sample was serially diluted with the standard dilute to make dilution 1:2, 1:4, 1:8, and 1:16. The concentrations of IgE in each diluted sample were measured. The recovery rate was calculated as the ratio of measured values at each dilution...
Table 1  Epidemiological and laboratory data of study subjects

<table>
<thead>
<tr>
<th>Group</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects enrolled (n)</td>
<td>24</td>
<td>9</td>
<td>13</td>
</tr>
<tr>
<td>Subjects analyzed (n)</td>
<td>23</td>
<td>8</td>
<td>12</td>
</tr>
<tr>
<td>Age</td>
<td>49 ± 16</td>
<td>34 ± 11</td>
<td>48 ± 22</td>
</tr>
<tr>
<td>Sex, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>13 (57%)</td>
<td>3 (38%)</td>
<td>5 (42%)</td>
</tr>
<tr>
<td>Female</td>
<td>10 (43%)</td>
<td>5 (62%)</td>
<td>7 (58%)</td>
</tr>
<tr>
<td>IgE</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive detection rate</td>
<td>78% †</td>
<td>63%</td>
<td>17%</td>
</tr>
<tr>
<td>JCP in nasal secretions (kUA/mL)</td>
<td>4.9 ± 9.9 †</td>
<td>0.50 ± 0.51</td>
<td>0.20 ± 0.49</td>
</tr>
<tr>
<td>JCP in serum (kUA/mL)</td>
<td>25 ± 28 †</td>
<td>9.1 ± 9.0 †</td>
<td>0.3 ± 1.3 × 10⁻⁷</td>
</tr>
<tr>
<td>Total IgE in serum (kU/mL)</td>
<td>247 ± 392</td>
<td>119 ± 93</td>
<td>79.0 ± 90.4</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± standard deviation. JCP, Japanese cedar pollen; JCP in nasal secretions, IgE specific to JCP in nasal secretion; JCP in serum, IgE specific to JCP in serum. † Significant difference between Groups 1 and 3. ‡ Significant difference between Groups 2 and 3.

to the expected values deduced based on the given respective dilution. A linear regression analysis was carried out to determine the extent of the relationship between the measured values and the expected values.

STATISTICAL ANALYSIS
Spearman’s rank correlation coefficient was used to evaluate correlation between two sets of variables. Data among the 3 groups were compared using the Kruskal-Wallis test. If a significant difference was found, post-hoc comparisons were carried out by the Mann-Whitney U test with Bonferroni’s correction. The chi-square test was used to examine differences with categorical variables. Values of P < 0.05 were considered significant. All statistical analyses were carried out using JMP version 5.1.1 software (SAS Institute, Cary, NC, USA).

RESULTS
SUBJECTS
The 46 subjects comprised 22 males and 24 females, with 24 subjects in Group 1, 9 in Group 2, and 13 in Group 3.

SUBJECT CHARACTERISTICS AND LABORATORY DATA
Table 1 shows the details of subject characteristics and all data concerning IgE. Of the 46 samples of nasal secretions obtained from 46 subjects, levels of JCP-specific IgE were successfully measured in 43. Measurement was technically impossible in remaining 3 samples due to excessively high viscosity, and these 3 subjects were subsequently eliminated from the analysis. As a result, Groups 1, 2, and 3 included 23, 8, and 12 subjects, respectively. No complications were seen in any subjects during the collection of nasal secretions. Of the 43 samples successfully measured, JCP-specific IgE was detected in 58%. Detection rates in each group were 78%, 63%, and 17% for Groups 1, 2, and 3, respectively.

Levels of JCP-specific IgE in nasal secretions were then compared among the 3 groups (Fig. 1). Group 1 showed significantly higher levels of JCP-specific IgE in nasal secretions than Group 3; however, no significant difference was observed between Groups 1 and 2. Additionally, no significant difference was observed between Groups 2 and 3. Levels of JCP-specific IgE in serum were also compared among the 3 groups. Groups 1 and 2 each showed significantly higher serum levels of JCP-specific IgE than Group 3, but no significant difference was observed between Groups 1 and 2. Additionally, no significant difference was observed in total IgE among the 3 groups (Table 1).

