

Specific Tear IgE in Patients with Moderate-to-Severe Autumnal Allergic Conjunctivitis

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Key Words

Allergic conjunctivitis • Immfast Check J1® • Immunochromatography • Tear • Specific IgE

Abstract

Background: Recently, a new commercial test for specific IgE based on immunochromatography (Immfast Check J1®) has been developed. We previously reported on the use of this kit for tear fluid samples during spring. In this study, we compared the level of specific IgE in tear fluid among patients with seasonal allergic conjunctivitis during spring or autumn and control subjects. **Methods:** A nonrandomized, cross-sectional study was conducted in patients with moderate-to-severe allergic conjunctivitis occurring in spring (n = 56, spring group) or autumn (n = 52, autumn group), as well as in age- and sex-matched healthy control subjects (n = 50, control group). Levels of specific IgE for cedar pollen, cat epithelium and *Dermatophagoides pteronyssinus* were measured in tear fluid with the Immfast Check J1. **Results:** Specific IgE scores for cedar pollen and *D. pteronyssinus* were significantly higher in the spring and autumn groups than in the control group (p < 0.0001). In the autumn group, the highest detection rate for a specific allergen was 96.2% for

D. pteronyssinus, followed by cedar pollen (86.5%) and cat epithelium (25.5%). In the spring group, the highest detection rate was 98.3% for cedar pollen, followed by *D. pteronyssinus* (51.7%) and cat epithelium (19.0%). **Conclusions:** These results suggest that both house dust mite allergen and outdoor allergens such as cedar pollen can be causes of allergic conjunctivitis during both spring and autumn in Japan. The Immfast Check J1 provides rapid measurement of specific IgE in tear fluid, which allows easy diagnosis of allergic conjunctivitis in an outpatient setting.

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Introduction

Allergic conjunctivitis is a common condition worldwide, and is characterized by ocular inflammation that is triggered by specific antigens. Detection and quantification of specific IgE antibodies in serum is currently the standard method for making a diagnosis of seasonal allergic conjunctivitis [1, 2]. Measurement of total IgE in tear fluid is also useful for diagnosing allergic conjunctivitis [3–12].

Table 1. Clinical profile of the subjects and results of the Immfast Check J1 test

	Control group	Spring group	Autumn group	p value
Number of patients	50	56	52	–
Gender				
Male	24 (48.0%)	27 (48.2%)	26 (50.0%)	NS ^a
Female	26 (52.0%)	29 (51.8%)	26 (50.0%)	
Mean age $\pm$ SD, years	22.2 $\pm$ 8.5	21.6 $\pm$ 10.1	20.4 $\pm$ 10.8	NS ^b
Immfast Check J1 test				
Test positive ¹	1	56	52	
Test negative	49	0	0	
Positive rate	2.0%	100.0%	100.0%	<0.00001 ^a
Sensitivity	–	100.0%	100.0%	
Specificity	–	98.0%	98.0%	
Positive predictive value	–	98.0%	98.0%	
Negative predictive value	–	100.0%	100.0%	
Positive likelihood ratio	–	50.0	50.0	

NS = Not significant.

^a χ^2 test of independence or Fisher's exact probability test. ^b Unpaired Student's t test.¹ At least one positive test for cedar pollen, cat dander or house dust mite (*D. pteronyssinus*) IgE.

Recently, a new commercial kit (Immfast Check J1®; Mitsubishi Kagaku Iatron Inc., Tokyo, Japan) has been introduced for the rapid immunoassay of specific serum IgE for cedar pollen, cat dander and house dust mites (*Dermaphagoides pteronyssinus*). Ikeda et al. [13] first reported on the use of this kit for tear fluid samples, detecting IgE for cedar pollen in 15 of 32 eyes, IgE for *D. pteronyssinus* in 11 of 32 eyes and IgE for cat dander in 1 eye of patients with allergic conjunctivitis during spring. We previously reported a detection rate of 96.9% (62/64) for cedar pollen IgE, 53.1% (34/64) for *D. pteronyssinus* IgE and 23.4% (15/64) for cat dander IgE in spring [14]. In Japan, allergic conjunctivitis shows two peaks, which are in spring and in autumn. Our previous study demonstrated that the highest positivity rate for a specific allergen was 52.5% for cedar pollen, followed by cypress pollen at 37.5% in spring [1]. Cedar pollen has been identified as the major allergen that causes allergic conjunctivitis in spring [1, 14]. However, no studies have investigated specific IgE levels in tear fluid of patients with allergic conjunctivitis during autumn.

