

ASSOCIATED DISEASES

Immunochromatographic Assay for Measurement of Total IgE in Tears, Nasal Mucus, and Saliva of Patients with Allergic Rhinoconjunctivitis

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Objective. Measurement of total immunoglobulin E (IgE) is clinically important for the diagnosis of allergic diseases. The total serum IgE level is normally measured because of its widespread use and convenience, but little attention has been paid to the measurement of local IgE concentrations. We evaluated whether the measurement of local production of IgE in tears, saliva, and nasal mucus was useful for the diagnosis of allergic rhinoconjunctivitis. **Methods.** A prospective, nonrandomized, cross-sectional study was conducted in 33 consecutive patients with seasonal allergic rhinoconjunctivitis (allergic group) and in 30 age- and sex-matched healthy control subjects (control group). The total IgE level was measured in tears, saliva, and nasal mucus from all subjects. Using a 4- or 5-point scale, symptoms (sneezing, rhinorrhea, nasal obstruction, ocular itching, and lacrimation) were assessed in each subject along with the activities of daily living (ADL) score and total symptom score for allergic conjunctivitis. **Results.** Total IgE could be assayed within 10 minutes of collection in all samples. The scores for all symptoms were higher in the allergic group than in the control group ($p < .00001$). The IgE scores for tear fluid samples ($p < .00001$) and undiluted saliva ($p = .00003$) were significantly higher in the allergic group than in the control group. The total IgE score of tear fluid samples was strongly correlated with the severity of symptoms of allergic conjunctivitis, including ocular itching ($r = 0.769, p < .00001$), tearing ($r = 0.560, p = .00035$), and ocular symptom score ($r = 0.329, p = .03452$). On the contrary, the total IgE scores for both saliva and nasal mucus were correlated with the severity of rhinitis-related symptoms, including sneezing (saliva $r = 0.897, p < .00001$; nasal mucus $r = 0.871, p = .00024$), nose blowing (saliva $r = 0.764, p < .00001$; nasal mucus $r = 0.829, p = .00080$), and nasal obstruction (saliva $r = 0.519, p = .00099$; nasal mucus $r = 0.745, p = .00429$). The ADL score was correlated with the total IgE level in each specimen (tear $r = 0.705, p < .00001$; saliva $r = 0.468, p = .00301$; nasal mucus $r = 0.479, p = .06816$). **Conclusions.** These results suggest that local production of IgE is closely correlated with local allergic symptoms. This rapid test for the measurement of local IgE is easy to perform on an outpatient basis and may be helpful in the diagnosis of allergic rhinitis and conjunctivitis.

Keywords allergic rhinoconjunctivitis, immunochromatography, nasal mucus, saliva, tear, total IgE

INTRODUCTION

The increasing number of persons with allergic rhinoconjunctivitis has become a growing social problem over recent years. The symptoms of seasonal allergy are mainly related to rhinitis and conjunctivitis. Allergic rhinitis is a common chronic condition that significantly impairs the patient's quality of life and its prevalence has increased markedly over the recent decades (1, 2). Allergic conjunctivitis is also one of the most common allergic diseases and has become increasingly prevalent (3–5). A close relation between allergic rhinitis and allergic conjunctivitis has been demonstrated by an epidemiological study (3) and the combination of allergic nasal and ocular symptoms (rhinoconjunctivitis) is extremely common.

The diagnosis of allergic rhinitis is normally based on the history (including symptoms of nasal congestion, sneezing, and clear rhinorrhea), an elevated total serum immunoglobulin E (IgE) concentration, and the detection

of an IgE response to allergens. On the contrary, a diagnosis of allergic conjunctivitis is based on the symptoms of ocular itching, hyperemia, mucus discharge, and tearing. Measurement of the total IgE level in tear fluid is also useful (6–8). Several research groups have reported a new immunochromatographic method for the measurement of total IgE in tears (9–11). In addition, a commercial kit (Allerwatch®, Hitachi Chemical Co., Ltd., Tokyo, Japan; Wakamoto Pharmaceutical Co., Ltd., Tokyo, Japan) has been developed and was recently released in Japan. This kit is currently being used for the diagnosis of allergic conjunctivitis in clinical trials. However, immunochromatographic measurement of the local IgE level in tears, saliva, and nasal mucus has not been done to date.

