

Relation Between Total Tear IgE and Specific Serum IgE in Seasonal Allergic Conjunctivitis

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Purpose: Recently, a new commercial test for total tear immunoglobulin E (IgE), based on immunochromatography (Allerwatch), was developed. We examined the relation between the total IgE level in tears and specific serum IgE.

Methods: A prospective, nonrandomized, cross-sectional study was conducted in 35 patients with allergic conjunctivitis (allergic group), 30 age- and sex-matched healthy control subjects (control group), and 8 patients with epidemic keratoconjunctivitis. In all subjects, the total tear IgE score was determined with the Allerwatch test (0, 1, and 2), and serum levels of total IgE and specific IgE for 12 inhaled allergens were measured with the Phadezym PRIST and CAP-RAST systems, respectively.

Results: The total tear IgE-positive rate was significantly higher in the allergic group than in the control and epidemic keratoconjunctivitis groups (100.0% vs. 0.0% vs. 0.0%; $P < 0.00001$). In the allergic group, the total tear IgE score was significantly correlated with the log-transformed total serum IgE level ($r = 0.61$) and with the serum levels of IgE for cedar pollen ($r = 0.35$), house dust ($r = 0.46$), *Dermatophagoides pteronyssinus* ($r = 0.49$), and acarus ($r = 0.36$). Multivariate logistic regression analysis showed that the log-transformed total serum IgE level was the only significant predictor of the total tear IgE score (odds ratio = 1.85, $P = 0.00008$).

Conclusions: The total tear IgE score, determined with the Allerwatch test, was correlated with the total and specific serum IgE levels. This rapid test is easy to perform, sensitive, and highly specific for the detection of ocular allergy on an outpatient basis.

Key Words: allergic conjunctivitis, Allerwatch, immunochromatography, serum, specific IgE, tear, total IgE

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Allergic conjunctivitis is a common condition worldwide, which is characterized by ocular inflammation triggered by specific antigen. Measurement of allergen-specific IgE levels in the serum^{1,2} and measurement of total tear IgE are useful tests for the diagnosis.^{3–5} Several research groups have reported on the measurement of the total tear IgE level by immunochromatography.^{6–8} Recently, a new commercial kit (Allerwatch; Hitachi Chemical, Co, Ltd and Wakamoto Pharmaceutical, Co, Ltd, Tokyo, Japan) has been developed and released that provides a rapid immunoassay for measuring total tear IgE. So far, little information is available regarding its clinical use or application in screening for allergic conjunctivitis. Accordingly, the objectives of the present study were as follows: (1) to assess the clinical value of the Allerwatch as a screening test for allergic conjunctivitis, (2) to compare the results obtained with this test between patients with seasonal allergic conjunctivitis and a control group in a cross-sectional study, and (3) to investigate the correlation between total tear IgE levels and allergen-specific serum IgE levels in patients with seasonal allergic conjunctivitis.

MATERIALS AND METHODS

Study Design

This was a prospective, nonrandomized, cross-sectional, consecutive case study conducted at the University 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 informed consent was provided by each subject.

Subjects

All subjects had a Schirmer I test score >15 mm and normal meibomian glands. None of them wore contact lenses, and none of them had been treated with a topical or systemic drug in the preceding 6 weeks. Patients with dry eyes or external ocular infection, including staphylococcal blepharitis and bacterial conjunctivitis, were excluded. Two groups were enrolled in this study (Table 1). Group 1, the allergic group, consisted of 35 consecutive outpatients suffering from acute seasonal allergic conjunctivitis, who were treated at our hospital between February and May 2009 (17 men and 18 women; mean age: 25.3 ± 14.1 years; range: 7–40 years). Group 2, the age- and sex-matched control group, comprised 30 healthy nonsmoking volunteers with no history of allergic diseases (14 men and 16 women; mean age: 25.7 ± 7.1 years;

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TABLE 1. Comparisons Among Healthy Control Subjects (Control Group), Patients With EKC (EKC Group), and Patients With Allergic Conjunctivitis (Allergic Group)

		Control Group	EKC Group	Allergic Group	P
No. patients		30	8	35	—
Age (yrs)		25.7 ± 7.1	24.1 ± 6.1	25.3 ± 14.1	NS*
Male/Female		14/16	4/4	17/18	NS†
	Grade	Control Group	EKC Group	Allergic Group	P
Allerwatch test					
Test positive	Grade 2	0	0	18	—
Test positive	Grade 1	0	0	17	<0.00001‡
Test negative	Grade 0	30	6	0	—
Sensitivity			100.0%		—
Specificity			100.0%		—
False negative rate			0.00%		—
False positive rate			0.00%		—
Positive predictive value			100.0%		—
Negative predictive value			100.0%		—
Positive likelihood ratio			— (∞)		—

Number of patients or mean ± SD.

