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Quantitative Analysis of Antigen Specific IgE in Tears in Comparison to Serum Samples

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SUMMARY We determined pollen specific IgE in tears and compared these results to the concentration of specific IgE in serum samples. We obtained tears (using Schirmer strips) and serum samples from subjects with Japanese cedar (*Cryptomeria japonica*) pollinosis, and tested for *C. japonica* pollen specific IgE using a quantitative ELISA. Time kinetic analyses through the pollen season showed that specific IgE levels in tears were found to increase earlier than those in sera and reached their maximum at the end of or after the pollen season, from March to early June. In the *C. japonica* pollen free season, July to December, the specific IgE levels in tears decreased, although the serum levels remained relatively high. These results indicate that the quantitative assay for specific IgE in tears might be useful to identify specific eye allergens.

The number of allergic patients is increasing, and Japanese cedar (*Cryptomeria japonica*) pollen is one of the major allergens for pollinosis in Japan. The pollen causes allergic rhinitis, conjunctivitis, and bronchitis. The prevalence of *C. japonica* pollinosis in the Japanese population was as high as 13.1% in the year 2001 as estimated by random sampling.[1]

Assays for antigen specific IgE in serum are valuable tools for the diagnosis of allergic diseases.[2] *C. japonica* pollen specific IgE was recently reported in 96.8% of sera from *C. japonica* pollinosis patients.[3] Another report showed that specific IgE for at least one of two allergens in *C. japonica* pollen, Cry j 1 and 2, was detected in all sera from *C. japonica* pollinosis patients.[4]

On the other hand, assays for antigen specific IgE in tears are rarely used for the diagnosis of allergic diseases, although the eye is one of the target organs for pollen allergens. To our knowledge, there are only four reports presenting quantitative results on specific IgE in tears.[5-8] One report showed that Schirmer strips absorbed a sufficient volume of tears to assay for specific IgE for allergens such as Japanese cedar pollen and house dust mite.[7] Another report evaluated the level of specific IgE for staphylo-

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coccal enterotoxin in tears obtained using Schirmer strips.[8]

These results led us to analyze the correlation of *C. japonica* pollen specific IgE levels in the extract of tears obtained using Schirmer strips with those in the serum. We recorded clinical symptoms and the pollen density in the air throughout the pollen season. Our results revealed that the specific IgE levels in tears increased just before the increase in the serum levels and also before the appearance of clinical eye symptoms. The difference in specific IgE levels between the pollen season and the pollen free season was higher in tears than in the serum. These results indicate that quantitative evaluation of pollen specific IgE in tears is useful to identify eye allergens.

MATERIALS AND METHODS

C. japonica pollen count

The *C. japonica* pollen density was counted in the air of 8 cities of Chiba prefecture, Japan, from the beginning of January to the end of April, 2002. The pollen grains were collected using Durham pollen samplers,[9] stained by gentian violet, and counted under a light microscope as described previously.[10] The results were expressed as the number of pollen grains/cm^2/day.

Subjects

Tears and sera were obtained from 31 volunteers, 20-55 years old, with or without pollinosis symptoms. They were randomly selected from the people living in the 8 cities where the *C. japonica* pollen were counted. Some of these individuals had received medical treatment. Tear and serum samples were obtained from each individual on the same day in all experiments. Informed consent was obtained from all of these individuals after the nature of the study had been fully explained.

To compare the seasonal change in *C. japonica* pollen specific IgE levels in tears to that in serum in detail, we selected 4 individuals for longitudinal studies from the volunteers mentioned above. These persons had a 3-18 years history of *C. japonica* pollinosis. They all showed pollinosis symptoms such as itchy eyes, watery eyes, sneezing, or runny nose during the *C. japonica* pollen season. However, none of them had received therapy such as desensitization or systemic steroid treatment. They agreed to provide serum and tear samples for 11 months (from January to November, 2002) almost every other week during the pollen season and at appropriate intervals after the season. Their eye and nose symptoms were graded on a scale from 0 to 3; 0 indicated no symptoms, and 1, 2 and 3 represented mild, moderate, and severe symptoms, respectively.