The objectives of this study were as follows: (1) to assess the clinical value of the Allerwatch® (a novel immunochromatographic test) when screening for allergic rhinitis and conjunctivitis by measuring the total IgE level in tear fluid, saliva, and nasal mucus; (2) to compare patients with seasonal allergic rhinoconjunctivitis and a control group in a cross-sectional study; and (3) to investigate the correlation between local IgE production and symptoms of allergic rhinoconjunctivitis graded for severity in patients with seasonal rhinoconjunctivitis.

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MATERIAL AND METHODS

Study Design

This was a prospective, nonrandomized, cross-sectional, consecutive case study conducted at the university hospital of 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 also obtained from each subject.

Subjects

All subjects had a Schirmer 1 test score greater than 15 mm and normal meibomian glands. None of them wore contact lenses or 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 or bacterial conjunctivitis, were excluded. Two groups were enrolled in this trial (Table 1). Group 1 was the allergic group, consisting of 33 consecutive outpatients suffering from acute seasonal allergic rhinitis and conjunctivitis, who were treated at our hospital between February and May 2009 (14 males and 19 females; mean age 29.5 ± 11.5 years; range 8–48 years). The criteria for inclusion in this study were (1) patients with acute seasonal allergic rhinoconjunctivitis, (2) duration of rhinoconjunctival symptoms (for 3 days or more), (3) symptoms of allergic conjunctivitis (ocular symptom score 1 or greater), and (4) symptoms of allergic rhinitis (sneezing and nasal symptom of nasal itching, rhinorrhea, or nasal obstruction). The mean duration of rhinoconjunctival symptom was 7.3 ± 3.4 days (range 3–14 days). All had positive skin prick test responses to Japanese cedar (*Cryptomeria japonica*) pollen using Phazet® (Pharmacia, Uppsala, Sweden) and positive specific serum IgE to cedar pollen using the CAP radioallergosorbent test (CAP RAST) system (Pharmacia). Group 2 was the age- and sex-matched control group, consisting of 30 healthy non-smoking volunteers with no history of allergic diseases (13 males and 17 females; mean age 29.6 ± 8.4 years; range 13–42 years). All had negative skin prick test results. All patients in the allergic group had active allergic rhinoconjunctivitis during the trial. Total IgE levels in nasal mucus, saliva, and tear fluid were compared between the two groups.

Definition of Allergic Rhinitis

Two definitions of allergic rhinitis were considered (12). Physician-diagnosed allergic rhinitis was defined by an

TABLE 1.—Patient characteristics.

	Control group	Allergic group	<i>p</i>
Number of patients	30	33	
Age (years)	29.6 ± 8.4	29.5 ± 11.5	NS*
Male/female	13/17	14/19	NS**

Note. Comparisons between the control group and the allergic group are shown. Data are the number of patients or mean $\pm$ standard deviation. NS = not significant. *Unpaired *t*-test, ** χ^2 test.

affirmative patient or parental answer to the question: "Have you ever being diagnosed by a physician as having allergic rhinitis?" Information on symptoms of allergic rhinitis was obtained by answering a questionnaire following a positive response to the question: "Have you ever had a problem with sneezing, or a runny or blocked nose, when you did not have a cold or the flu?"

The scores for nasal allergic symptoms and severity were calculated in accordance with the Practical Guidelines for the Management of Allergic Rhinitis in Japan 2005 (13). Patients assessed each symptom on a 5-point scale as shown in Table 2, 0 = absent; 1 = mild; 2 = moderate; 3 = severe, and 4 = very severe, as follows: sneezing and rhinorrhea (0 = none, 1 = 1–5 episodes, 2 = 6–10 episodes, 3 = 11–20 episodes, 4 = >20 episodes), and nasal obstruction (0 = none, 1 = present, no mouth breathing; 2 = marked, frequent mouth breathing; 3 = severe, considerable mouth breathing; 4 = complete, mouth breathing all day).