*One-way ANOVA and Scheffe multiple comparison test.

† χ^2 test of independence or Fisher exact probability test.

‡The Kruskal–Wallis test.

NS, not significant.

range: 15–37 years). Group 3, the infectious group, comprised 8 patients with epidemic keratoconjunctivitis (EKC group; 4 men and 4 women; mean age: 24.1 ± 6.1 years; range: 16–32 years). Diagnosis of EKC was made on the basis of the anamnesis and the presence of follicular conjunctivitis, preauricular adenopathy, and punctate keratitis. In 8 patients with EKC, all conjunctival swabs were positive for adenoviral antigen by the rapid immunochromatographic assay kit (Adenochek; Santen Pharmaceutical, Co, Ltd, Osaka, Japan).

Definition of Allergic Conjunctivitis

Allergic conjunctivitis was diagnosed by a single ophthalmologist (T.M.), according to the published guidelines for the diagnosis and treatment of conjunctivitis.⁹ Patients were enrolled if they had moderate or severe allergic conjunctivitis according to the classification proposed by The Japanese Ophthalmological Society.¹⁰

Determination of Total IgE in Tear Fluid

Determination of total IgE was performed with the Allerwatch test according to the manufacturer's instructions. In brief, the filter strip was placed at the lower fornix of the right eye and was removed when the tear fluid had wetted the strip to the control line. Then, the strip was immersed in 100 μ L of reaction buffer in a 2.0-mL Eppendorff tube for 10 minutes at room temperature. The primary antibody was gold-labeled antihuman IgE. After this gold-labeled antibody bounded to the IgE in the tear fluid impregnating the test strip, it was captured by the mouse antihuman IgE antibody immobilized at the test line on the strip. Gold-labeled antihuman IgE was also immobilized as a control line on each strip. The intensity of red color developing at the test line was dependent on the total IgE content of the sample. At normal IgE levels (<2.0 kU/L), no line developed, as stated in the manufacturer's instructions.

The results of the test were classified according to the following 3 grades: grade 0 = no detectable IgE (no line), grade 1 = a low total IgE level (test line weaker than the control line), and grade 2 = a high total IgE level (test line similar to or stronger than the control line). The results were determined by naked eye observation and then were confirmed using Scion Image software (Scion Corporation, Frederick, MD). According to their total tear IgE scores, patients were divided into the following 3 groups: group 0 (control group, Allerwatch test negative, n = 30), group 1 (allergic patients with a test score of 1, n = 17), and group 2 (allergic patients with a test score of 2, n = 18).

Allergens

A battery of 12 RASTs were performed with the following allergens: an equal mixture of 5 types of animal epithelium (ie, epithelium or dander of cats, dogs, guinea pigs, rats, and mice), house dust (Greer Laboratories, Lenoir, NC), *Dermatophagoides pteronyssinus*, acarus, moth, *Candida*, *Alternaria tenuis*, Japanese cedar (*Cryptomeria japonica*) pollen, Japanese cypress (*Chamaecyparis obtusa*) pollen, ragweed, mugwort, and orchard grass. This test panel included the allergens that are the main causes of allergic diseases in Japan.

Determination of Serum Total and Specific IgE Levels

Determination of serum total IgE was carried out with the Phadezym IgE PRIST kit (Pharmacia, Uppsala, Sweden). Blood was also withdrawn for assay of 12 specific IgEs by the Phadiatop CAP-RAST (Pharmacia) test. Assays were carried out on undiluted serum samples. Detection of antibodies for the 12 allergens was done in duplicate with the

