

Relationship Between Total Tear IgE and Specific Serum IgE in Autumnal Allergic Conjunctivitis

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Purpose: A new commercial immunochromatographic test (Allerwatch) has recently been developed for determining total immunoglobulin E (IgE). We previously reported on the use of this kit for analyzing tear fluid samples collected during the spring. In this study, we examined the relationship between the total IgE level in tears and the specific serum IgE levels in patients with autumnal allergic conjunctivitis.

Methods: A prospective, nonrandomized cross-sectional study was conducted in 36 patients with autumnal allergic conjunctivitis (autumnal allergic group), in 32 age-matched and sex-matched healthy control subjects (control group), and in 10 patients with epidemic keratoconjunctivitis. Total tear IgE score was determined in all subjects using the Allerwatch test (0, 1, and 2), whereas the serum levels of total IgE and specific IgE for 12 inhaled allergens were measured using the Phadezym PRIST and CAP-RAST systems, respectively.

Results: Positivity rates for total tear IgE were significantly higher in the autumnal allergic group as compared with the control and epidemic keratoconjunctivitis groups (88.9% vs. 0.0% vs. 0.0%, $P < 0.00001$). In the autumnal allergic group, there was a significant correlation between the total tear IgE score and the log-transformed total serum IgE level ($r = 0.84$). Significant correlations were also seen with the IgE serum levels for *Dermatophagoides pteronyssinus* ($r = 0.53$), house dust ($r = 0.51$), acarus ($r = 0.49$), cedar pollen ($r = 0.33$), and mugwort ($r = 0.33$). Multivariate logistic regression analysis showed that the only significant predictor of the total tear IgE score was the log-transformed total serum IgE level (odds ratio = 2.76, $P < 0.00001$).

Conclusions: The correlation between the Allerwatch total tear IgE score and the specific IgE serum levels against house dust mites suggests that house dust mite allergens might be the primary cause of allergic conjunctivitis during autumn in Japan. The Allerwatch methodology can provide rapid measurements of total IgE in the tear fluid, which makes it possible to easily diagnose allergic conjunctivitis in an outpatient setting.

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

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Allergic conjunctivitis is a common worldwide condition that is characterized by ocular inflammation triggered by specific antigens. Measurements of allergen-specific immunoglobulin E (IgE) levels in the serum^{1,2} and measurements of total tear IgE have proven to be useful diagnostic tests.^{3–5} Recent development of a rapid immunoassay for measuring total IgE has now made it possible to measure total IgE tear levels.^{6–11}

Two allergic conjunctivitis peaks are observed in Japan, with one each occurring in the spring and autumn.^{1,2} We previously reported finding a strong correlation between the total tear IgE score and specific serum IgE antibodies for cedar pollen in patients with allergic conjunctivitis during the spring.¹² However, there have yet to be any reported studies that have investigated the association between the total tear IgE and serum-specific IgE levels in patients with allergic conjunctivitis during the autumn. Thus, the present cross-sectional study was designed to evaluate the relationship between the total tear IgE levels and the serum-specific IgE levels during the autumn season. We also compared the total tear and specific serum IgE levels among patients with autumnal allergic conjunctivitis, control subjects without conjunctivitis, and patients with infectious conjunctivitis.

MATERIALS AND METHODS

Study Design

This prospective, nonrandomized cross-sectional case study was conducted at the university hospital of the University of Tokyo Graduate School of Medicine and its affiliated hospitals. The study was approved by our institutional review board and performed in accordance with the Helsinki Declaration of 1975 and its 1983 revision. Informed consent was obtained from each subject before enrollment in the study.