Activities of Daily Living Score

The activities of daily living (ADL) score was calculated from information provided by patients or family members

TABLE 2.—Symptom scores for allergic rhinitis and ADL.

Symptoms	Severity	Score
Sneezing		Number of sneezing attacks per day
	No symptoms	0 = 0
	Mild	1 = 1–5
	Moderate	2 = 6–10
	Severe	3 = 11–20
	Most severe	4 > 20
Nose blowing		Number of nose blowings per day
	No symptoms	0 = 0
	Mild	1 = 1–5
	Moderate	2 = 6–10
	Severe	3 = 11–20
	Most severe	4 > 20
Nasal obstruction		Frequency of symptom
	No symptoms	0 = None
	Mild	1 = Present (no mouth breathing)
	Moderate	2 = Marked (frequent mouth breathing)
	Severe	3 = Severe (considerable mouth breathing)
	Most severe	4 = Complete (mouth breathing all day)
ADL score		Severity of ADL*
	No symptoms	0 = No problems
	Mild	1 = Mild symptoms not causing difficulties with ADL
	Moderate	2 = Moderate symptoms and some difficulties with ADL
	Severe	3 = Severe symptoms and major difficulties with ADL
	Very severe	4 = Very severe symptoms

Note. ADL, activities of daily living.

*Difficulties with daily activities such as working, housekeeping, studying, reading, going outdoors, and sleeping.

about daily activities, such as working, housekeeping, studying, reading, doing sports, going outdoors, sleeping, and having a conversation (14). The ADL score ranged from 0 to 4: 0 = no problems, 1 = mild symptoms not causing difficulties with ADL, 2 = moderate symptoms causing some difficulties with ADL, 3 = severe symptoms causing major difficulties with ADL, and 4 = very severe symptoms (Table 2).

Definition of Allergic Conjunctivitis

Allergic conjunctivitis was diagnosed by an ophthalmologist (T.M.) according to the guidelines of diagnosis and treatment of conjunctivitis (15). This involved slit lamp examination to detect findings, such as conjunctival follicles, papillae, and redness, a history of seasonal symptoms (such as ocular itching and tearing) without proliferative lesions, and positive RAST test and skin-prick tests to cedar pollen. We excluded patients with atopic conjunctivitis and infectious conjunctivitis.

Symptoms of allergic conjunctivitis were evaluated on the following scale proposed by Abelson et al. (16): ocular itching (0 = none, 1 = slight, with an occasional tingling sensation; 2 = moderate, but no need to rub the eyes; 3 = severe, with a need to rub eye; and 4 = unbearable, with a compulsive desire to rub the eyes) and lacrimation (0 = none, 1 = slight; 2 = moderate, with occasional tears; and 3 = severe, with tears running down the cheeks).

In addition, the total symptom score for allergic conjunctivitis was calculated for nine symptoms (ocular itching, conjunctival injection, discharge, tearing, foreign body sensation, overall discomfort, lid swelling, burning, and photophobia) as follows: score 0 = none, score 1 = 1–2 symptoms, score 2 = 3–4 symptoms, score 3 = 5–6 symptoms, and score 4 = 7–9 symptoms (Table 3).

Determination of Total IgE

Determination of total IgE was performed using the Allerwatch[®] kit according to the manufacturer's instructions. Before collection of tear fluid, saliva, and nasal mucus, the subjects did not eat or drink for at least 1 hour. To collect tears, a filter strip was placed in the lower fornix of the right eye and was removed when the strip had been wetted to the control line. Unstimulated saliva and whole nasal mucus samples were collected using sterile polyethylene pipettes or with a capillary tube, while taking great care to avoid touching the oral and nasal cavities, and were transferred into 2.0 ml Eppendorf tubes. Then a filter strip was placed into each Eppendorf tube containing samples and was removed when the sample reached the control line. If the strip could not absorb a sample due to its high viscosity, the sample was diluted twofold with phosphate-buffered saline and filtered through a 0.45- μ m pore filter (Millipore, Tokyo, Japan). Subsequently, each strip wetted with a sample was immersed in 100 μ l of reaction buffer solution in a 2.0-ml Eppendorf tube for 10 minutes at room temperature. During the test, IgE in

TABLE 3.—Modified grading system for allergic conjunctivitis*.