radioimmunoassay version of the Pharmacia CAP-RAST system, and the manufacturer's instructions were strictly followed. In brief, test sera and standards were incubated at room temperature (approximately 20°C) with the ImmunoCAP for 30 minutes, after which the solid phase was washed and a combination of specific polyclonal and monoclonal anti-IgE (Fc) antibodies (labeled with ^{125}I) was added. After further incubation for 150 minutes, the ImmunoCAP solid phase was washed again, and the bound radioactivity was measured with a gamma counter. Each specific IgE assay was calibrated against a World Health Organization standard over the range of 0.35–100 kU/L, and the results were read from the standard curve. The upper and lower cutoff points were 100 kU/L and 0.35 kU/L, respectively, as for the Phadebas RAST. Results were expressed as kU/L or as classes 0–6 for a standard RAST (class 0 = <0.35 kU/L, class 1 = 0.35–0.69 kU/L, class 2 = 0.70–3.49 kU/L, class 3 = 3.50–17.49 kU/L, class 4 = 17.50–49.99 kU/L, class 5 = 50.00–100.00 kU/L, and class 6 = >100 kU/L). The solid-phase antigens in the ImmunoCAP were optimized to achieve maximum binding with a low background, even when undiluted test sera were used. IgE levels (international units per milliliter) were log transformed to normalize the distribution of data.

Statistical Analyses

The 2-tailed unpaired *t* test was performed to compare the mean values, whereas frequencies were compared by χ^2 analysis or Fisher exact test. One-way analysis of variance (ANOVA) and Scheffe multiple comparison test were used to compare the mean values among the 3 groups. Differences of semiquantitative measurements were examined by the 2-tailed Mann–Whitney *U* test for 2 groups and the Kruskal–Wallis 1-way ANOVA by ranks for 3 groups. Relations between continuous variables were investigated by calculating Pearson 2-tailed correlation coefficients. Sensitivity, specificity, and

positive and negative predictive values of the Allerwatch test were calculated by standard methods. The forward stepwise multiple logistic regression method was used to select factors significantly associated with the total tear IgE level. Explanatory variables, including each of the specific serum IgEs, were considered for inclusion in the model if they were associated with total tear IgE by correlation coefficient analysis. The level of significance was set at $P < 0.05$. Analyses were done with the Stat View statistical software package (Abacus Concepts, Berkeley, CA).

RESULTS

The Allerwatch test was positive in all the patients with allergic conjunctivitis (100.0%) and was negative in all 8 patients with EKC (0.0%) and all controls (0.0%, Table 1). There was a significant difference of the positive rate [$\chi^2 = 73.0$, degree of freedom (df) = 2, $P < 0.00001$, χ^2 test] and the grade (1.51 ± 0.50 vs. 0.00 ± 0.00 , vs. 0.00 ± 0.00 , $P < 0.00001$, Kruskal–Wallis test) among the allergic, EKC, and control groups (Table 1). The sensitivity, specificity, and positive and negative predictive values of the test are shown in Table 1.

When the positivity rates for specific IgE antibodies targeting the 12 test allergens were determined by CAP-RAST (Fig. 1), the highest positive rate was 94.3% for IgE to cedar pollen. This was followed by cypress pollen (68.6%) and ragweed (28.6%), all of which are outdoor allergens. In contrast, the positivity rates for IgE to mugwort (17.1%) and orchard grass (17.1%) were low. Among the indoor allergens, the highest positivity rate was 57.1% for IgE to house dust and *D. pteronyssinus*, followed by acarus IgE (54.3%) and moth IgE (37.1%).

The log (total serum IgE) value was significantly higher in the allergic group than in the control and EKC groups