Subjects

All subjects were given a Schirmer 1 test, with the Schirmer paper test strips placed over the lid margin at the junction of the lateral and middle third of the lower eyelid for 5 minutes. All enrolled subjects had a Schirmer 1 test score

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>15 mm and had normal meibomian glands. None of subjects wore contact lenses or had been treated with either a topical or a systemic drug during the preceding 6 weeks. Patients with dry eye syndrome, atopic blepharoconjunctivitis, atopic keratoconjunctivitis, and vernal keratoconjunctivitis, and also those with a history of cataract surgery, corneal refractive surgery, or infectious conjunctivitis, were excluded from the study. In addition, we also excluded all subjects older than 40 years. As seen in Table 1, 3 groups were examined in this study. The autumnal allergic group was designated as group 1 and consisted of 36 consecutive outpatients with acute seasonal allergic conjunctivitis that only occurred during autumn. This group consisted of 18 men and 18 women (mean age: 29.5 ± 8.5 years, range: 6–39), with all treatments performed between August and October 2010. All patients were given cedar pollen and *Dermatophagoides farinae* skin prick tests (1:20 wt/vol; Torii Pharmaceutical Co, Ltd, Tokyo, Japan) and were positive to at least 1 of the 2 allergens. Group 2 was the age-matched and sex-matched control group, and comprised 15 men and 17 women (mean age: 28.8 ± 7.4 years, range: 18–39) who were healthy nonsmoking volunteers with no history of allergic diseases. Group 3 was the infectious group, and comprised 5 men and 5 women (mean age: 27.3 ± 5.5 years, range: 18–35) who had been diagnosed with epidemic keratoconjunctivitis (EKC group). EKC diagnosis was made on the basis of anamnesis and the presence of follicular conjunctivitis, preauricular adenopathy, and punctate keratitis. Conjunctival swabs from all 10 EKC patients were positive for the adenoviral antigen, as determined by a rapid immunochromatographic assay kit (Adenochek; Santen Pharmaceutical Co, Ltd, Osaka, Japan).

Definition of Allergic Conjunctivitis

A single ophthalmologist (T.M.) diagnosed the allergic conjunctivitis based on symptoms such as ocular itching and tearing, and also on the results of a slit-lamp examination (conjunctival follicles, papillae, and erythema) in accordance with the published guidelines for the diagnosis of conjunctivitis.¹³ Patients with moderate or severe allergic conjunctivitis who were enrolled in the study were classified according to the criteria proposed by Shoji et al.¹⁴

Determination of Total IgE in Tear Fluid

Determination of total IgE was performed using the Allerwatch (Hitachi Chemical, Tokyo) test in accordance with the manufacturer's instructions. Briefly, the filter strip was placed at the lower fornix of the right eye and then removed when the tear fluid reached the control line of the strip. The strip was then immersed in 100 μ L of reaction buffer in a 2.0-mL Eppendorf tube for 10 minutes at room temperature. The primary antibody was gold-labeled antihuman IgE. After the binding of the gold-labeled antibody to the IgE in the tear fluid impregnating the test strip, it was then captured by mouse antihuman IgE antibody that was immobilized at the test line on the strip. As a control, gold-labeled antihuman IgE was also immobilized on each strip. The intensity of the red color that developed at the test line was dependent on the total IgE content of the sample. As stated in the manufacturer's instructions, no lines are visible when normal IgE levels (<2.0 kU/L) are present. Test results were separated into 1 of 3 grades, with 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) as shown in Figure 1. Initial

TABLE 1. Comparisons Between Healthy Control Subjects, Patients With EKC, and Patients With Autumnal Allergic Conjunctivitis

	Control Group	EKC Group	Autumnal Allergic Group	P
No. patients	32	10	36	
Age (yr)	28.8 $\pm$ 7.4	27.3 $\pm$ 5.5	29.5 $\pm$ 8.5	NS*
Male/female	15/17	5/5	18/18	NS†
Allerwatch test				
Test positive				
Grade 2	0	0	18	
Grade 1	0	0	14	<0.00001‡
Test negative				
Grade 0	32	10	4	
Sensitivity (%)	88.9			
Specificity (%)	100.0			
False negative rate (%)	11.1			
False positive rate (%)	0.0			
Positive predictive value (%)	100.0			
Negative predictive value (%)	91.3			
Positive likelihood ratio	– (∞)			

Values presented correspond to the number of patients or the mean $\pm$ SD.
*One-way analysis of variance and Scheffe multiple comparison test.
†Chi-square test of independence or Fisher exact probability test.
‡Kruskal–Wallis test.
NS, not significant.

