Histamine Release Test and Measurement of Antigen-Specific IgE Antibody in the Diagnosis of Allergic Conjunctival Diseases

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Although systemic allergic laboratory tests for the quantification of allergen-specific serum IgE antibody have been widely used, in these tests a high titer of serum specific IgE does not necessarily indicate evidence of allergy. We evaluated the diagnostic value of the glass microfiber-based histamine release test (HRT) using small amounts of whole blood, in 36 cases of allergic conjunctival diseases: 17 cases of allergic conjunctivitis and 19 of atopic keratoconjunctivitis. The patients were evaluated by HRT, capsulated hydrolic carrier polymer (CAP)-RAST, and conjunctival provocation test (CPT) against ten allergens. The positive rates for all allergens were higher in CAP-RAST than

in HRT. The mean concordance of HRT with CAP-RAST results was 0.789. The mean concordance of HRT with CPT was 0.892 and that of CAP-RAST with CPT was 0.693. A significantly higher concordance was observed in HRT than CAP-RAST for Japanese cedar and mite antigen. The mean sensitivity, specificity, and efficiency of HRT were higher than those of CAP-RAST. These results indicate that CAP-RAST is good for the screening of allergens and that HRT has an advantage in the confirmation of clinical allergens in allergic conjunctival diseases because of its high sensitivity, specificity, efficiency, and higher concordance with CPT. *J. Clin. Lab. Anal.* 15:71–75, 2001. © 2001 Wiley-Liss, Inc.

Key words: histamine release test; allergic conjunctival diseases; CAP-RAST; allergic conjunctivitis; atopic keratoconjunctivitis; conjunctival provocation test

INTRODUCTION

Allergic conjunctival diseases are usually diagnosed clinically, based on typical clinical findings. Conjunctival allergic laboratory tests, such as conjunctival cytology, conjunctival provocation test (CPT), and the tear test, are highly specific and useful for differential diagnosis, because these tests are applied to the specimens obtained from the inflammatory site itself. However, this type of test has some problems. Patients may experience pain, itching, or irritation on sample collection, and CPT may in some cases cause anaphylactic shock. In contrast, most systemic allergic laboratory tests are based on the quantification of allergen-specific serum IgE such as radioallergosorbent test (RAST) (1), capsulated hydrolic carrier polymer (CAP)-RAST system (2), and multiple allergosorbent test (MAST) (3). RAST is completed within a few hours and MAST is suitable for multiple-allergen screening. However, in these tests a high titer of serum-specific IgE does not necessarily indicate evidence of allergy (4). Therefore, more reliable systemic allergic laboratory tests that are suitable for determining the treatment including immune therapy and protection against allergens—especially in the pollen season—are needed.

The histamine-release test using the glass-fiber method (HRT) (5,6) is based on the measurement of histamine level,

which reflects the activity of mast cells or basophils, but it can be performed safely and easily in vitro using peripheral whole blood. To date, no study has been reported on the usefulness of HRT in the diagnosis and treatment of allergic conjunctival diseases. In this study, we evaluated the usefulness of HRT in comparison with CPT and CAP-RAST in patients with allergic conjunctival diseases.

MATERIALS AND METHODS

Patients

We studied 36 patients with allergic conjunctival diseases who presented to the Department of Ophthalmology, Yokohama City University Hospital, Yokohama, Japan, between June 1997 and May 1999. Informed consent was obtained from all patients before sample collection and CPT.