CORRELATIONS BETWEEN JCP-SPECIFIC IgE IN NASAL SECRETIONS AND JCP-SPECIFIC AND TOTAL IgE IN SERUM
A significant moderate association was observed between JCP-specific IgE in nasal secretions and JCP-specific IgE in serum overall for the 43 subjects (r = 0.79, P < 0.001) (Fig. 2A). When all data were stratified by subject group and analyzed separately, a significant positive correlation was observed only for Group 1 (r = 0.78, P < 0.001) (Fig. 2B). No significant correlations were seen for Groups 2 and 3 (Fig. 2C, D). A similar significant moderate association was observed between JCP-specific IgE in nasal secretions and total IgE in serum in all 43 subjects (r = 0.65, P < 0.001).

SPIKE AND RECOVERY TESTS
The recovery rates of each sample were 102%, 105%, 108%, and 109%. 

Comparison of JCP-specific IgE levels in nasal secretions among the 3 groups. Individual data are shown. Since the majority of values from the 3 groups were clumped at the bottom of the graph when data were shown on a linear scale, data are shown on a logarithmic scale to make the graph more understandable. Each dot is shown if its value is greater than zero, because zero cannot be shown on the log scale. Horizontal bars represent means for each group. *P < 0.005.

LINEARITY OF DILUTION TESTS
The recovery rates of each sample at dilution of 1 : 2, 1 : 4, 1 : 8, and 1 : 16 were 106%, 106%, 110%, and 119% respectively. Adjusted coefficient of determination was 0.999 and the equation obtained was: y = 0.159 + 1.04x, where y was the measured values and x was expected values. Coefficient correlation was 0.999 and P-value was below 0.001.

DISCUSSION
This study was intended to measure allergen-specific IgE in nasal secretions in patients with allergic rhinitis caused by JCP. It had two major findings. First, JCP-specific IgE was detected in most symptomatic subjects and in more than half of asymptomatic but sensitized subjects, and levels of JCP-specific IgE both in nasal secretions and in serum correlated significantly in symptomatic subjects. Second, nasal secretions were able to be obtained easily and safely using a simple suctioning and collection device.

JCP-specific IgE was detected in most Group 1 (symptomatic) subjects. Previous studies have detected allergen-specific IgE in nasal secretions in patients with AR induced by several aeroallergens. The high detection rate of JCP-specific IgE in nasal secretions from symptomatic patients in our study was consistent with those results. In addition, our study showed that 63% of Group 2, which represented asymptomatic but sensitized subjects, had JCP-specific IgE in nasal secretions. To the best of our knowledge, analysis of allergen-specific IgE in nasal secretions from asymptomatic sensitized subjects has not been reported previously. Furthermore, although levels were quite low, a small portion of Group 3, representing asymptomatic and non-sensitized subjects, also showed positive IgE in nasal secretions. A possible explanation for this observation in Group 3 is that JCP-specific IgE may have been present in serum at levels below the threshold of detection. Another possibility is production of JCP-specific IgE only in the nasal mucosa. However, no definite explanation for this observation can be drawn.

A significant moderate correlation between the level of allergen-specific IgE in nasal secretions and serum was observed for the overall subject population in our study. With stratification by subject group, only Group 1 showed a significant correlation, representing a novel finding in this field. The overall correlation might be attributable to the high correlation in Group 1, particularly since Group 1 included 53% of all subjects. Generally, quantitative specific IgE levels in serum have been shown to be useful in predicting the probability of AR, and specific IgE serum concentration is associated with symptom severity in children with seasonal AR. Our data imply the possible utility of measuring allergen-specific IgE in nasal secretion as an alternative diagnostic biomarker of allergen-specific IgE in serum. On the other hand, although JCP-specific IgE was detected from nasal se-
IgE in Nasal Secretions

Fig. 2 Correlation between JCP-specific IgE in nasal secretions and serum in all subjects and within each group. A) All subjects; B) Group 1; C) Group 2; D) Group 3. P values are shown. Values for the coefficient of correlation and trend lines are displayed for statistically significant correlations. In Graph D, six dots with values of 0 on the X-axis and 0.34 on the Y-axis overlapped exactly; these dots are jittered slightly for the purposes of depiction.

creations in 63% of subjects in Group 2, no significant correlation was seen between the level of JCP-specific IgE in nasal secretions and that in serum. It is possible to make a speculative hypothesis as follows on the basis of those observations: JCP-specific IgE is secreted into nasal secretions at a certain concentration if a patient is sensitized to JCP. After developing symptoms of AR, much more JCP-specific IgE is released into nasal secretions, reaching a level corresponding to the level in serum. Since our study was conducted on a practical clinical basis, detailed cellular and molecular mechanisms are beyond the reach of this study.