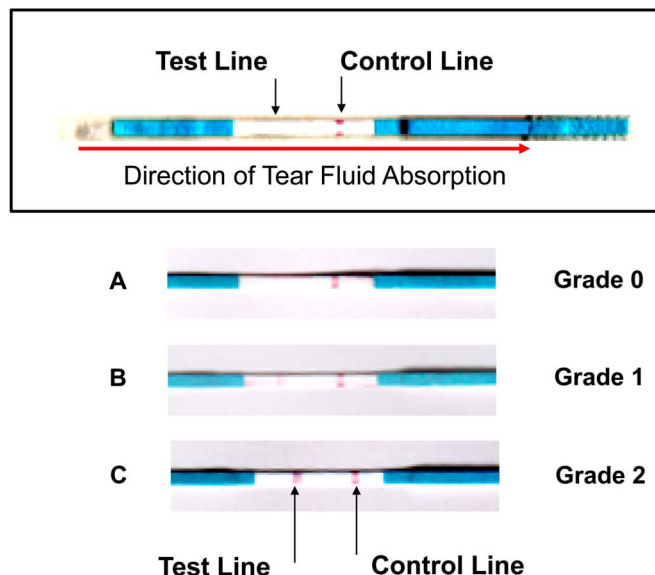


FIGURE 1. Photographs showing the results of the Allerwatch test. Results were graded according to the manufacturer's instructions as follows: grade 0 was no line (A), grade 1 was a weaker color than that of the control line (B), and grade 2 was similar to or stronger than the control line (C). Red arrow indicates the tear absorption direction.

results were based on naked eye observations and then confirmed using Scion Image software (Scion Corporation, Frederick, MD). The quantification measure was defined as the ratio between density values of a central area of 1.0 mm² in the test line versus the same area in the control line using the Scion Image. The 3 grades were defined as follows: grade 0: <0.2 times control level; grade 1: 0.2 to 1 times control level; and grade 2: >1 times control level.

Patients were divided into 3 groups based on their total tear IgE scores. In group 0, the allergic patients were classified as negative by the Allerwatch test ($n = 4$), whereas group 1 allergic patients had a test score of 1 ($n = 14$) and group 2 allergic patients had a test score of 2 ($n = 18$).

Allergens

A battery of 12 radio allergosorbent tests (RASTs) were performed using 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. The allergens included in this test panel are considered to be the main causes of allergic diseases in Japan.

Determination of Serum Total IgE and Specific IgE Levels

Determination of serum total IgE was carried out using the Phadezym IgE PRIST kit (Pharmacia, Uppsala, Sweden). In addition, blood was also collected for an assay of 12

specific IgEs in the Phadiatop CAP-RAST test (Pharmacia). All assays were carried out using undiluted serum samples. Detection of the antibodies for the 12 allergens was performed in duplicate using the radioimmunoassay version of the Pharmacia CAP-RAST system. The manufacturer's instructions were strictly followed in all tests. Briefly, test sera and standards were incubated at room temperature ($\sim 20^\circ\text{C}$) for 30 minutes with ImmunoCAP, after which the solid phase was then washed with a combination of specific polyclonal and monoclonal anti-IgE (Fc) antibodies (labeled with ^{125}I). After a further 150-minute incubation, the ImmunoCAP solid phase was washed once again, with the bound radioactivity then measured with a gamma counter. After calibration against a World Health Organization standard over the range of 0.35 to 100 kU/L for each specific IgE assay, results were calculated from the standard curve. For the Phadebas RAST, the upper and lower cutoff points were 100 and 0.35 kU/L, respectively. Results were expressed as either kilounits per liter or as a class for the 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). Solid-phase antigens in the ImmunoCAP were optimized to achieve a 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 the data.