Symptoms	Score
Itching	0 = None 1 = Slight (occasional tingling sensation) 2 = Moderate (no need to rub eyes) 3 = Severe (with a need to rub eyes) 4 = Unbearable (compulsive desire to rub eyes)
Tearing	0 = None 1 = Slight 2 = Moderate (occasional tears) 3 = Severe (tears running down cheeks)
Total ocular symptom score	The number of symptoms of allergic conjunctivitis (1, ocular itching; 2, conjunctival injection; 3, discharge; 4, tearing; 5, foreign body sensation; 6, overall discomfort; 7, lid swelling; 8, burning; 9, photophobia) 0 = 0 1 = 1–2 2 = 3–4 3 = 5–6 4 = 7–9

Note. *Abelson and associates (16).

the sample first reacts with a gold-labeled polyclonal anti-human IgE antibody. Then anti-human IgE bound to total IgE migrates through the immunochromatographic membrane and is captured by monoclonal mouse anti-human IgE antibody immobilized at the test line on the strip. Gold-labeled anti-human IgE antibody is also immobilized at a control line on the same membrane. The red color of the test line depends on the total IgE level (Figure 1). With normal values below 2.0 kU/L, the test line is invisible. The results were divided into the following three categories: grade 0 was no visible test line; grade 1 (low total IgE level) was a weaker test line than the control line; and grade 2 (high total IgE level) was a test line similar to or stronger than the control line. If highly viscous samples failed to reach the control line, the assay was considered invalid. Results were determined by visual observation and confirmed using Scion Image software (Scion Corporation, Frederick, MD, USA).

Statistical Analysis

The unpaired *t*-test was performed to compare the mean values. Frequency analysis was performed with χ^2 or Fisher's exact test. The Mann–Whitney *U* test was used for the comparison of semiquantitative measurements between the two groups. Sensitivity, specificity, and positive and negative predictive values were calculated with standard formulas. Correlations among the variables were investigated by calculating Spearman's rank-order correlation coefficients and partial correlation coefficients. The differences between correlation coefficients were tested by Fisher's *Z* transformation. The level of significance

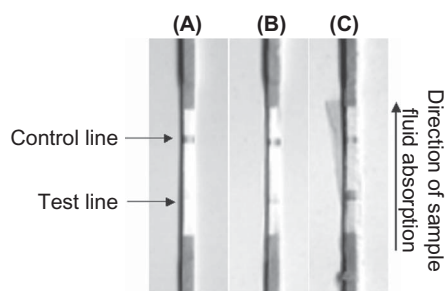


FIGURE 1.—Photographs showing the results of Allerwatch® tests. The results were graded according to the manufacturer's instructions on a scale of 0–3 as follows: grade 0, no visible test line (A); grade 1, weaker test line than the control line (B); and grade 2, test line similar to the control line or stronger than the control line (C). Arrow indicates the direction of tear absorption.

was set at $p < .05$. Calculations were done with the Stat View statistical software package (Abacus Concepts, Berkeley, CA, USA).

RESULTS

Symptom scores for allergic rhinitis and conjunctivitis are shown in Figure 2. The symptom scores of the allergic group were higher for all items than those of the control group (all $p < .00001$, Mann–Whitney U test).