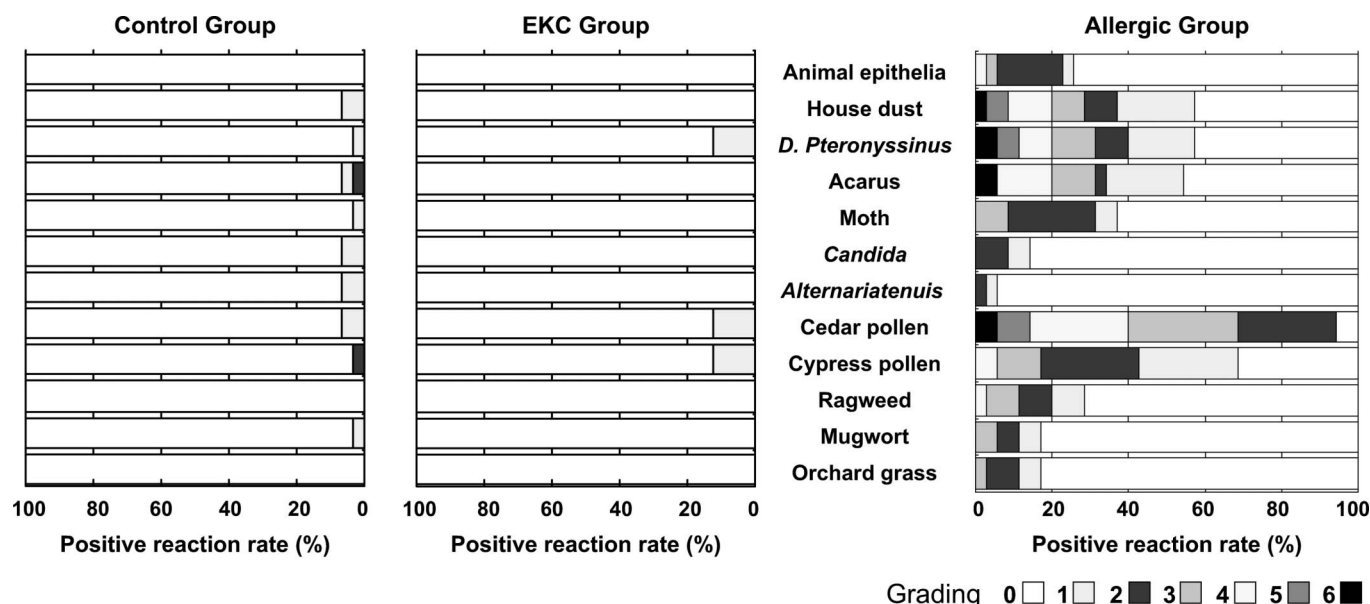


FIGURE 1. Frequency and grade of IgE positivity. Frequency of IgE antibodies for 12 allergens determined by CAP-RAST in the control group (n = 30), EKC group (n = 8), and the allergic group (n = 35).

(2.18 ± 0.50 vs. 1.21 ± 0.25 , vs. 0.90 ± 0.35 , $P < 0.00001$, 1-way ANOVA and Scheffe multiple comparison test, Fig. 1). Significant differences were found among the 3 groups ($P < 0.00001$, Scheffe multiple comparison test, Fig. 2).

Specific IgE levels were also compared among the groups identified by the total tear IgE score (Fig. 3). The grades of specific serum IgE antibodies for house dust, *D. Pteronyssinus*, acarus, moth, cedar pollen, and cypress pollen were higher in group 2 than in group 1 ($P = 0.01828$, 0.00616 , 0.04955 , 0.04955 , 0.00020 , and 0.00715 , respectively, 2-tailed Mann–Whitney U test). There were significant differences among the 3 groups with respect to the grades of specific serum IgE antibodies for house dust and cedar pollen ($P = 0.00015$ and 0.04278 , respectively, Kruskal–Wallis test).

Table 2 shows the correlations between total tear IgE and each log (serum IgE) value and the multivariate odds ratios for total tear IgE in the allergic group. The total tear IgE score was significantly correlated with log (total serum IgE) ($r = 0.615$, $P < 0.00001$, Pearson correlation coefficient analysis). When all the variables were considered simultaneously, the only significant predictor of the total tear IgE score was log (total serum IgE) according to multivariate logistic regression analysis with stepwise selection of variables (odds ratio = 1.85 , $P = 0.00008$).

DISCUSSION

We found that the Allerwatch test showed very high sensitivity and specificity. In addition, the total tear IgE level was significantly higher in the allergic group than in the control and EKC groups and was strongly correlated with total serum IgE and some allergen-specific serum IgE levels in the

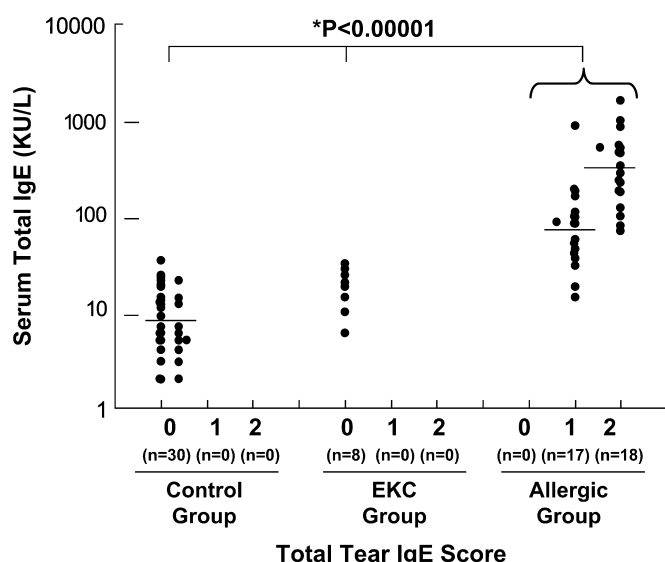


FIGURE 2. Relation between total tear and serum IgE levels in the control, EKC, and allergic groups. The total tear IgE score was determined with the Allerwatch test (0–2), and the serum total IgE level was measured by the Phadezym PRIST kit. *One-way ANOVA and Scheffe multiple comparison test: log (serum total IgE level) was compared among the 3 groups.