Statistical Analyses

Chi-square test or Fisher exact test was used to test differences between the proportions or the categorical variables. One-way analysis of variance (ANOVA) and Scheffe multiple comparison test were used to compare the mean values among the 3 groups. Semiquantitative measurement differences were examined by the Kruskal–Wallis 1-way ANOVA by ranks for the 3 groups. Pearson 2-tailed correlation coefficient calculations were used to investigate the relationships between the continuous variables. Sensitivity, specificity, and positive and negative predictive values of the Allerwatch test for diagnosing allergic conjunctivitis were calculated using standard formulae. Factors significantly associated with the total tear IgE level were selected using the forward stepwise multiple logistic regression method. 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 a correlation coefficient analysis. The level of significance was set at $P < 0.05$. Analyses were performed using the StatView statistical software package (Abacus Concepts, Berkeley, CA).

RESULTS

The Allerwatch test was positive in 32 of the 36 allergic conjunctivitis patients (88.9%) and was negative in all 10 EKC patients (0.0%) and in all 32 controls (0.0%; Table 1). There was a significant difference for the positivity rate ($\chi^2 = 63.3$, $df = 2$, $P < 0.00001$, χ^2 test) and the grade (1.39 ± 0.68 vs. 0.00 ± 0.00 vs. 0.00 ± 0.00 , $P < 0.00001$, Kruskal–Wallis test) among the

allergic, EKC, and control groups, respectively (Table 1). The sensitivity, specificity, and positive and negative predictive values of the test are shown in Table 1.

The positivity rates for the specific IgE antibodies targeting the 12 test allergens were determined by CAP-RAST (Fig. 2). Among all the outdoor allergens, IgE to cedar pollen had the highest positivity rate, with a value of 59.5%, followed by cypress pollen (48.6%), ragweed (46.0%), mugwort (37.8%), and orchard grass (29.7%) IgEs. Among the indoor allergens, the highest positivity rate was observed for the IgE to house dust and *D. pteronyssinus* (54.1%), followed by acarus IgE (42.2%) and moth IgE (35.1%).

A significantly higher log (total serum IgE) value was observed for the autumnal allergic group as compared with the control and EKC groups (2.08 ± 0.56 vs. 0.91 ± 0.38 vs. 1.24 ± 0.26 , $P < 0.00001$, 1-way ANOVA and Scheffé multiple comparison test; Fig. 2). The differences among the 3 groups were also found to be significant ($P < 0.00001$, Scheffé multiple comparison test; Fig. 3).

Specific IgE levels were also compared among the groups identified by the total tear IgE score (Fig. 4). Significant differences among groups 1, 2, and 3 in the autumnal allergic group were found for the specific serum IgE antibody grades for house dust (0.25 ± 0.39 vs. 0.50 ± 0.91 vs. 2.28 ± 1.94 , $P = 0.00876$), *D. pteronyssinus* (0.25 ± 0.39 vs. 0.43 ± 0.90 vs. 2.50 ± 1.95 , $P = 0.00198$), and acarus (0.00 ± 0.00 vs. 0.50 ± 0.98 vs. 2.00 ± 1.94 , $P = 0.02162$, Kruskal–Wallis test; Fig. 4).

Table 2 shows the correlations between the total tear IgE and each log (serum IgE) value, and the multivariate odds ratios for the total tear IgE in the autumnal allergic group. The total tear IgE score was significantly correlated with the log (total serum IgE) value ($r = 0.838$, $P < 0.00001$, Pearson correlation coefficient analysis). When all variables were considered simultaneously by the multivariate logistic regression analysis with stepwise selection of variables, the only significant predictor of the total tear IgE score was the log (total serum IgE) value (odds ratio = 2.76, $P < 0.00001$).

DISCUSSION

Both the specific IgE levels and the positivity frequency for the IgE specific for house dust, *D. pteronyssinus*, and cedar

pollen were found to be higher in the autumnal allergic group. The total tear IgE level was also significantly higher in the autumnal allergic group as compared with the control and EKC groups. In addition, there was also a strong correlation with the serum IgE levels specific for house dust, *D. Pteronyssinus*, acarus, and cedar pollen in the autumnal allergic group. These results suggest that these allergens are the causes of allergic conjunctivitis during the autumn season.