Samples of tears and saliva were obtained from all patients, but the collection rate for nasal mucus was low (control group: 0%, allergic group: 33%) (Table 4). Using undiluted samples, the highest success rates for the measurement of total IgE was obtained with tear fluid (control group: 100.0%; allergic group: 100.0%), followed by saliva (control group: 86.7%; allergic group: 72.7%), and then nasal mucus (control group: 0% (no samples); allergic group: 45.5%) as shown in Table 4. The success rate was improved by diluting samples and this increased it to 100.0% for saliva [$\chi^2 = 4.2857$, degrees of freedom (df) = 1, $p = .05620$, Fisher's exact test] in the control group, and significantly increased it to 100% for both

saliva ($\chi^2 = 10.4211$, df = 1, $p = .00104$, Fisher's exact test) and nasal mucus ($\chi^2 = 8.2500$, $p = .00619$, Fisher's exact test) in the allergic group (Table 4).

The highest positive rate was 84.8% for tears, followed by saliva (66.7%) and nasal mucus (60.0%) when undiluted samples were tested in the allergic group (Table 4), but there was no significant difference between the three samples ($\chi^2 = 3.2454$, df = 2, $p = .19737$, Fisher's exact test). When diluted samples from the allergic group were tested, the positive rate was 63.6% for saliva and 63.6% for nasal mucus (Table 4). There was no significant difference of the positive rates between undiluted and diluted saliva ($\chi^2 = 0.0560$, df = 1, $p = .81290$, χ^2 test) or undiluted and diluted nasal mucus ($\chi^2 = 0.0194$, df = 1, $p = .88924$, Fisher's exact test) samples.

Total IgE scores for tears and undiluted saliva were significantly higher in the allergic group than in the control group (tears: $p < .00001$, and undiluted saliva: $p = .00003$, Mann–Whitney U test) (Figure 3).

The sensitivity and specificity of the test for detecting IgE in undiluted samples were highest for tears (sensitivity = 0.848, specificity = 1.000), followed by saliva (sensitivity = 0.667, specificity = 0.962) and nasal mucus samples (sensitivity = 0.600, specificity = could not be calculated) (Table 5). Correlation analysis was performed among each total IgE score in the allergic group. Total diluted saliva IgE score was significantly correlated with total diluted nasal mucus IgE score ($r = 0.798$, $p = .00325$). On the contrary, no correlation was found between total tear IgE score and diluted saliva ($r = 0.081$, $p = .65285$) or diluted nasal mucus IgE score ($r = -0.239$, $p = .47841$).

As shown in Table 6, the total IgE scores for undiluted samples were correlated with the symptom scores. Total IgE scores for tears were strongly correlated with the severity of symptoms of allergic conjunctivitis, including ocular itching ($r = 0.769$, $p < .00001$) and tearing ($r = 0.560$, $p = .00035$), and were also correlated with the ocular symptom score ($r = 0.329$, $p = .03452$). On

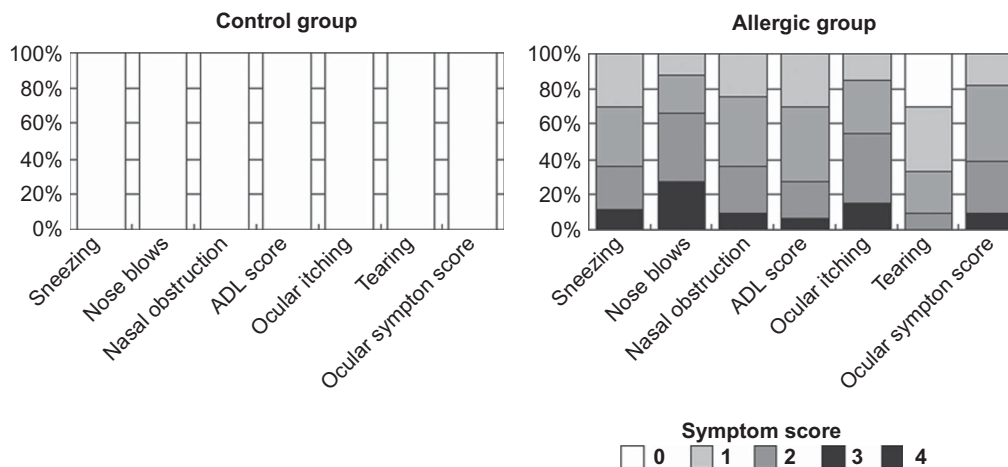


FIGURE 2.—Comparison of symptom scores between the control and allergic groups. The scores for allergic rhinitis, activities of daily living (ADL), and allergic conjunctivitis were graded as shown in Tables 2 and 3. All symptom scores were higher in the allergic group than in the control group ($p < .00001$, Mann–Whitney U test).