The present cross-sectional study was designed to evaluate the relationship between levels of specific IgE in tear fluid and allergic conjunctivitis during autumn by using the Immfast Check J1 to measure IgE. We also compared patients with seasonal allergic conjunctivitis during spring or autumn and control subjects without conjunctivitis.

Subjects and Methods

Study Design

This was a prospective, nonrandomized, cross-sectional, consecutive case study conducted at the hospital of the University of Tokyo Graduate School of Medicine. The study was performed in accordance with the Helsinki Declaration of 1975 and its 1983 revision. Institutional Review Board approval was obtained and each subject provided informed consent.

Subjects

All subjects had a Schirmer 1 test score greater than 15 mm and normal meibomian glands. Schirmer paper test strips are placed over the lid margin at the junction of the lateral and middle third of the lower eyelid for 5 min. Five millimeter lengths of Schirmer paper were used for the measurement of specific tear IgE as mentioned later. Therefore, we excluded patients with dry eye syndrome. None of the subjects had used topical ophthalmic medications or systemic drugs during the preceding six weeks. Furthermore, we excluded patients with dry eye syndrome, atopic blepharoconjunctivitis, atopic keratoconjunctivitis and vernal keratoconjunctivitis, as well as patients wearing contact lenses and those with a history of cataract surgery, corneal refractive surgery or infectious conjunctivitis. Patients older than 40 years were excluded because of the increased likelihood of atopic blepharoconjunctivitis or atopic keratoconjunctivitis.

Three groups were enrolled (table 1). The spring group consisted of 56 outpatients suffering from acute seasonal allergic conjunctivitis just in spring, who were treated at our hospital and its affiliated hospitals between February and April 2009 (27 males and 29 females with a mean age of 21.6 $\pm$ 10.1 years; range 6–40 years). The autumn group consisted of 52 patients with acute sea-

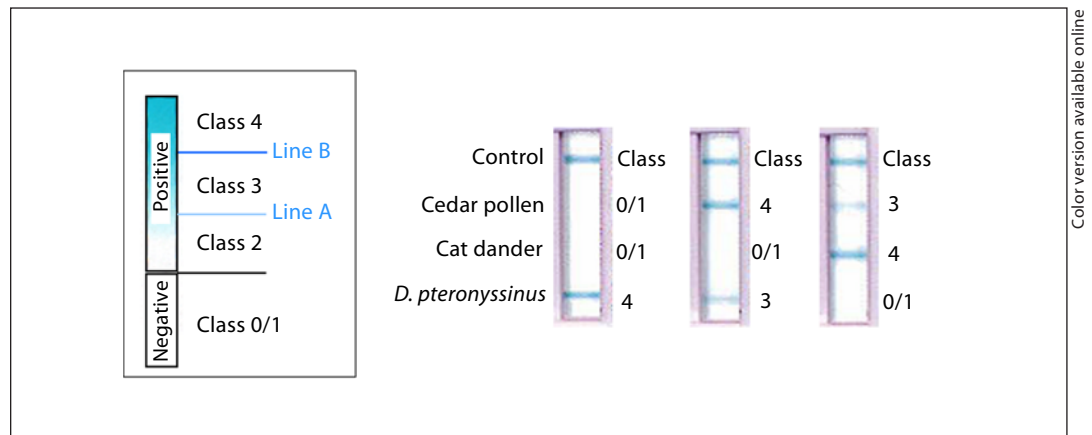


Fig. 1. Photographs showing the results of the Immufast Check J1 test. Results are graded according to the manufacturer's instructions on a scale of 0–4 as follows: class 0/1, negative; class 2, weaker than line A; class 3, similar to or stronger than line A and weaker than line B; class 4, similar to or stronger than line B. Positive results were determined by visual observation and confirmed by using Scion Image software.

sonal allergic conjunctivitis just in autumn, who were treated between August and October 2009 (26 males and 26 females with a mean age of 20.4 ± 10.8 years; range 6–39 years). Skin prick tests to cedar pollen and *Dermatophagoides farinae* were performed in all patients (1:20 wt/vol; Torii Pharmaceutical Co. Ltd., Tokyo, Japan) and were positive to at least 1 of 2 allergens in both spring and autumn groups. The control group comprised 50 healthy control subjects with no history of allergic disease and with negative skin prick tests (24 males and 26 females with a mean age of 22.2 ± 8.5 years; range 8–39 years).