Among the indoor allergens, specific IgE levels and the positivity rates were higher for house dust, *D. pteronyssinus*, and acarus in the autumnal allergic group (Fig. 2). Outdoor allergens are more closely related to seasonal allergic conjunctivitis, whereas indoor allergens seemed to be more frequently associated with perennial allergic conjunctivitis. During this season, there is a proliferation of mites because of the high temperatures and high humidity. Thus, mold and house dust mites can cause allergic conjunctivitis not only during the spring but also during the autumn because of the year-round mild and wet climate that is found in Japan.

Among the outdoor allergens, the positivity rates and specific IgE levels were high for cedar, cypress, and ragweed pollens in the autumnal allergic group (Fig. 2). There are 2 periods of high cedar pollen levels that occur every year in Japan. Fujishima et al.¹⁵ reported that the scattering of cedar pollen occurred not only during the spring but was present throughout the year, especially in the autumn in Japan. As a consequence, cedar pollen is able to cause allergic conjunctivitis both during the spring and the late autumn. In addition, the mugwort and ragweed pollen season runs from August through October, although the levels of these pollens are much lower than those noted during the early seasons. Our results demonstrated that the total tear IgE levels were correlated with the levels of specific serum IgEs for the major indoor and outdoor allergens responsible for the autumnal allergic conjunctivitis. Therefore, the Allerwatch test is a practical alternative for clinical evaluation of the local allergic reactions that affect the conjunctiva in patients with autumnal 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, there was a correlation between the specific IgE

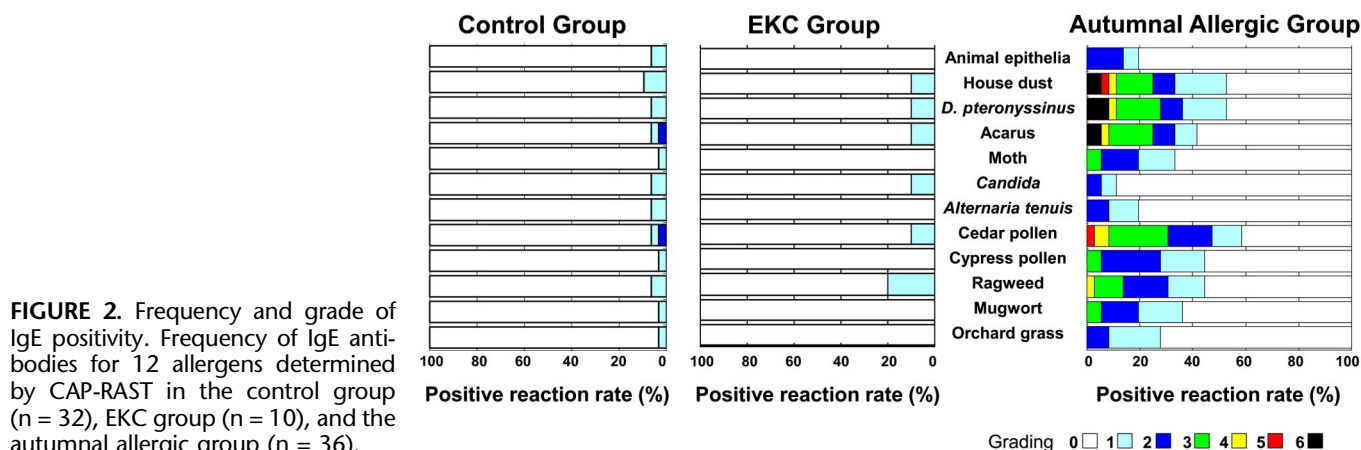


FIGURE 2. Frequency and grade of IgE positivity. Frequency of IgE antibodies for 12 allergens determined by CAP-RAST in the control group (n = 32), EKC group (n = 10), and the autumnal allergic group (n = 36).