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Seventeen of the subjects enrolled had allergic conjunctivitis (AC) (8 male and 9 female) with a mean age of 41.8 (range 15–78) years, and 19 had atopic keratoconjunctivitis (AKC) (9 male and 10 female) with a mean age of 19.9 (8–38) years. AC and AKC were diagnosed according to the guidelines of diagnosis and treatment of conjunctivitis proposed previously (7), which are summarized in Table 1. Patients with AC who showed seasonal or perennial symptoms such as ocular itching and tearing without proliferative lesions had positive CAP-RAST or skin-prick test to one or several allergens including grass pollen. Ten patients (59%) had allergic rhinitis (AR) but none of the patients had bronchial asthma (BA) among the patients with AC. AKC patients had atopic dermatitis (AD) and conjunctival and/or corneal involvement. The patients with AKC met the diagnostic criteria of AD proposed by Hanifin and Rajka (8). Out of 19 patients with AKC, 13 (68%) and 4 cases (21%) had AR and BA, respectively. None of the patients was receiving systemic or local steroids or anti-allergic drugs on sample collection. To avoid the influence of systemic treatment, a blood sample was obtained with 7-day suspension of oral medication of anti-histaminergic agents, anti-allergic agents, theophylline, β stimulant, and corticosteroids.

Histamine Release Test

HRT was performed with an HRT kit (Lucica HRT, Asahi Chemical Industry Co., Ltd., Tokyo, Japan) as described previously (9). In this study, pollen (Japanese cedar, grass [*Dactylis glomerata*], ragweed, and mugwort), dog dander, cat dander, mite (*Dermatophagoides pteronyssinus*), house dust, *Candida albicans*, and *Alternaria tenuis* were selected as antigens. Glass-fiber-prepared 96-well microtiter plates were used, and each allergen was prepared to create a 6-step dilution system where each allergen was diluted serially 3.5-fold. Then, 25 μ l of heparinized blood was added and histamine released from basophils and mast cells was trapped by the glass-fiber, which resulted in a reaction with o-phthalaldehyde. Fluorescence intensity (excitation at 355 nm, emission at 450 nm) was measured. The net histamine release was calculated by subtracting the value of the negative control. A result greater than the cut-off value (15 ng/ml) was defined as significant. The results of HRT were scored as 0 to 4 as described previously (9). Scores greater than or equal to class 1 were defined as positive.

CAP-RAST

Specific serum IgE antibody against the same allergen in HRT was measured by CAP-RAST kit (Pharmacia, Uppsala, Sweden), and the results were scored as class 0 to 6, and scores greater than or equal to class 1 were defined as positive as described previously (10).

Conjunctival Provocation Test

Conjunctival provocation test (CPT) was carried out according to the method described previously (11) in 22 patients. Three dilutions (10, 100, and 1,000 A.U./ml) of seven kinds of commercially available allergen extract solutions—Japanese cedar; grass, ragweed, mugwort, cat dander, *Dermatophagoides pteronyssinus*, and house dust (Torii Yakuhin Co., Ltd., Tokyo, Japan)—were made at the time of testing, starting with the basic solution. One drop of saline was administered to the conjunctival sac of the tested eye to test individual nonspecific reactivity. (For ethical reasons, a positive control—histamine—was not used.) After five minutes, the existence of three symptoms (conjunctival hyperemia, tearing, and ocular itching) was determined. If the number of symptoms was greater than that in the opposite eye where no allergen had been challenged, the result was regarded as positive. Additional CPTs were eventually performed in the same patients with different antigens at weekly intervals.

Statistical Analysis

The diagnostic sensitivity, specificity, and efficiency were calculated by standard methods (12). The relationship between the classes for each test was analyzed by Spearman's rank correlation test. The concordance between two tests was analyzed by chi-squared and Fisher tests. In each analysis, significance was set at a level of probability below 0.05.

RESULTS

Positive Rate for Each Allergen in Each Test

The positive rate for each allergen is shown in Table 2. In HRT, the allergen that showed the highest positive rate was mite (0.694), followed by house dust (0.611), Japanese cedar (0.528), and cat dander (0.361), while the positive rates of other allergens were below 0.2. In CAP-RAST, the positive

TABLE 1. Diagnostic inclusion criteria for allergic ocular disorders^a

Disease	Conjunctival signs	Age group	Current pollen exposure and confirmed pollen allergy	History/presence of atopic dermatitis
AC	Hyperemia, swelling, and discharge without proliferative lesions	Any	Required	No
AKC	Conjunctival erythema or giant papillae with lid eczematous lesions	Any	Not required	Yes

^aAC, allergic conjunctivitis; AKC, atopic keratoconjunctivitis.