Preparation of tear samples

We obtained tears using Schirmer strips as described in previous reports[8] with modifications. Briefly, a Schirmer strip (Schirmer Tear Production Measuring Strip [Scaled], Menicon Co., Ltd, Nagoya, Japan) was placed for 4 minutes on the inferior fornix of each conjunctiva. After removing the strips from both eyes, the strips were dried, and the material in the 10 mm tip of the strips was extracted by incubation for 2 hours at room temperature in 120 μl of 10 mM phosphate-buffered saline (pH 7.4). The extracts were stored at -20°C until use. In our preliminary experiments, most IgE (mean ± SD, 92 ± 6%) was confirmed to be in the 10 mm tip of the strip, including the semi-circle part which directly attached to the surface of the conjunctiva, regardless of the tear volume. The tear extract is referred to as tear sample in this paper.

Assay for *C. japonica* pollen specific IgE

The *C. japonica* pollen specific IgE was assayed by AlaSTAT® system (Diagnostic Products Corporation, Los Angels, CA, USA) using the antigen preparation provided in the kit. AlaSTAT® is an enzyme-linked immunosorbent assay (ELISA) system using biotinylated antigen with reference to the WHO Second International Reference Preparation for Human Serum IgE, 75/502. The specific IgE in serum and tear samples were assayed in duplicate, and the average values were presented as U/ml. The results were converted to class scores according to the manufacturer's instructions when it was needed: class 0 (negative), < 0.35 U/ml; class 1, 0.35-0.69 U/ml; class 2, 0.70-3.49 U/ml; class 3, 3.50-17.49 U/ml and class 4-6, 17.50 or more.

Statistical analysis

The statistical significance was evaluated using Wilcoxon's signed-rank test. The correlation of the class score of the specific IgE in tears with that in the serum was evaluated by Spearman's rank order correlation coefficient test.

RESULTS

C. japonica **pollen count**

The *C. japonica* pollen densities in the air of the 8 cities described were counted for 4 months from the beginning of January to the end of April, 2002. The time kinetics of the *C. japonica* pollen count in these cities were essentially similar to each other, and Tomisato showed the highest, Funabashi the lowest, and Sakura an intermediate accumulated count. Fig. 1 shows the time kinetics of the *C. japonica* pollen counts in these representative 3 cities. More than 99% of the pollen were counted in 60 days from February 7th to April 7th in these 8 cities. We defined these 60 days as *C. japonica* pollen season in this paper.

Seasonal change in *C. japonica* **pollen specific IgE levels in tears and sera from 4 pollinosis patients**

We obtained tear samples and sera from the 4 pollinosis patients for 11 months. Fig. 2 shows the kinetics of the specific IgE levels in the tear samples and sera in relation to the pollen season together with their clinical symptoms. The *C. japonica* pollen specific IgE levels in the sera increased just after the beginning of the pollen season except for patient #1. The increase in the specific IgE level in the serum from patient #1 was delayed for 2 weeks, although his clinical symptom scores increased immediately after the beginning of the pollen season similar to the other patients. On the other hand, the levels in the tear samples from the 4 patients increased earlier than those in the sera just before the appearance of eye symptoms, although the increase was gradual. After an initial slow increase for a month or so, the specific IgE levels in the tear samples increased rapidly. The levels in the tear samples from patient #4 were higher than those in his serum from March to June.

Although the eye symptom scores decreased immediately after the pollen season, the specific IgE levels in the tear samples and the sera from these 4 patients seemed to reach their maximum sometimes between the late phase of the pollen season and 2 months after the season. In July, the specific IgE in the tear samples from 2 of the 4 patients (#1, #4) turned to be negative (< 0.35 U/ml), although the sera still showed over 5U/ml.

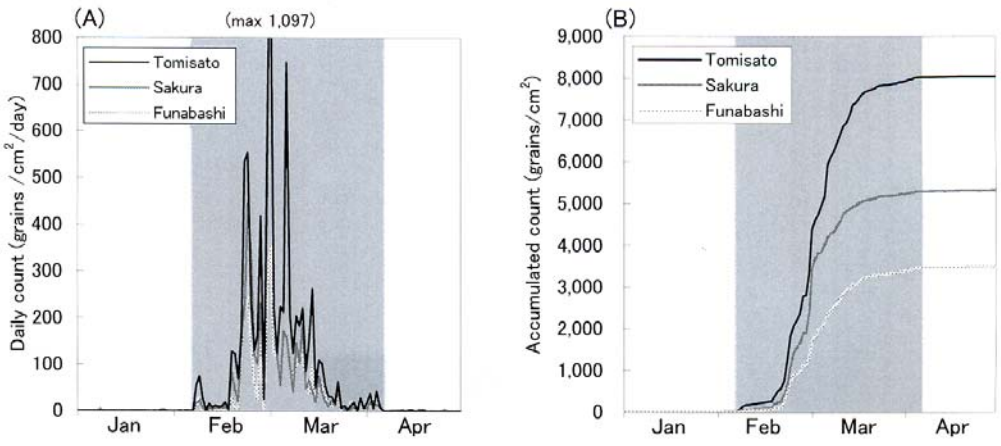

Fig. 1 Daily (A) and accumulated (B) *C. japonica* pollen counts in 3 representative cities. The gray area indicates the 60 days of the *C. japonica* pollen season (Feb. 7-Apr. 7) in which more than 99% of the pollen were counted.