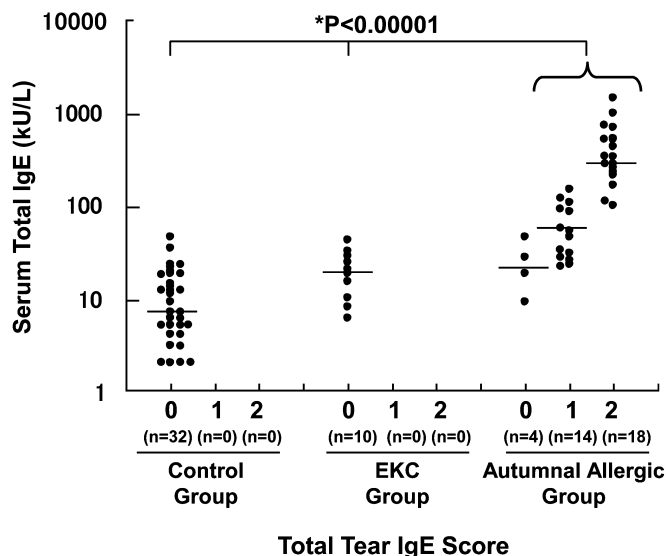


FIGURE 3. Relationship between the total tear IgE and serum IgE levels in the control, EKC, and autumnal allergic groups. The total tear IgE score was determined using the Allerwatch test (0–2), whereas the serum total IgE level was measured using the Phadezym PRIST kit. *One-way ANOVA and Scheffe multiple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

levels for house dust mites and cedar pollen and the total tear IgE score. A possible reason for why only total serum IgE was found to be a predictive factor might be related to the total IgE level. This level represents the sum of all the antigen-specific IgEs and is overwhelmingly higher than any of the specific IgE levels. However, the levels for the specific IgEs for cedar pollen and house dust mites, which are the major causes of allergic conjunctivitis, exhibited a correlation with the total tear IgE score, with the scores higher in group 2 compared with groups 0 and 1 (Fig. 4). Therefore, simultaneous calculations of the total tear IgE and the specific serum IgE levels may be important in determining the best allergic conjunctivitis treatment. Measurement of the total tear IgE level is also useful for the diagnosis of ocular allergy and for evaluating the response to treatments. Alternatively, measurements of specific IgE levels can also play a significant clinical role because these can be useful in identifying the major allergens responsible for the allergic symptoms.

Compared with the circulating serum IgE, local production of IgE is a more important contributor to the severity of allergic conjunctivitis.^{5,16,17} The aqueous portion of the tear film is secreted by the main and accessory lacrimal glands. Because of the blood–tear barrier, tear IgE levels are normally very low.¹⁸ Allergic sensitization and/or an allergic response can induce local IgE production, which leads to increases in the total tear IgE as a result of the elevated lacrimal IgE levels being filtered at the blood–tear barrier.¹⁹ Kari et al²⁰ demonstrated there is a good correlation between the conjunctival allergen provocation test and the lacrimal IgE level. Several research groups have also reported that there is a correlation between the total tear IgE and manifestations of ocular allergy.^{5,16,17} Our current results also showed that there is