TABLE 4.—Rates of sample collection and IgE measurement in the control and allergic groups.

Control group										
Rate	Tear fluid		Saliva				Nasal mucus			
	Undiluted		Undiluted		Diluted (twofolds)		Undiluted		Diluted (twofolds)	
	<i>N</i>	(%)	<i>N</i>	(%)	<i>N</i>	(%)	<i>N</i>	(%)	<i>N</i>	(%)
Sample collection rate										
	30/30	(100.0)	30/30	(100.0)	—	—	0/30	(0.0)	—	—
IgE detection rate										
	30/30	(100.0)	26/30	(86.7)	30/30	(100.0)	—	—	—	—
Test positive rate										
	0/30	(0.0)	1/26	(3.9)	1/33	(3.3)	—	—	—	—
Allergic group										
Sample collection rate										
	33/33	(100.0)	33/33	(100.0)	—	11/33	(33.3)	—	—	—
IgE detection rate										
	33/33	(100.0)	24/33	(72.7)	33/33	(100.0)	5/11	(45.5)	11/11	(100.0)
Test positive rate										
	28/33	(84.8)	16/24	(66.7)	21/33	(63.6)	3/5	(60.0)	7/11	(63.6)

Note. N = Number of patients.

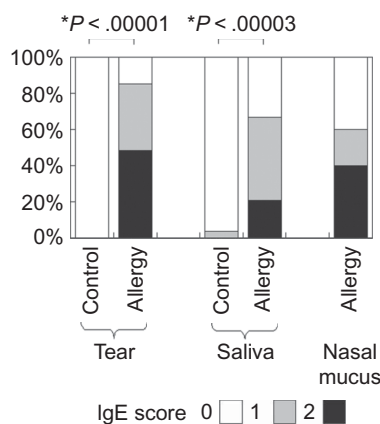


FIGURE 3.—Comparison of total IgE scores in undiluted samples between the control and allergic groups. The total IgE score was graded as described in Methods and Figure 1. *Mann–Whitney *U* test.

the contrary, both total saliva and nasal mucus IgE scores were correlated with the severity of symptoms of rhinitis, including sneezing (saliva $r = 0.897$, $p < .00001$; nasal mucus $r = 0.871$, $p = .00024$), nose blowing (saliva $r = 0.764$, $p < .00001$; nasal mucus $r = 0.829$, $p = .00080$), and nasal obstruction (saliva $r = 0.519$, $p = .00099$; nasal mucus $r = 0.745$, $p = .00429$), whereas no correlation was found between the total IgE levels in saliva or nasal mucus and the ocular symptom score (Table 6).

The correlation between the ADL score and total IgE in tear fluid ($r = 0.705$, $p < .00001$) was stronger than that for total IgE in undiluted saliva ($r = 0.468$, $p = .00301$) or undiluted nasal mucus ($r = 0.479$, $p = 0.06816$) (Table 6). However, there were no significant differences between the correlations (ADL and tear IgE vs. ADL and undiluted saliva IgE, $p = .1528$; and ADL and tear IgE vs. ADL

and undiluted nasal mucus IgE, $p = .1646$; Fisher's z statistics).

DISCUSSION

There have been several recent reports about immunochromatographic analysis of total IgE in tear fluid (9–11), but similar studies of total IgE in saliva or nasal mucus have not been reported so far, to our knowledge. We believe that this study is the first attempt to measure total IgE in tears, saliva, and nasal mucus simultaneously in patients with allergic rhinitis and conjunctivitis using a commercial kit.

Tears and saliva were obtained from all patients, but the sample collection rate was low for nasal mucus (control group: nil; allergic group: 33.3%). To measure physiological IgE levels accurately, we obtained whole nasal mucus directly from the nasal cavity without washing with normal saline or using a nebulizer, cotton swabs, or filter paper disks. These methods can increase the success rates of sample collection, but the accuracy of the estimated total IgE level may be questionable compared with that obtained by direct collection from the nasal cavity.

