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Correlations between allergen-specific IgE serum levels in patients with allergic conjunctivitis in spring

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Abstract *Purpose:* The purpose of this study was to evaluate the relation between various specific class E immunoglobulins (IgE) in serum and allergic conjunctivitis in spring. *Methods:* Forty patients with allergic conjunctivitis and fifty healthy volunteers were selected for this trial. Total IgE and specific IgE to twelve inhalant allergens were measured using the CAP system. *Results:* Specific IgE levels caused by cedar and cypress pollen, house dust, *Dermatophagoides pteronyssinus*, acarus, and moth allergens were higher in the allergy group than in the control group. No correlation was proven between the specific IgE levels and the total IgE level. The highest positivity rate for a specific allergen in patients with allergic conjunctivitis was 52.5% for cedar pollen, followed by cypress pollen at 37.5%. *Conclusions:* These results suggest that cypress pollen was the largest cause of allergic conjunctivitis and that the total IgE level had no correlation with each specific IgE.

Key words Allergic conjunctivitis; CAP-RAST system; cedar pollen; specific IgE; total IgE

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Introduction Ocular allergic disease includes seasonal allergy such as pollenosis, year-round allergy, and atopic conjunctivitis. The increasing number of cases of allergic conjunctivitis has resulted in a growing social concern in recent years.

As allergic conjunctivitis is one of the most common diseases in Japan and can be caused by various allergens, it is crucial to detect the causal allergens. The clinical symptoms of the allergic state are season (pollen)-dependent and can appear in the forms of conjunctivitis, rhinitis, or bronchial asthma.¹⁻³ Other antigens, however, have been ignored and little information is available about fungi-, house dust-, and mite-induced IgE antibodies in patients with allergic conjunctivitis. The sensitized antigen must therefore be located in each individual patient in order to diagnose the allergic conjunctivitis.

The aim of this study was to evaluate the relation between specific IgE in serum and allergic conjunctivitis in spring. Using the CAP radioallergosorbent (CAP-RAST) system, we investigated the correlation between total and specific IgE in patients with allergic conjunctivitis raised against various allergens that have been reported to cause allergic diseases.

Materials and methods

SUBJECTS This study was performed in accordance with the Helsinki Declaration of 1975 and its 1983 revision. All participants provided written informed consent before being tested. Forty consecutive outpatients with allergic conjunctivitis, who had been treated at the Okamoto Eye Clinic between February and June 2002, were selected for this trial. PhadiatopTM (Pharmacia, Uppsala, Sweden) and the determination of total IgE levels were performed in duplicate on sera obtained from each of the subjects in the two groups. Group One, the allergic conjunctivitis group, consisted of 40 patients suffering from acute seasonal allergic conjunctivitis (13 M, 27 F, mean age: 59.9 ± 15.0 , range: 19–77). Group Two, the control group, was made up of 50 healthy, non-smoking volunteers with no history of allergic diseases. All had negative skin-prick test results using Phazet[®] (Pharmacia), and none wore contact lenses or had been treated with a topical or systemic drug in the preceding six weeks (23 M, 27 F, mean age: 41.3 ± 10.7 , range: 23–62). IgE levels were compared between these two groups.

ALLERGENS In all, twelve RAST were performed using the following allergens: a mixture in equal parts of 5-animal epithelia (cat epithelium/dander, dog, guinea pig, rat, mouse), house dust, *Dermatophagoides pteronyssinus*, acarus, moth, *Candida*, *Alternaria tenuis*, Japanese cedar (*Cryptomeria japonica*) pollen, Japanese cypress (*Chamaecyparis obtusa*) pollen, ragweed, mugwort, and orchard grass. These groups include the allergens that are the main causes of allergy in Japan.

DEFINITION OF ALLERGIC CONJUNCTIVITIS Allergic conjunctivitis was diagnosed according to the guidelines of the diagnosis and treatment of conjunctivitis.⁴ Allergic conjunctivitis was diagnosed by slit-lamp examination looking for clinical symptoms such as conjunctival follicle, papillae, redness, and seasonal symptoms such as ocular

itching and tearing without proliferative lesions. Allergic conjunctivitis was diagnosed by two doctors (TM, SO).