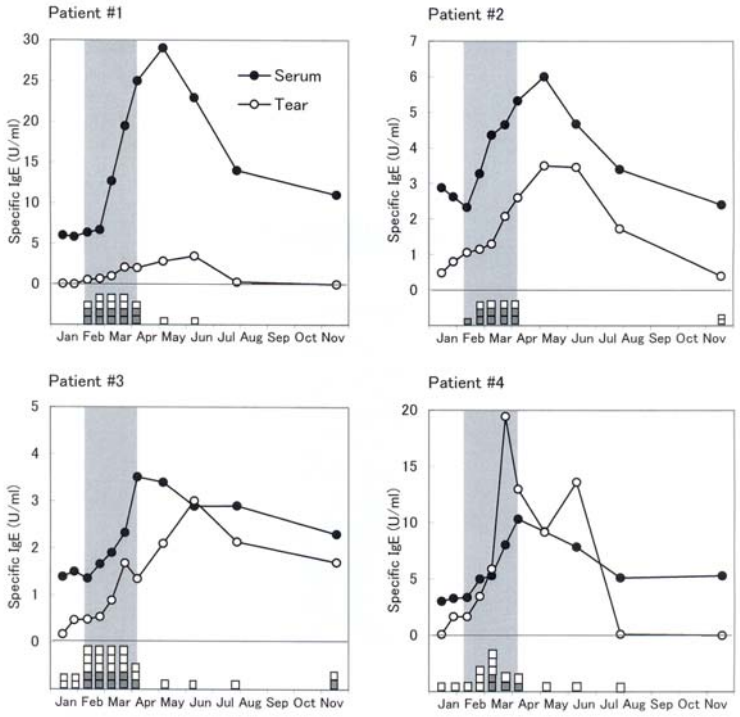

Fig. 2 Time kinetics of *C. japonica* pollen specific IgE levels in tear samples (○) and serum (●) in correlation with clinical symptoms. The numbers in the blocks indicate the symptom scores of the eyes (■) and nose (□), respectively. The gray area indicates the *C. japonica* pollen season (Feb. 7-Apr. 7).

Seasonal change of *C. japonica* pollen specific IgE levels from 31 individuals

In the experiment mentioned above, *C. japonica* pollen specific IgE levels both in tear samples and sera were shown to be relatively high from March to May, and low from July to November. To confirm the seasonal change of the specific IgE levels, we assayed tear samples and sera from 31 individuals including the above 4 patients. The samples were obtained from each individual from March to May and also from July to December 2002.

A seasonal change in the specific IgE levels was observed both in tear samples and sera ($p < 0.001$, Fig. 3). In the sera, the average showing of specific IgE levels of 7.5 U/ml from March to May decreased to 3.1 U/ml from July to December. From the 31 sera, 27 sera were positive for *C. japonica* pollen specific IgE (> 0.35 U/ml) from March-May, and only 3 from these 27 individuals turned to be negative from July-December. The average specific IgE level of these 3 sera was 0.52 U/ml from March-May, which was weakly positive. In tear samples, however, the average specific IgE level (4.3 U/ml) from March-May decreased to 0.4 U/ml from July-December. The specific IgE levels of 15 tear samples classified positive from March-May turned to be negative from July-December, and none of the 31 samples had a higher level than 2.0 U/ml from July-December. These results indicated that the seasonal change in *C. japonica* pollen specific IgE was more apparent in the tear sample than in the serum.