When undiluted samples were used, the detection rate of IgE was lower for nasal mucus (45.5%) and saliva (72.7%) compared with tears (100.0%) in the allergic group (Table 4). This lower detection rate for nasal mucus and saliva was probably influenced by the high viscosity of these specimens that made absorption by the test filter more difficult. The detection rate increased from 72.7% to 100.0% for saliva and from 45.5% to 100.0% for nasal mucus after diluting the samples with phosphate-buffered saline. In this study, we did not compare the positive rates of diluted and undiluted samples from the same patient. Therefore, further investigation of the influence of sample dilution on measurement of the total IgE level is necessary.

TABLE 5.—Test results obtained with the Allerwatch® kit.

Concentration Group	Tear IgE		Saliva IgE				Nasal mucus IgE			
	Undiluted		Undiluted		Diluted (twofolds)		Undiluted		Diluted (twofolds)	
	Allergy	Control	Allergy	Control	Allergy	Control	Allergy	Control	Allergy	Control
Test										
Positive	28	0	16	1	21	1	3	0	7	0
Negative	5	30	8	25	12	29	2	0	4	0
Coefficient of variation	0.544	—	0.829	5.385	0.870	5.385	0.894	—	0.853	—
Sensitivity	0.848		0.667		0.636		0.600		0.636	
Specificity	1.000		0.962		0.967		—		—	
False rate										
Positive	0.000		0.038		0.033		—		—	
Negative	0.152		0.333		0.364		0.400		0.364	
Predictive value										
Positive	1.000		0.941		0.955		—		—	
Negative	0.857		0.758		0.707		—		—	
Positive likelihood ratio	— (∞)		17.33		19.09		—		—	

TABLE 6.—Correlation coefficients for comparisons of total IgE and various allergic scores in the allergic group.

	Tear IgE		Saliva IgE		Nasal mucus IgE	
	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>
Sneezing	0.126	.24305	0.897	<.00001	0.871	.00024
Nose blowing	0.432	.00602	0.764	<.00001	0.829	.00080
Nasal obstruction	0.602	.00011	0.519	.00099	0.745	.00429
ADL score	0.705	<.00001	0.468	.00301	0.479	.06816
Ocular itching	0.769	<.00001	0.422	.00726	0.673	.01168
Tearing	0.560	.00035	0.600	.00011	0.742	.00449
Ocular symptom score	0.320	.03452	−0.349	.97690	−0.793	.99820

Note. Correlation coefficients were calculated by Spearman's rank correlation method. ADL = activities of daily living.

With tear samples, the Allerwatch® kit showed a very high sensitivity (84.8%) and specificity (100.0%). According to the manufacture's data, the sensitivity and specificity were 73.6% and 100.0%, respectively. The data of manufacture contain the patients with relatively mild symptoms of allergic conjunctivitis compared with that of the current study and could partly explain these differences in sensitivity. Sirbikaite et al. reported that the sensitivity and specificity of another diagnostic kit for allergic conjunctivitis (Lacrytest®, ADIATEC S.A, Nantes, France) were 93.8% and 89.7%, respectively (9). These results are similar to those reported previously for the Lacrytest® (9, 10).

The sensitivity of the test using undiluted samples was higher for tears (84.8%) than saliva (66.7%) or nasal mucus (60.0%, Table 5). Likewise, false negative rate was higher for saliva (33.3%) or nasal mucus (40.0%) than for tears (15.2%), although positive false rate is very low in both tears (0.0%) and undiluted saliva (3.8%).