Definition of Allergic Conjunctivitis

Allergic conjunctivitis was diagnosed by a single ophthalmologist (T.M.), based on symptoms such as ocular itching and tearing, as well as the results of slit lamp examination (conjunctival follicles, papillae and erythema) according to published guidelines for the diagnosis of conjunctivitis [15]. Patients with moderate or severe allergic conjunctivitis according to the criteria proposed by Shoji et al. [16] were enrolled. The critical clinical observations of allergic conjunctivitis were classified into the 100-point-grade group, 10-point-grade group or 1-point-grade group, according to the clinical severity of allergic conjunctivitis, and 5 critical findings were identified in each grade group [16]. Each positive observation in the 100-point-grade group represented 100 points. Each positive observation in the 10-point-grade group represented 10 points and each positive observation in the 1-point-grade group represented 1 point. Finally, the 5-5-5 exacerbation grading scale was determined by the number of total points scored from clinical observations [16]. The 100-point-grade group was determined based on the presence of the following observations: active giant papillae, gelatinous infiltrates of the limbus, exfoliative corneal epitheliopathy, shield ulcer and papillary proliferation at lower tarsal conjunctiva. The 10-point-grade group was determined based on the presence of the following observations: blepharitis, papillary proliferation with velvety ap-

pearance, Horner-Trantas, edema of bulbar conjunctiva and superficial punctate keratopathy. The 1-point-grade group was determined based on the presence of the following observations: papillae at upper tarsal conjunctiva, follicular lesion at lower tarsal conjunctiva, hyperemia of tarsal conjunctiva, hyperemia of bulbar conjunctiva and lacrimal effusion. Furthermore, the severity classes were divided into mild, moderate or severe, according to the criteria by Shoji et al. [16] as follows: mild, clinical observation classified in the 10- or 100-point-grade group is absent; moderate, less than two clinical observations classified in the 10-point-grade group are observed and those in the 100-point-grade group are absent; severe, more than three clinical observations classified in the 10-point-grade group are observed and those in the 100-point-grade group are absent [16].

Measurement of Specific IgE in Tear Fluid

Measurement of specific IgE was performed with the Immufast Check J1 immunochromatographic assay kit, which is designed for direct measurement of IgE antibodies against cedar pollen, cat dander, and house dust mites (*D. pteronyssinus*). Measurement was performed according to the manufacturer's instructions, as described previously [17, 18]. In brief, tear fluid samples were obtained with Schirmer test strips (Schirmer Tear Production Measuring Strips; Showa Yakuhin Kako, Tokyo, Japan), and 5 mm lengths of filter paper soaked with tears were placed into test tubes containing 100 μ l of reaction buffer solution. After mixing the tears with the reaction buffer by gentle pipetting for 10 s at room temperature, the mixture was transferred to an Immufast Check J1 cassette. The enzyme substrate, 200 μ l of a mixture of 5-bromo-4-chloro-indolylphosphate and nitroblue tetrazolium (BCIP/NBT), was added to the substrate reservoir pad immediately after application of the sample. Specific IgE antibodies for cedar pollen, cat epithelium and *D. pteronyssinus* in the samples reacted with alkaline phosphatase-labeled mouse anti-human IgE antibody (ALP-labeled anti-IgE Ab) in the reaction buffer. ALP-labeled an-