Correlation of *C. japonica* pollen specific IgE levels in tears and sera

We then evaluated the correlation of *C. japonica* pollen specific IgE levels in tear samples with the serum levels of the 31 individuals mentioned above. Data on specific IgE levels are commonly evaluated in terms of class scores. We, therefore, classified the quantitative data on serum and tear samples into class scores as described in Materials

and Methods. Class 0 (< 0.35 U/ml) means negative in specific IgE up to 6 which is the highest grade containing more than 100 U/ml. The class scores of the specific IgE levels in both samples from March-May correlated well to each other (Fig. 4A, Spearman's rank correlation coefficient was 0.75, the concordance rate was 97%). However, from July-December, the specific IgE levels in all tear samples were classified into class 2 or lower although those in 9 of the 31 sera were classified into class 3, and the specific IgE levels in tear samples and sera did not correlate to each other (Fig. 4B, Spearman's rank correlation coefficient was 0.37, and the concordance rate was 58%). These results indicate that CJ pollen specific IgE levels in tear samples decreased more markedly than those in sera during the pollen free season.

DISCUSSION

Since antigen specific IgE was first detected in tears,[5-11] there were several reports suggesting that the detection of antigen specific IgE in tears is useful for the diagnosis of allergic diseases with eye symptoms.[7,8,12-14] Many reports examining specific IgE in tears obtained the tears using micro-capillary devices, and assayed qualitatively.[14-18] Two recent reports[7,8] obtained the tears using Schirmer strips, and suggested a clinical application of their method. We assayed *C. japonica* pollen specific IgE in tears obtained using Schirmer strips throughout the pollen season, and evaluated the results in comparison with

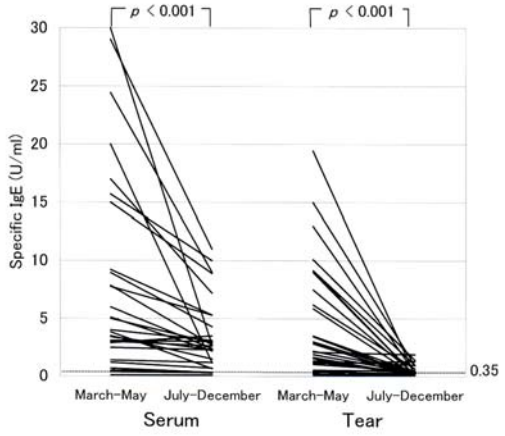

Fig. 3 Seasonal changes of *C. japonica* pollen specific IgE levels in sera (left) and tear samples (right) from 31 individuals. From some individuals including the 4 patients shown in Fig. 2, two or more samples were obtained from March-May and July-December, respectively. In these cases, data are presented as average.

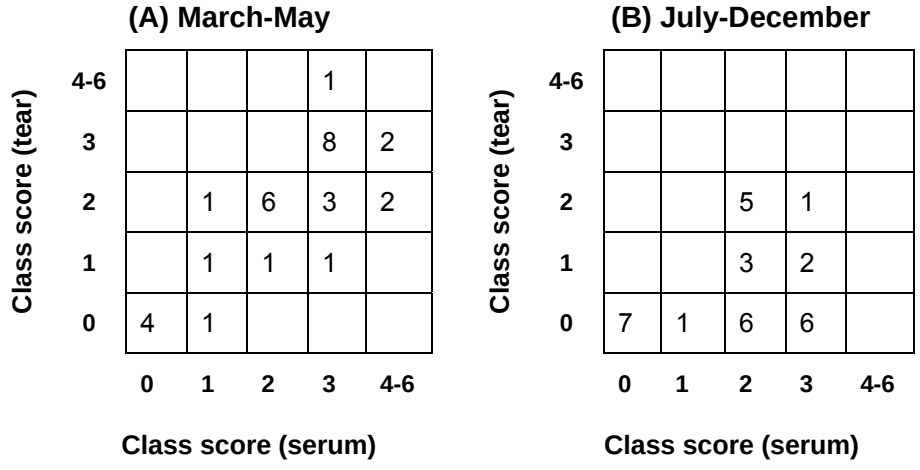

Fig. 4 Correlation of *C. japonica* pollen specific IgE levels in tears with those in sera obtained from March-May (A) and from July-December (B). Specific IgE levels were presented in class scores, and classes 4, 5, and 6 were treated as one class score. Figures in the above boxes represent the number of samples with the corresponding class score of *C. japonica* pollen specific IgE in serum (abscissa) and tears (ordinate).

the concentration of specific IgE in serum and with the pollen density. We obtained tear samples by extracting the material absorbed in the 10 mm tip of the Schirmer strip directly attached to the conjunctiva, because in preliminary experiments most of the IgE in the strip was confirmed to be in the tip. There remains a possibility that the tip of the Schirmer strips may directly absorb IgE and/or IgE-containing cells from the conjunctiva, which is the main site for allergic inflammation in the eye.