Second, nasal secretions were obtained easily and safely using a simple suctioning and collection device, the ATOMS tap⡴. Unlike measurement of allergen-specific IgE in serum, standard methods for collecting nasal secretions and measuring levels of allergen-specific IgE in nasal secretions have yet to be established. Several methods have been employed in the literature to detect IgE in nasal secretions. For instances, some authors have collected nasal secretions by nasal lavage,7 while others have employed a device using the allergen-coupled cellulose derivative for measurement of IgE.13,14 We used the ATOMS tap⡴ in the present study to obtain nasal secretions. The ATOMS tap⡴ is a device originally designed for collecting middle ear effusion for the diagnosis of otitis media and has received formal approval for use as a medical device in Japan. By simply connecting the unit to a conventional suction device, it is instantly ready for use. The tip is thin enough to be inserted into a congested nasal cavity or the narrow nasal cavity of a child. No prior steps are needed before collecting nasal secretions. The device enables rapid and convenient collection of nasal secretions and is applicable even for children.

We were able to obtain measurements for almost all samples, with the exception of 3 exceptionally viscous samples. The methodology used in this study is applicable to daily clinical practice. Another advantage of our method is the ability to measure allergen-specific IgE among the wide IgE repertoire offered by clinical laboratories. The CAP- immunoassay is the test designed and validated to measure allergen-specific IgE in the serum. Unlike the serum, nasal secretion contains many substances including IgA and mucin. These substances may interfere with the assay and may affect the results. Spike and recovery tests demonstrated that the assay was not affected
the matrices of nasal secretions. Linearity of dilution tests proved the linearity of the assay. We believe that these two tests validated the accuracy of the assay in our study. Currently, possible clinical implications of measuring local IgE in nasal secretions include the measurement of specific IgEs from children, from whom collection of a blood sample can be difficult. Moreover, our methodology may contribute to further investigation of local IgE by providing a useful tool to obtain nasal secretions. However, the reproducibility and repeatability of our measurement method have not been confirmed, and additional fundamental studies are needed to validate the present approach.

The significance of local IgE in AR has yet to be investigated. Practical Guideline for the Management of Allergic Rhinitis in Japan do not mention local IgE, and it is not currently used as an examination for AR. Moreover, the Allergic Rhinitis and its Impact of Asthma (ARIA) criteria issued in 2008 refers to nasal-specific IgE and states that, based on current data, the concept of local allergic reaction in the nose without systemic IgE release is not fully supported and the measurement of IgE in nasal secretions cannot be routinely proposed. However, a growing number of articles have addressed local IgE in AR. A diagnostic flow-chart to detect forms of allergy different from the common IgE-mediated hypersensitivity, and incorporating local IgE, has been proposed.

Some limitations must be considered when interpreting the present results. Measurement of allergen-specific IgE in nasal secretions and serum was carried out only once during the study period. In seasonal AR, levels of allergen-specific IgE in serum depend on the amount of allergen to which the patient has been exposed. Our result might depict only part of the phenomenon, not the entire situation. Ideally, repeated measurement of allergen-specific IgE in both serum and nasal secretions over the year seems necessary to accurately evaluate the association between levels in these different compartments. Since the number of enrolled subjects was too small to reach a definitive conclusion, larger-scale studies are needed to confirm our result.

In conclusion, we measured JCP-specific IgE in the nasal secretions of subjects with and without Japanese cedar pollinosis using a novel methodology. A significantly high correlation was observed between levels of IgE in nasal secretions and in serum in symptomatic subjects. Although the current understanding of local IgE is insufficient and much remains to be studied, our data might offer some contributions and clinical implications regarding local IgE in AR.

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