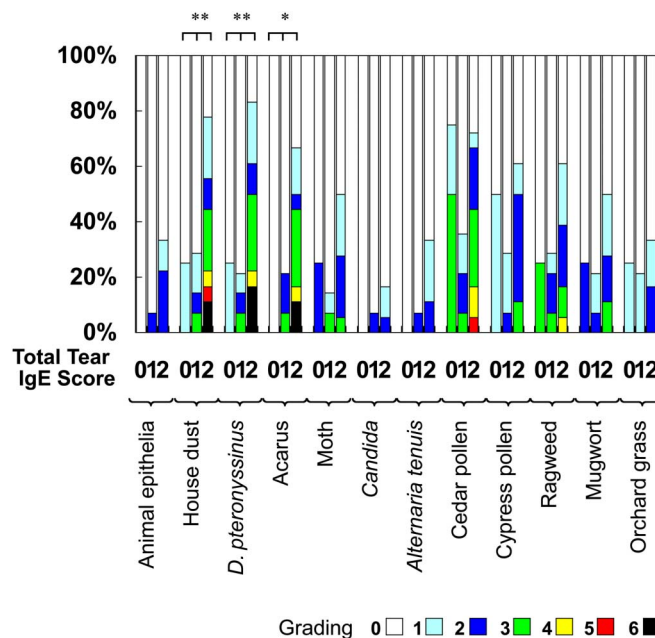


FIGURE 4. Comparison of specific serum IgE levels among the groups identified by the total tear IgE score in the autumnal allergic group. The total tear IgE score was determined using the Allerwatch test (0–2), whereas the specific serum IgE level was measured using the CAP-RAST system. The autumnal allergic group was divided into 3 subgroups based on their total tear IgE scores. In group 0, the total tear IgE score was equal to 0 ($n = 4$), whereas in groups 1 and 2, the total tear IgE scores were equal to 1 ($n = 14$) and 2 ($n = 18$), respectively. The Kruskal–Wallis test was used to determine the grades of the specific serum IgEs among the 3 groups (* $P < 0.05$, ** $P < 0.01$).

a good correlation between the total tear IgE level and the serum total or specific IgE levels. These findings suggest that immunochromatographic measurement of tear IgE should be able to provide important information on local allergic inflammation that ultimately can assist in the conventional serological diagnosis of allergic conjunctivitis.

The Allerwatch kit showed a very high sensitivity (88.9%) and specificity (100.0%). According to the manufacturer's data, the sensitivity and specificity were 73.6% and 100.0%, respectively. Sirbikaite et al⁶ reported that the sensitivity and specificity of another diagnostic kit for allergic conjunctivitis (Lacrytest; ADIATEC SA, Nantes, France) were 93.8% and 89.7%, respectively. These results are similar to those reported previously for the Lacrytest.^{6,7}

One limitation of this study is that the patients selected all had moderate to severe allergic conjunctivitis. Therefore, the sensitivity of the Allerwatch test will need to be further evaluated in patients with mild allergic conjunctivitis. Furthermore, we excluded patients with atopic keratoconjunctivitis from the study because this research focused on seasonal allergic conjunctivitis. Inada et al⁸ reported that the total IgE score was significantly higher in atopic and vernal keratoconjunctivitis than in seasonal and perennial allergic conjunctivitis. In the future, we will investigate the relationship between the

TABLE 2. Correlations Between Total Tear IgE and Log (specific serum IgE) Values, and Multivariate Odds Ratios for Total Tear IgE in the Autumnal Allergic Group (n = 36)

Variables	Correlation Coefficients		Multivariate Analysis	
	r (95% CI)	P	OR (95% CI)	P
Total IgE	0.84 (0.70 to 0.92)	<0.00000	2.76 (0.79 to 1.25)	<0.00001
Animal epithelium	0.21 (−0.12 to 0.51)	0.21282	—	—
House dust	0.51 (0.22 to 0.72)	0.00141	—	—
<i>Dermatophagoides pteronyssinus</i>	0.53 (0.25 to 0.73)	0.00083	—	—
Acarus	0.49 (0.20 to 0.71)	0.00216	—	—
Moth	0.25 (−0.08 to 0.54)	0.13705	—	—
<i>Candida</i>	0.21 (−0.13 to 0.50)	0.22993	—	—
<i>Alternaria tenuis</i>	0.15 (−0.18 to 0.46)	0.37208	—	—
Cedar pollen	0.33 (0.01 to 0.60)	0.04967	—	—
Cypress pollen	0.31 (−0.02 to 0.58)	0.06421	—	—
Ragweed	0.31 (−0.02 to 0.58)	0.06393	—	—
Mugwort	0.33 (0.01 to 0.60)	0.04511	—	—
Orchard grass	0.21 (−0.13 to 0.50)	0.22700	—	—

To obtain a normal distribution, specific serum IgE data were converted to log IgE values before the statistical analyses. Correlations between the total tear IgE levels were then calculated using 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; r, Pearson correlation coefficient.

total IgE level in tears and the specific serum IgE levels in patients with atopic keratoconjunctivitis.

In conclusion, our study confirmed that the total tear IgE score, as measured by immunochromatography, was strongly correlated with serum total IgE and specific serum IgE levels for house dust mites and cedar pollen in patients with autumnal allergic conjunctivitis. Immunochromatographic measurement of the total tear IgE also proved to be useful as a rapid point-of-care assay for distinguishing between allergic and infectious causes in patients with acute conjunctivitis.

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