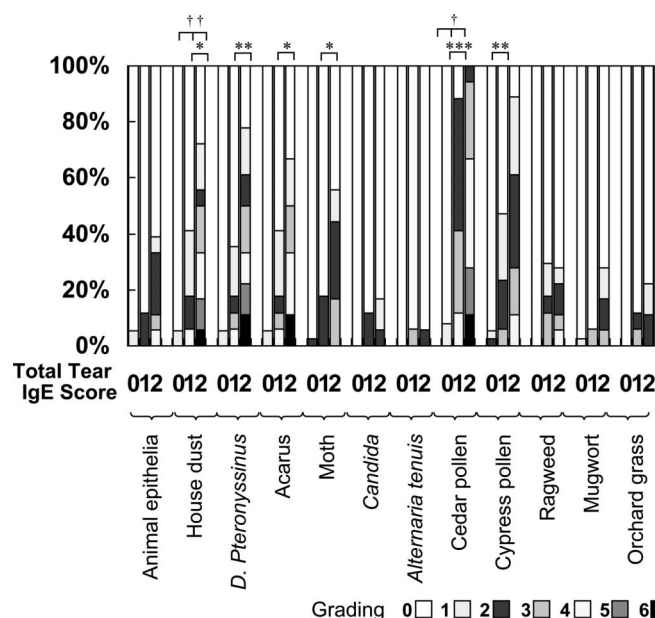


FIGURE 3. Comparison of specific serum IgE levels among the control, EKC, and allergic groups. The total tear IgE score was determined with the Allerwatch test (0–2), and specific serum IgE was measured by the CAP/RAST system. The control and EKC groups were group 0 (total tear IgE score = 0, $n = 38$). The allergic group was divided into 2 subgroups according to their total tear IgE scores: group 1 (total tear IgE score = 1, $n = 17$) and group 2 (total tear IgE score = 2, $n = 18$). The grades of specific serum IgEs were compared between group 1 and group 2 by using the Mann–Whitney U test (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$) and among the 3 groups with the Kruskal–Wallis test († $P < 0.05$, †† $P < 0.001$).

allergic group. Therefore, carrying out the Allerwatch test on tear samples is useful for making a clinical diagnosis of allergic conjunctivitis and strongly supports the data provided by conventional serological tests.

Specific IgE levels and the positivity rate for specific IgE to cedar and cypress pollens were high in the allergic group (Fig. 1), suggesting that these allergens are major causes of seasonal allergic conjunctivitis in Japan. There are 2 periods of high pollen levels every year in Japan. The cedar season runs from the end of January to the beginning of April, whereas the cypress season runs from the beginning of March until the end of May. In addition, the mugwort and ragweed pollen season runs from August until October, although the levels of these pollens are much lower than those of the first seasons. Thus, the symptoms of seasonal allergic conjunctivitis, which occur from spring to summer, are generally caused by cedar and cypress pollens.

Among the indoor allergens, the positivity rates and specific IgE levels were high for house dust, *D. pteronyssinus*, and acarus in the allergic group (Fig. 1). Outdoor allergens are more closely related to seasonal allergic conjunctivitis, whereas indoor allergens might be more frequently associated with perennial allergic conjunctivitis. Hence, mold and house dust mites cause allergic conjunctivitis, not only during spring but also during summer and winter because of the year-round

TABLE 2. Correlations Between Total Tear IgE and Log (Specific Serum IgE) Values and Multivariate Odds Ratios for Total Tear IgE in the Allergic Group (n = 35)