TABLE 2. Positive rate for each allergen in HRT and CAP-RAST, and correlation and concordance between HRT and CAP-RAST against each tested antigen^a

	HRT	CAP-RAST	Correlation (rank correlation coefficient)	Concordance
Japanese cedar	0.528	0.611	0.539 ^b	0.750
Grass	0.139	0.306	0.356 ^c	0.778
Ragweed	0.083	0.167	0.921 ^b	0.917
Mugwort	0.139	0.278	0.301	0.750
Dog dander	0.194	0.389	0.505 ^b	0.750
Cat dander	0.361	0.528	0.511 ^b	0.722
Mite	0.694	0.778	0.710 ^b	0.750
House dust	0.611	0.694	0.581 ^b	0.778
<i>Candida albicans</i>	0.194	0.361	0.571 ^b	0.778
<i>Alternaria tenuis</i>	0.111	0.194	0.908 ^b	0.917
Mean	0.305	0.431	0.590	0.789

^an=36.^bP < 0.01.^cP < 0.05.

rate for mite was the highest (0.778), and those of house dust (0.694), Japanese cedar (0.611), and cat dander (0.528) were also high. The positive rates for all allergens were higher in CAP-RAST than in HRT. The mean positive rates of HRT and CAP-RAST were 0.305 and 0.431. The mean positive rates of HRT for the ten allergens in AC and AKC were 0.206 and 0.391, respectively, and those of CAP-RAST in AC and AKC were 0.382 and 0.482, respectively. The mean positive rate of CPT for seven allergens tested in AC and AKC were 0.222 and 0.443, respectively. All of the mean positive rates in AKC with the three methods were higher than those in AC.

Correlation and Concordance for Each Allergen Between HRT and CAP-RAST

The correlation between HRT and CAP-RAST is shown in Table 2. A statistically significant correlation was observed between these tests for all allergens except mugwort antigen. Among the allergens tested, a good correlation was observed for ragweed (rank correlation coefficient: 0.921), *Alternaria tenuis* (0.908), and mite (0.710). The concordance of results (positive or negative) between HRT and CAP-RAST is also shown in Table 2. High concordance between 0.722 and 0.917 was observed for all ten antigens tested. Mean concordance was 0.789.

Concordance Between CPT and HRT or CAP-RAST in Each Allergen

The concordance between CPT and HRT or CAP-RAST is displayed in Table 3. In general, HRT showed a higher concordance with CPT than CAP-RAST. A significantly higher concordance with CPT was observed in HRT than CAP-RAST for Japanese cedar and mite antigen. Although the concordance between HRT and CPT was higher than that between CAP-RAST and CPT for mugwort, cat dander, and grass, the differences were not significant.

Sensitivity, Specificity, and Efficiency for Each Allergen of HRT and CAP-RAST in CPT

As shown in Table 4, the sensitivity of HRT for all allergens except for grass was equal to or higher than that of CAP-RAST. Specificity of HRT for all allergens was higher than that of CAP-RAST, and a significant difference in sensitivity was observed between HRT and CAP-RAST for mite antigen. The efficiency of HRT was higher than that of CAP-RAST for all seven allergens, and significantly higher efficiency of HRT than that of CAP-RAST was observed for Japanese cedar and mite antigen. Overall, HRT had better sensitivity, specificity, and efficiency in CPT than CAP-RAST. Mean sensitivity, specificity, and efficiency of HRT for seven tested allergens were 0.766, 0.872, and 0.870, respectively; in contrast, those of CAP-RAST were 0.696, 0.612, and 0.688, respectively.

DISCUSSION

It has been reported that the rates of positive RAST and multiple sensitivity for inhalant allergens are significantly higher in patients with coexisting atopic respiratory disease (13). Therefore, the systemic allergic features of AD may

TABLE 3. Concordance between CPT and HRT or CAP-RAST for each allergen^a

	CPT/HRT	CPT/CAP-RAST	P
Japanese cedar	0.909	0.636	0.030*
Grass	0.955	0.773	0.082
Ragweed	0.909	0.773	0.158
Mugwort	0.909	0.682	0.056
Cat dander	0.818	0.591	0.070
Mite	0.864	0.591	0.036*
House dust	0.864	0.818	0.294
Mean	0.892	0.693	N.C.