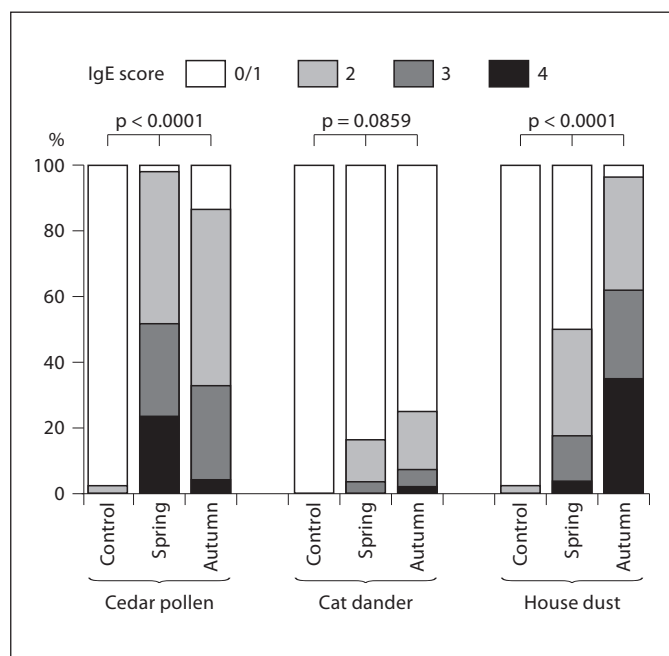


Fig. 2. Comparison of the grade of positivity for each specific IgE in tear fluid among the control, spring and autumn groups. Positivity for IgE antibodies directed against cedar pollen, cat dander and house dust mites (*D. pteronyssinus*) was determined by the Immufast Check J1 test. The specific IgE score was graded as described in the Subjects and Methods section and figure 1. p values were determined according to the Kruskal-Wallis test.

ti-IgE was bound to specific IgE for cedar pollen, cat dander or *D. pteronyssinus* at different capture sites on the immunochromatography membrane. *D. pteronyssinus* antigen (Ag) was immobilized at the first line on the membrane, cat epithelium Ag at the second line and cedar pollen Ag at the third line. When the substrate solution (BCIP/NBT) reached the control line on the membrane, it reacted with ALP in the ALP-labeled specific IgE-Ab complex. Reaction of ALP with BCIP/NBT resulted in the formation of a blue-purple complex at each test line.

Twenty minutes after sample application, the color of the test lines was assessed according to the manufacturer's instructions. The intensity of the blue color of the test lines varied according to the level of each specific IgE (fig. 1). The assay was calibrated in the range of 0.35–100 kU/l, and results were classified into four categories according to a semiquantitative scale, as described in the manufacturer's instructions: class 0/1 (negative, 0.35–0.69 kU/l); class 2 (weaker than line A, 0.70–3.49 kU/l); class 3 (similar to or stronger than line A and weaker than line B, 3.50–17.49 kU/l); class 4 (similar to or stronger than line B, ≥ 17.50 kU/l) (fig. 1). Positive results were determined by visual observation and confirmed by using Scion Image software (Scion Corporation, Frederick, Md., USA).

Statistical Analysis

Mean values were compared with the unpaired t test. Frequency analysis was performed with the χ^2 or Fisher exact test. The

Kruskal-Wallis test was used for comparison of semiquantitative measurements among the three groups. Sensitivity, specificity and positive as well as negative predictive values of this kit for diagnosing allergic conjunctivitis during spring or autumn season were calculated with standard formulae. The level of significance was set at $p < 0.05$. Calculations were done with the Stat View statistical software package (Abacus Concepts, Berkeley, Calif., USA).

Results

The Immfast Check J1 gave positive results for any of the three allergenic strips in all patients from the spring group (100.0%) and the autumn group (100.0%), as well as in 1 control subject (2.0%; table 1). The difference was significant ($p < 0.00001$, Fisher's exact test; table 1). The sensitivity, specificity, positive and negative predictive values as well as positive likelihood ratio are shown in table 1.