Variables	Correlation Coefficients			Multivariate Analysis		
	OR	95% CI	P	OR	95% CI	P
Total IgE	0.61	0.35–0.79	0.00009	1.85	−0.17 to 0.77	0.00008
Animal epithelium	0.24	−0.10 to 0.53	0.16689	—	—	—
House dust	0.46	0.15–0.69	0.00551	—	—	—
<i>D. pteronyssinus</i>	0.49	0.19–0.71	0.00279	—	—	—
Acarus	0.36	0.03–0.62	0.03487	—	—	—
Moth	0.46	0.15–0.69	0.00528	—	—	—
<i>Candida</i>	0.10	−0.24 to 0.42	0.55599	—	—	—
<i>A. tenuis</i>	−0.01	−0.34 to 0.32	0.94337	—	—	—
Cedar pollen	0.35	0.02–0.61	0.03727	—	—	—
Cypress pollen	0.24	−0.10 to 0.53	0.17009	—	—	—
Ragweed	0.12	−0.23 to 0.43	0.50797	—	—	—
Mugwort	0.27	−0.07 to 0.55	0.11498	—	—	—
Orchard grass	0.08	−0.26 to 0.40	0.64605	—	—	—

Before statistical analysis, specific serum IgE data were converted to log IgE values to obtain a normal distribution. Then, correlations between total tear IgE levels were calculated with the 2-tailed Pearson product moment formula. Independent determinants of total tear IgE were investigated by multiple logistic regression analysis.

CI, confidence interval; OR, odds ratio.

mild and wet climate in Japan. The total tear IgE level was correlated with the levels of specific serum IgEs for these major indoor and outdoor allergens responsible for seasonal allergic conjunctivitis. Therefore, the Allerwatch test is a practical alternative for the clinical evaluation of local allergic reactions affecting the conjunctiva in patients with seasonal allergic conjunctivitis.

Multivariate analysis identified total serum IgE as an independent predictor of total tear IgE. Although none of the specific IgEs were identified as independent predictors of total tear IgE, some of the specific IgE levels were correlated with the total tear IgE score. A possible reason why only total serum IgE was found to be a predictive factor is that the total IgE level represents the sum of all antigen-specific IgEs and is overwhelmingly higher than any 1 specific IgE level. However, the levels of specific IgE for cedar pollen and house dust mites, which are major causes of allergic conjunctivitis, were correlated with the total tear IgE score and were higher in group 2 than in group 1 (Fig. 3). Therefore, the simultaneous determinations of total tear IgE and specific serum IgE levels may be important for the treatment of allergic conjunctivitis. Measurement of the total tear IgE level is useful for the diagnosis of ocular allergy and for the evaluation of the response to the treatment. On the other hand, measurement of specific IgE levels also has clinical significance and is useful for identification of the major allergens responsible for allergic symptoms.

Compared with circulating serum IgE, local production of IgE is a more important contributor to the severity of allergic conjunctivitis.^{5,11,12} The aqueous portion of the tear film is secreted by the main and accessory lacrimal glands. Tear IgE levels are normally very low as a result of the blood–tear barrier.¹³ Allergic sensitization and/or an allergic response induce local IgE production, after which total tear IgE

increases because of the elevated level of lacrimal IgE being filtered through the blood–tear barrier.¹⁴ Kari et al¹⁵ demonstrated a good correlation between the conjunctival allergen provocation test and the lacrimal IgE level. Several research groups have reported a correlation between the total tear IgE and the manifestations of ocular allergy.^{5,11,12} Our results also showed a good correlation between the total tear IgE level and the serum total or specific IgE levels, suggesting that the immunochromatographic measurement of tear IgE can provide important information about local allergic inflammation that adds to the conventional serological diagnosis of allergic conjunctivitis.

In previous studies, total tear IgE levels were analyzed by enzyme-linked immunosorbent assay and immunoblotting.^{5,11,12} However, measurement of IgE by a standard enzyme-linked immunosorbent assay takes longer than with the Allerwatch test, since we could assay total tear IgE within 15 minutes, including the time for tear fluid collection. Therefore, the determination of the total tear IgE level by the Allerwatch test is a reliable, sensitive, reproducible, and practical method for the clinical evaluation of local ocular allergic reactions compared with the measurement of serum total IgE.

One limitation of the present study is that we selected patients with moderate to severe allergic conjunctivitis, so we need to reevaluate the sensitivity of the Allerwatch test in patients with mild allergic conjunctivitis. In conclusion, this study demonstrated that the total tear IgE score (measured by immunochromatography) was strongly correlated with serum total IgE and some specific serum IgE levels in patients with allergic conjunctivitis. The Allerwatch test for the measurement of total tear IgE is useful as a rapid point-of-care assay for distinguishing between allergic and infectious causes in patients with acute conjunctivitis.

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