In the present experiments, the *C. japonica* pollen specific IgE levels in the tears of 4 pollinosis patients started to increase just before the increase in their clinical symptom scores and also before the serum specific IgE levels. Some reports showed that specific IgE levels in nasal lavage reached the maximum 4 days after the allergen provocation with diesel exhaust particles.[19,20] The local immune response in the conjunctiva could be elicited by a small amount of *C. japonica* pollen in the air before the pollen season. Patient #3 showed eye symptoms and the specific IgE level in the tear samples remained high even in November. There could be two explanations for this case: 1) a small amount of *C. japonica* pollen was dispersed in late autumn as suggested by one report,[21] 2) this particular patient was exposed to other allergens cross-reactive to *C. japonica* pollen.

The tear samples of 15 individuals which were positive for *C. japonica* pollen specific IgE from March-May turned to be negative from July-December (Fig. 3). Thirteen of these 15 individuals, however, were positive for *C. japonica* pollen specific IgE in their sera even from July-December. Among these 13 individuals only 2 persons had eye symptoms. Their eye symptoms might not have been caused by cedar pollen, as they also were positive for specific IgE for mite antigen not only in the serum but also in the tears (data not shown).

Many patients showed several different allergic symptoms and also had specific IgE for different allergens in the serum. It is often difficult to identify the specific allergen for a local allergic reaction. It is possible that body fluids in the local areas such as tears for the eyes and snivel for the nose contain a higher concentration of an specific IgE responsible for the local reaction than fluids in other areas or the serum. An assay for specific IgE in tears, therefore, would be beneficial for patients having specific IgE against different kinds of allergens with severe eye symptoms to identify the causative allergen of the symptoms. The volume of tears obtained by the Schirmer strip allows us to assay for 4 different kinds of allergens by AlaSTAT®. In addition, it is easier for eye doctors working in a small medical office to obtain tear samples using a strip than collecting blood.

There are many reports discussing the origin of specific IgE in tears.[5,7,13,14,17,22] In our preliminary experiment, the ratio of *C. japonica* pollen specific IgE to total IgE in tear samples was much higher (2-38 times) than that in the serum, which is corresponding to a previous report.[5] In addition, in one of our patients (#4) the specific IgE levels in the tear samples were even higher than those in the serum for a certain period of time. These results suggest that the specific IgE in tears might have been produced locally in addition to being transferred from the serum.

The pollen specific IgE levels both in tear samples and sera reached their maximum sometimes between the late phase of the pollen season and 2 months after the season. The seasonal change in *C. japonica* pollen specific IgE levels in the sera of our present study was similar to that in a previous report, which showed that *C. japonica* pollen specific IgE levels in serum reached the minimum in February and the maximum in May.[22] Ragweed pollen specific IgE levels in the serum were also shown to reach the maximum levels more than 2 months after the pollen season in one study,[23] and 4 weeks after the provocation in another study.[24] Repeated exposures to *C. japonica* pollen antigen for more than 60 days may cause a continuous increase in the specific IgE levels in tears and serum throughout the pollen season. *C. japonica* pollen allergen remaining in tissues may continue to stimulate specific IgE production for more than one month.

When *C. japonica* pollen specific IgE levels in tear samples were relatively high at the end of or after the pollen season, March - May, they correlated well to those in the serum. However, in July-December, many tear samples had low specific IgE levels, and more than half of the tear samples were

negative (< 0.35 U/ml). Thus, the levels in the tear samples did not correlate to those in the sera from July-December. specific IgE for other pollens than *C. japonica* pollen were also reported not to be detected in tears from many pollinosis patients during the pollen free season, although the specific IgE in the serum were positive.[14,17]

When we examined the correlation of *C. japonica* pollen specific IgE levels in tears to that in the serum, we tentatively applied the grading used for serum specific IgE levels to classify the specific IgE level in tears, simply because the data for specific IgE levels in serum are commonly evaluated in terms of class scores in clinical examinations, and it is convenient to compare both data by the same standard. It is well known that the titer of specific IgE does not always correlate with the symptoms, because an allergic reaction consists of many steps of cell-to-cell interactions. We have to evaluate the clinical significance of the specific IgE levels in tears in correlation with clinical eye symptoms by studying many patients with eye symptoms before we formally grade the specific IgE levels in tears in class scores.

Our results so far suggest that the quantitative assay for specific IgE in tears is a valuable tool to identify the environmental allergen causing the eye symptoms.

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