For specific antibodies targeting cedar pollen and *D. pteronyssinus*, significant differences in the frequency of positive results were found among the control, spring and autumn groups (cedar pollen: 2.0 vs. 98.3 vs. 86.5%, $p < 0.0001$; *D. pteronyssinus*: 2.0 vs. 51.7 vs. 96.2%, $p < 0.0001$, Fisher's exact test), as well as in the grade of antibody positivity (cedar pollen: 0.04 ± 0.28 vs. 2.71 ± 0.88 vs. 2.10 ± 0.99 , $p < 0.0001$; *D. pteronyssinus*: 0.04 ± 0.28 vs. 1.21 ± 1.29 vs. 2.88 ± 1.01 , $p < 0.0001$, Kruskal-Wallis test) (table 1; fig. 2). For specific antibodies to cat dander, the positive results were significantly more common in the spring and autumn groups than in the control group ($p = 0.0030$ and 0.0002 , respectively, Fisher's exact test). There was also a significant difference in the frequency of positive results for cat dander among the three groups (0.0 vs. 19.0 vs. 25.0%, $p = 0.0011$, Fisher's exact test), but no difference in the grade of positivity among the three groups ($p = 0.0859$, Kruskal-Wallis test; fig. 2).

Discussion

The frequency of positive results and the grade of positivity for specific IgE targeting house dust mites and cedar pollen were both high in tear fluid samples from the autumn group, suggesting that these allergens are important causes of allergic conjunctivitis during autumn. Allergens can be divided into two categories according to their source: indoor or outdoor. Indoor allergens are more closely related to perennial allergic

conjunctivitis, while outdoor allergens are more frequently associated with seasonal allergic conjunctivitis. House dust mites and animal dander (usually from cats) are major indoor allergens. In this study, the frequency of specific IgE-targeting house dust mites was high in the autumn group (96.2%). This finding supports our previous observations on serum house dust mite-specific IgE in autumn [2].

In contrast, cedar pollen is a typical outdoor allergen in Japan. In this study, the detection rate of specific IgE-targeting cedar pollen was high in both the spring group (98.3%) and the autumn group (86.5%), although the grade of positivity was higher in spring than in autumn. This result suggests that outdoor allergens like cedar pollen are an important cause of allergy in Japan. There are two main cedar pollen seasons in Japan. Fujishima et al. [19] reported that cedar pollen is found during all seasons in Japan, but especially in autumn. Consequently, cedar pollen can cause allergic conjunctivitis during both spring and autumn.

In this study, the Immfast Check J1 showed a very high sensitivity (100.0% in both the spring and autumn groups) and specificity (98.0% in both groups). The level of each specific antibody in tear fluid was significantly higher in the spring and autumn groups than in the control group. Accordingly, the Immfast Check J1 is useful for detection of allergen-specific IgE in tear fluid samples from patients with allergic conjunctivitis. Our detection rates were similar to or higher than those reported by Ikeda et al. [13], probably because our allergic group consisted of patients with moderate-to-severe allergic conjunctivitis. Additionally, the detection rate of each allergen in the spring group was similar to that of patients with spring

time allergic conjunctivitis in our previous study performed in another population [14]. These results suggest that this test achieves highly reproducible results with regard to the diagnosis of allergic conjunctivitis.

Immunochromatographic analysis of specific IgE in tear fluid has several advantages over an enzyme-linked immunosorbent assay and CAP-RAST, which are standard methods of serum IgE analysis. First, measuring IgE with the Immfast Check J1 requires less time than standard assays. Detection of allergen-specific IgE in serum is normally performed with an enzyme-linked immunosorbent assay or CAP-RAST [20–24], but these assays are time consuming. With the Immfast Check J1, we could determine specific IgE levels in tear fluid within 20 min. This could be of considerable benefit to patients because it allows allergic conjunctivitis to be diagnosed during the first visit. Second, this is a noninvasive test for allergic conjunctivitis since it simply involves touching a piece of filter paper to the lower fornix of the conjunctiva. Therefore, we conclude that the Immfast Check J1 is a rapid, sensitive and noninvasive method for confirmation of allergic conjunctivitis.

One limitation of the present study is that we selected patients with moderate-to-severe allergic conjunctivitis, so we could not evaluate the sensitivity of the kit in patients with mild conjunctivitis.

In conclusion, our study confirmed that specific IgE for house dust mites and Japanese cedar pollen in tear fluid is closely associated with autumnal allergic conjunctivitis. Immunochromatographic measurement of the specific IgE level in tear fluid is a rapid and easy method for diagnosing allergic conjunctivitis.

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