One possible explanation for this difference in sensitivity could be that the samples are derived from different glands. Additionally, the samples of saliva and nasal mucus may contain various impurity compared with tear samples. The aqueous portion of the tear film is secreted by the main lacrimal gland and the accessory lacrimal glands. Nasal secretions are primarily derived from the parasympathetically innervated submucosal seromucous glands, as well as several other sources that include anterior serous glands, goblet cells, lacrimal glands, epithelial transport of ions, and transudation from blood vessels. Approximately 90% of all saliva is secreted by the three pairs of major salivary glands, which are the parotid, submandibular, and sublingual glands. In addition, the nasal and oral cavities communicate with the lacrimal sac, indicating that saliva and nasal mucus IgE may be influenced by tear IgE although there was no significant correlation between total tear IgE score and total saliva or nasal mucus IgE score. Thus, the lacrimal gland is the main contributor to tear film secretion, whereas nasal and oral

mucosal secretions may be influenced by various factors. Differences in the mechanism of secretion of IgE may influence the test results.

The total IgE score for tear fluid was correlated with the severity of conjunctival and ocular symptoms. On the contrary, the total IgE scores for saliva and nasal mucus were correlated with the severity of rhinitis-related symptoms. ADL scores were better correlated with the total IgE score for tears than with the scores for saliva or nasal mucus. Kari et al. reported that there was no correlation between the total IgE levels in serum and tears of patients with allergic conjunctivitis (17). They also demonstrated a good correlation between the results of the conjunctival allergen provocation test and lacrimal IgE (17). Several research groups have reported a significant correlation between symptoms of ocular allergy and both total IgE in tears and allergen-specific IgE in tears, whereas only a poor correlation has been found between specific and/or total IgE in serum and the manifestations of ocular allergy (8, 18–20). We also examined the relationship between total tear IgE and serum IgE in patients with allergic conjunctivitis, and there was a moderate correlation between the total tear IgE score and log (total serum IgE) (21). These reports and our results suggest local production of IgE is the chief contributor to the manifestations of allergic conjunctivitis. Therefore, measurement of locally produced total IgE with the Allerwatch® is a more practical method for the clinical evaluation of local allergic reactions compared with the measurement of total serum IgE.

Advantages of the Allerwatch® include the following: Less time is required for the measurement of IgE levels than when using a standard enzyme-linked immunosorbent assay. We could assay the total IgE level of tears within 15 minutes, including the time for sample collection. This is of considerable benefit to patients because allergic conjunctivitis can be diagnosed at the first visit to a clinic. The strip is ready for use immediately after unpacking and the sterile strip is simply touched to the lower fornix of the conjunctiva. Therefore, we conclude that the Allerwatch® is a rapid, sterile, and noninvasive method for examination of allergic conjunctivitis.

The major limitation of this study is that we selected patients with both allergic rhinitis and conjunctivitis. The correlations among total IgE levels of tears, saliva, and nasal mucus may be higher in patients with both nasal and ocular symptoms because of linkage through mutual relationships than in patients with only nasal or ocular symptom. In fact, total saliva IgE score was correlated with total nasal mucus IgE score, although total tear IgE score has no correlation with saliva or nasal mucus IgE score. Thus, the association between total IgE in each specimen and the various allergic scores was probably also higher than otherwise expected. Further research comparing total IgE scores in patients with allergic rhinitis or allergic conjunctivitis alone would clarify the association of local IgE levels with the severity of each allergic symptom. Second, although detection of eosinophils in

body fluids/tissues is used for the diagnosis of allergic conditions, we did not investigate eosinophils in the collected samples. Detection of eosinophils is a complicated procedure and the detection rate is low, being about 60% in conjunctival scrapings from patients with mild allergic conditions (22). Therefore, rapid measurement of local IgE is preferable for diagnosis of allergic diseases instead of the traditional eosinophil count. Third, although the reproducibility of the assay is reliable as described in the manufacturer's protocol, further evaluation of the reproducibility of the assay in the same subject is needed.

In summary, this study was the first attempt to measure total IgE in tears, saliva, and nasal mucus simultaneously using a commercial kit. Scores for local levels of total IgE showed a good correlation with clinical symptoms. We have demonstrated that the Allerwatch® is a rapid and easy test for diagnosing allergic rhinitis and conjunctivitis and has advantages for determining the severity of allergic diseases.

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DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

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