DETERMINATION OF TOTAL AND SPECIFIC IgE IN SERUM The determination of total IgE was performed with the Phadezym IgE PRIST® (Pharmacia). Blood was drawn for an assay on specific IgEs in serum using the Pharmacia CAP system. Assays were carried out on undiluted sera against each of the 12 allergens. The assays were carried out in duplicate and the radioimmunoassay version of the Pharmacia CAP system was used.⁵⁻⁷ 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 min, then washed. IgE (Fc)-specific combinations of polyclonal and monoclonal anti-IgE labeled with ¹²⁵I were added. After a second incubation step of 150 min, the ImmunoCAP was washed and the bound radioactivity counted in a gamma counter. The specific IgE assay was calibrated against a WHO standard for IgE, in the range 0.35–100 kU/l. Results were read from a standard curve. The upper and lower cutoff points were 100 kU/l and 0.35 kU/l as for the Phadebas RAST, and the results could be expressed either as kU/l or as classes (0–6, as for RAST, but where class 0 is <0.35 kU/l, class 1 is 0.35–0.69 kU/l, class 2 is 0.70–3.49 kU/l, class 3 is 3.50–17.49 kU/l, class 4 is 17.50–49.99 kU/l, class 5 is 50.00–100.00 kU/l, and class 6 is >100 kU/l). The solid-phase allergens in the ImmunoCAP were optimized to give maximum binding with low background levels, even when undiluted test sera were used.

STATISTICAL ANALYSIS The unpaired t-test was performed to compare mean values. The level of significance was set at $p < 0.05$. The relation between continuous variables was investigated by means of Pearson's correlation coefficient and partial correlation coefficient. The correlation coefficient was evaluated by Fisher's Z transformation and the Bartlett test. Calculations were done using the Stat View statistical software package (Abacus Concepts, Berkeley, CA, USA).

Results Total IgE was higher in the allergy group than in the control group (Table 1). Specific IgE antibody levels were high against the year-round house dust, *D. pteronyssinus*, acarus, and moth allergens. Specific IgE antibody levels were also high against the seasonal cedar and cypress pollen allergens, but showed no significant difference against ragweed, mugwort, and orchard grass (Table 1).

The positive frequency of specific IgE antibodies against the 12 allergens was determined using CAP-RAST (Fig. 1). The highest rate was 52.5% for cedar pollen, followed by cypress pollen (37.5%), moth (30.0%), and *D. pteronyssinus* (30.0%). In contrast, the positive rates for *Candida*, *Alternaria*, and animal epithelia were low.

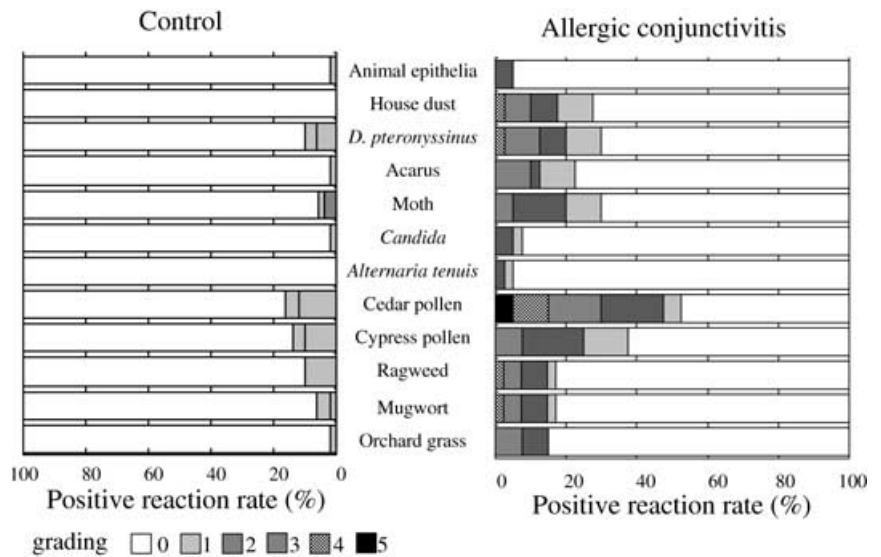
Specific IgE antibody levels were compared between the 21 cedar-positive and the 19 cedar-negative patients. All specific IgE levels were higher in the cedar pollen-positive patients, but this increase was not always statistically significant. Antibodies produced in reaction to *D. pteronyssinus*, acarus, moth, and cypress pollen showed a highly significant difference between the positive and negative groups (Table 2).

TABLE 1. Comparisons of various specific IgEs between the control group and the allergic conjunctivitis group.

	Control	Allergic conjunctivitis	t-test
Number of patients	50	40	
Age (years)	41.3 ± 10.7	59.9 ± 15.0	
Male/Female	23/27	13/27	
	IgE level	IgE level	
Total IgE	10.32 ± 7.06	147.88 ± 252.70	0.0016
Animal epithelia	0.11 ± 0.14	0.18 ± 0.35	NS
House dust	0.11 ± 0.06	1.11 ± 3.08	0.049
<i>D. pteronyssinus</i>	0.18 ± 0.20	1.25 ± 3.23	0.047
Acarus	0.11 ± 0.13	0.76 ± 1.75	0.028
Moth	0.13 ± 0.13	0.58 ± 1.07	0.013
<i>Candida</i>	0.13 ± 0.10	0.18 ± 0.18	NS
<i>Alternaria tenuis</i>	0.12 ± 0.06	0.16 ± 0.14	NS
Cedar pollen	0.34 ± 0.49	7.97 ± 16.4	0.0059
Cypress pollen	0.25 ± 0.40	0.72 ± 1.26	0.031
Ragweed	0.28 ± 0.46	1.51 ± 6.73	NS
Mugwort	0.16 ± 0.15	0.40 ± 0.94	NS
Orchard grass	0.12 ± 0.14	0.20 ± 0.35	NS