^an=22; P < 0.05; N.C., not calculable.

TABLE 4. Sensitivity, specificity, and efficiency of HRT and CAP-RAST in CPT^a

	HRT			CAP-RAST		
	Sensitivity	Specificity	Efficiency	Sensitivity	Specificity	Efficiency
Japanese cedar	0.923	0.889	0.909 ^b	0.692	0.556	0.634
Grass	0.400	0.941	0.818	0.600	0.765	0.727
Ragweed	0.667	0.947	0.909	0.667	0.789	0.773
Mugwort	0.667	0.947	0.909	0.333	0.737	0.682
Cat dander	0.875	0.786	0.818	0.750	0.500	0.591
Mite	0.909	0.818 ^b	0.864 ^b	0.909	0.273	0.591
House dust	0.923	0.778	0.864	0.923	0.667	0.818
Mean	0.766	0.872	0.870	0.696	0.612	0.688

^an=22.^bP < 0.05.

underlie the higher positive rate observed in AKC (Table 3). The fact that HRT, CAP-RAST, and CPT showed a similar pattern in the two diseases indicates that in spite of the wide clinical spectrum of allergic conjunctival diseases, these three diagnostic tests are stable in ocular allergic disorders.

As a sensitization test, CPT has been considered to be simple, fast, and low-cost, and it could replace skin tests in patients with skin disorders (14,15). In allergic conjunctival diseases, skin tests can be supported by the finding of increased systemic total and specific IgE to confirm the diagnosis. However, a group of patients with only allergic conjunctivitis exists in whom skin test and RAST show a low correlation (15). Therefore, we selected only CPT for provocation test in this study.

In this study, the positive rates for all allergens were higher in CAP-RAST than in HRT. In contrast, the mean concordance of HRT with CPT was higher than that of CAP-RAST with CPT, and the mean sensitivity, specificity, and efficiency of HRT in CPT were higher than those of CAP-RAST. Non-specific binding to cellulose may explain the positive CAP-RAST results in patients without a positive reaction to the same allergen in CPT (11). Another possible explanation is that allergic individuals sometimes demonstrate IgE sensitization without ever having had symptoms, and others whose symptoms have resolved may retain detectable IgE antibodies (16). In this study, the fact that the antigenic materials in HRT and CAP-RAST were not identical for some allergens might also be the cause of discrepancies in the results between the two tests.

The detection of histamine in peripheral blood has been carried out in the laboratory (17,18). Measurement of released histamine by allergen stimulation is a direct diagnostic test for type I allergy. In contrast to previous methods, HRT requires only a 25- μ l whole-blood sample. Using a 96-well microtiter plate, HRT enables multiple testing of up to 10 allergens. HRT, in addition, eliminates the necessity for leukocyte separation and histamine extraction by the use of glass-fiber that selectively binds histamine, but not histidine, spermidine, or other interfering materials (5). This is an important explanation for why HRT has better specificity, with

fewer false positive reactions, than do conventional measurements of antigen-specific IgE antibody such as CAP-RAST or MAST (6). However, HRT in its current form has several problems. Because whole blood is used as the sample in HRT, the result is influenced by blocking antibodies in the serum, and thus, the result obtained does not directly reflect the histamine released by basophils (19).

In conclusion, commercially produced allergy screening tests such as these are increasingly being marketed for general use in clinical practice, but our observations show that the results must be interpreted with caution, and these tests should be used with particular care in immune therapy for allergic conjunctival diseases. The results of this study also indicate that CAP-RAST is good for screening of allergens. In contrast, the advantages mentioned here suggest that HRT is a useful diagnostic method for the confirmation of clinical allergens in allergic conjunctival diseases, because of its high sensitivity, specificity, efficiency, and higher concordance with CPT.

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