Comparisons between the control group and the allergic conjunctivitis group are shown. Number of patients or mean ± standard deviation. NS, not significant.

Fig. 1. Frequency of IgE antibodies and grading of IgE. Frequency of IgE antibodies against 12 allergens determined by CAP-RAST in allergic conjunctivitis group (n = 40) and control group (n = 50).



Correlation analysis was performed between each specific IgE antibody level (Table 3). No correlation could be demonstrated between specific IgE levels and total IgE level. Using both Pearson's correlation coefficient and partial correlation coefficient, positive significant correlations were only observed between house dust-*D. pteronyssinus* and cedar-cypress pollen.

It was confirmed by the Bartlett test that there was correlation between some variables in the conjunctivitis group ($\chi^2 = 871.1$, $p < 0.0001$), but none in the control group.

	<i>Cedar pollen (+)</i> (<i>N</i> = 21)			<i>Cedar pollen (-)</i> (<i>N</i> = 19)			<i>t</i> -test
	<i>IgE</i> level	<i>N</i>	(%)	<i>IgE</i> level	<i>N</i>	(%)	
Total <i>IgE</i>	176.29 ± 180.32			116.47 ± 310.84			NS
Animal epithelia	0.26 ± 0.46	2	(9.5%)	0.09 ± 0.07	0	(0.0%)	NS
House dust	1.99 ± 4.05	10	(47.6%)	0.13 ± 0.09	1	(5.3%)	NS
<i>D. pteronyssinus</i>	2.23 ± 4.23	11	(52.4%)	0.16 ± 0.09	1	(5.3%)	0.041
Acarus	1.33 ± 2.27	8	(38.1%)	0.12 ± 0.09	1	(5.3%)	0.027
Moth	0.91 ± 1.39	9	(42.9%)	0.21 ± 0.19	3	(15.8%)	0.037
<i>Candida</i>	0.23 ± 0.22	3	(14.3%)	0.13 ± 0.08	0	(0.0%)	NS
<i>Alternaria tenuis</i>	0.19 ± 0.18	2	(9.5%)	0.13 ± 0.06	0	(0.0%)	NS
Cedar pollen	15.05 ± 20.09	21	(100.0%)	0.14 ± 0.08	0	(0.0%)	0.0034
Cypress pollen	1.28 ± 1.53	15	(71.4%)	0.11 ± 0.08	0	(0.0%)	0.0027
Ragweed	2.75 ± 9.11	6	(28.6%)	0.14 ± 0.08	1	(5.3%)	NS
Mugwort	0.67 ± 1.24	7	(33.3%)	0.10 ± 0.07	0	(0.0%)	NS
Orchard grass	0.28 ± 0.47	4	(19.0%)	0.11 ± 0.08	0	(0.0%)	NS

IgE levels (mean ± standard deviation) or number (*N*) of *IgE*-positive patients. Numbers in parentheses are percentages. NS, not significant.

Discussion In-vitro tests for *IgE* constitute a technically valid method for the detection of allergen-specific *IgE* antibodies in serum.^{8,9} In patients suffering from diseases with a possible allergic cause, it would be helpful to have a rapid and easy screening test to diagnose an allergy. Taking a medical history has not proved to have sufficient diagnostic value, except in patients suffering from seasonal symptoms.¹⁰

There was no correlation between the each specific *IgE* level and the total *IgE* level. Therefore, the total *IgE* level can be used to determine the parameters of an allergy, but not the *IgE* level caused by each specific allergen. In this study, the specific *IgE* levels and the positive frequency of specific *IgE* against house dust, *D. pteronyssinus*, acarus, moth, cedar pollen, and cypress pollen was found to be high, suggesting that we should consider these allergens to be causes of allergic conjunctivitis in Japan. There are two continuous pollen seasons with high pollen levels in Japan. The cedar season runs from the end of January to the beginning of April and the cypress season from the beginning of March until the end of May. In addition, there is the mugwort and ragweed pollen season from August until October, although the pollen levels of these plants are much lower than those of the first two. The symptoms of seasonal allergic conjunctivitis, which develop from spring to summer, are generally caused by cedar and cypress pollen. The year-round allergens also cause allergic conjunctivitis from spring to early autumn. This is not only because it is the pollen season of various plants such as ragweed, mugwort, and orchard grass, but also because mites proliferate in the high temperatures and high humidity of this season.

There was no significant difference in specific *IgE* levels between the control and allergic conjunctivitis groups with regard to ragweed, mugwort, and orchard grass. This may be because these allergens are only seen from summer through fall.

TABLE 2. Comparison of *IgE*/specific *IgE* between cedar pollen-positive and -negative allergic conjunctivitis patients.

TABLE 3. Correlation coefficients between allergens in allergic conjunctivitis patients.

	IgE	Epithelia	House dust	<i>D. pteronyssinus</i>	Acarus	Moth	<i>Candida</i>	<i>Alternaria</i>	Cedar	Cypress	Ragweed	Mugwort
Epithelia	0.021	0.312										
House dust	0.134	0.337	0.997									
<i>D. pteronyssinus</i>	0.143	0.704	0.402	0.462								
Acarus	0.175	0.909	0.271	0.290	0.600							
Moth	0.140	0.809	0.278	0.297	0.498	0.793						
<i>Candida</i>	-0.003	0.699	0.365	0.416	0.757	0.528	0.615					
<i>Alternaria</i>	0.088	0.113	0.000	0.014	0.067	0.288	0.143	0.110				
Cedar	0.209	0.266	0.173	0.193	0.233	0.426	0.266	0.282	0.860			
Cypress	0.251	0.017	-0.028	-0.027	-0.010	0.054	0.039	-0.064	0.254	0.327		
Ragweed	0.123	0.569	0.643	0.615	0.255	0.616	0.447	0.129	0.123	0.214	0.296	
Mugwort	0.131	0.753	0.324	0.305	0.337	0.766	0.598	0.231	0.137	0.171	0.129	0.860
Orchard grass	0.131	0.753	0.324	0.305	0.337	0.766	0.598	0.231	0.137	0.171	0.129	0.860

Correlation coefficients were calculated using the Pearson product moment formula.

Patients who were positive for cedar pollen had a high IgE level for cedar and cypress pollen, moth, house dust mite, and acarus. Therefore, there is multiple antigen sensitivity in this group.

Although there are many kinds of specific IgEs which correlate with each other, only house dust, *D. pteronyssinus*, and cedar and cypress pollen showed a significant correlation using both Pearson's and the partial correlation coefficient. *D. pteronyssinus* and acarus are sarcoptes, which cause a long pathologic antigen-antibody reaction in the human body. A high correlation between house dust and *D. pteronyssinus* indicates that mites occupy an important position in house dust. The elimination of house dust will, therefore, lead to elimination of the mite. Our results have clinical significance. Consequently, we can employ the correlation results shown in this study to advise patients on which allergens to avoid and treat in advance in the season with high positive antigen levels.

References

- 1 Suzuki M, Itoh H, Sugiyama K, et al. Causative allergens of allergic rhinitis in Japan with special reference to silkworm moth allergen. *Allergy*. 1995;50:23-27.
- 2 Plebani M, Faggian D, Borghesan F. Full automation in allergy testing: measurement of specific IgE by the ENEA System. *Allergy*. 1995;50:229-233.
- 3 Kerkhof M, Schouten JP, de Monchy JG. The association of sensitization to inhalant allergens with allergy symptoms: the influence of bronchial hyperresponsiveness and blood eosinophil count. *Clin Exp Allergy*. 2000;30:1387-1394.
- 4 Ben Ezra D. Guidelines on the diagnosis and treatment of conjunctivitis. *Ocul Immunol Inflamm*. 1994;2(Suppl):17-26.
- 5 Axen R, Drevin H, Kober A, et al. A new laboratory diagnostic system applied to allergy testing (abstract). *Allergy Proc*. 1998;9:503.
- 6 Duc J, Peitrequin R, Pécoud A. Value of a new screening test for respiratory allergy. *Allergy*. 1988;43:332-337.
- 7 Ewan PW, Coote D. Evaluation of a capsulated hydrophilic carrier polymer (the ImmunoCAP) for measurement of specific IgE antibodies. *Allergy*. 1990;45:22-29.
- 8 Lockey R, Lichtenstein L, Bloch K, et al. Position statement. The use of in vitro tests for IgE antibody in the specific diagnosis of IgE-mediated disorders and in the formulation of allergen immunotherapy. *J Allergy Clin Immunol*. 1992;90:263-267.
- 9 Terr AI. In vitro tests for immediate hypersensitivity. *Annu Rev Med*. 1998;39:135-145.
- 10 Pécoud A, Bonstein HS, Frei PC. Value of the case history in diagnosis of allergic state and the detection of allergens. *Clin Allergy*. 1983;13:141-147.

