

# Clinical Evaluation of Total IgE in Tears of Patients with Allergic Conjunctivitis Disease Using a Novel Application of the Immunochromatography Method

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## ABSTRACT

**Background:** The determination of total IgE in tears is useful as a diagnostic tool in allergic conjunctivitis disease (ACD). We evaluated the efficacy of this diagnostic tool for ACD, which is a clinically applicable novel immunochromatographic method to determine total IgE in tears.

**Methods:** The subjects comprised 4 groups: 15 patients with vernal keratoconjunctivitis (VKC group), 8 patients with atopic keratoconjunctivitis (AKC group), 18 patients with allergic conjunctivitis (AC group), and 7 normal healthy volunteers as a control (control group). Tears were sampled using filter paper, and the total IgE in tears was determined by immunochromatography assay. Semiquantitative determination was carried out by examining the intensity of the colored line using an immunochromatoreader (IgE index). The relationship between IgE indices in tears and total IgE levels in serum or between IgE indices and the clinical scores of ACD was examined.

**Results:** The positive ratio obtained by this novel application of the immunochromatography assay was 38 of the 41 in the patients with ACD and none in the 7 controls. IgE indices for the VKC group, AKC group and AC group were  $27.5 \pm 15.6$ ,  $19.8 \pm 15.8$ , and  $4.0 \pm 3.1$  (mean  $\pm$  SD), respectively. IgE indices in tears showed significant correlation with both total IgE levels in serum ( $P < 0.001$ ,  $r = 0.76$ ) and clinical scores of ACD ( $P < 0.001$ ,  $r = 0.57$ ).

**Conclusions:** The novel application of the immunochromatography assay to assess the total IgE in tears is a useful clinical tool to investigate ACD.

## KEY WORDS

allergic conjunctivitis disease, immunochromatography, immunoglobulin E

## INTRODUCTION

Allergic conjunctivitis diseases (ACD) are among the major ocular surface diseases mainly caused by the type I allergic reaction. ACD are classified into 3 clinical types: vernal keratoconjunctivitis (VKC), atopic keratoconjunctivitis (AKC), and allergic conjunctivitis (AC). Although major clinical symptoms and findings of ACD are itching, conjunctival hyperemia, and pap-

illary proliferation, these are not specific clinical entities of ACD.<sup>1</sup> In terms of etiologic factors, IgE, a specific granular protein biosynthesized by infiltrated eosinophils and cytokines released from infiltrated lymphocytes, plays a major role in the etiology of ACD. However, the current clinical available method to diagnose ACD is limited to identifying eosinophils in tears or conjunctival scratch specimens.<sup>1</sup>

IgE is a subclass of immunoglobulin and is in-

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**Table 1** Number and characteristics of the subjects

|                                    | Control      | AC Group        | AKC group      | VKC group       |
|------------------------------------|--------------|-----------------|----------------|-----------------|
| No. of subjects                    | 7            | 18              | 8              | 15              |
| Sex (Male : Female)                | 2 : 5        | 8 : 10          | 6 : 2          | 13 : 2          |
| Age (Years) (Mean $\pm$ SD)        | 30 $\pm$ 8.7 | 27.2 $\pm$ 10.6 | 25.1 $\pm$ 8.4 | 17.5 $\pm$ 10.0 |
| Treatment                          |              |                 |                |                 |
| None                               | 7            | 9               | 0              | 1               |
| Anti-allergic drug (AAD) only      | 0            | 9               | 6              | 1               |
| AAD + corticosteroid (CS)          | 0            | 0               | 0              | 3               |
| AAD + immunosuppressive drug (ISD) | 0            | 0               | 2              | 8               |
| AAD + CS + ISD                     | 0            | 0               | 0              | 2               |

VKC, vernal keratoconjunctivitis; AKC, atopic keratoconjunctivitis; AC, allergic conjunctivitis.

involved in the pathophysiology of anaphylaxis.<sup>2</sup> IgE is detected in circulatory blood and somatic tissues. IgE in tissues binds to the IgE receptor, Fc $\epsilon$ RI, expressed mainly on mast cells.<sup>3</sup> Clinical examinations to detect IgE are classified into two methods: determination of total IgE and antigen-specific IgE in serum. Total IgE in serum reflects systemic atopic diathesis and antigen-specific IgE indicates the presence of a sensitized antigen. For allergic disorders such as bronchial asthma, allergic rhinitis and AC, organ-specific immune reaction is considered to be a critical in the pathogenesis. To investigate the organ-specific immunological disorders in allergic disease, examination of total IgE in various body secretions such as tears, nasal discharge or perspiration might be a better marker to assess the tissue-specific pathophysiology rather than serum IgE examination. Therefore, an examination of total IgE in tears has been useful not only for the diagnosis but also for the assessment of ACD severity.<sup>4</sup> Moreover, a clinically applicable examination kit for total IgE in tears may be a useful tool for diagnosing patients with ACD. By the novel application of the immunochromatography assay as a diagnostic tool, we have developed a new method which can be used clinically for detecting the total IgE in tears, and we have investigated its efficacy clinically.

## METHODS

### SUBJECTS

Subjects were 41 patients (41 eyes) with ACD (ACD group) who were treated at the Department of Ophthalmology, University Hospital, Nihon University, between May and July, 2008. Demographic data are shown in Table 1. The enrollment criteria were patients without any allergic treatment or undergoing topical anti-allergic treatment only. Patients undergoing systemic anti-allergic drug treatment including corticosteroid therapy and immunotherapy were excluded in this study. The topical regimen for each patient is shown in Table 1. Patients in the ACD group were classified into 3 groups; 15 patients (15 eyes) with VKC (VKC group), 8 patients (8 eyes) with AKC

(AKC group) and 18 patients (18 eyes) with AC (AC group). The AC group was further subclassified into seasonal (SAC) and perennial (PAC) groups. Each patient was classified into one of the 3 groups by slit-lamp microscopic findings based on diagnostic criteria described in the Guidelines of the Japanese Ophthalmological Society for Allergic Conjunctivitis Diseases.<sup>5</sup> Then each patient underwent an examination for allergen-specific IgE antibodies in serum such as house-dust-mite or Japanese cedar pollen using the AlaSTAT (Mitsubishi-Kagaku, Iatron, Tokyo, Japan) method and was identified to be positive to allergen specific IgE antibodies. As a control, 7 healthy volunteers without allergic disorders were recruited for this study. Testing was carried out in the right eye of each subject.

This study was approved by the Internal Review Board, Nihon University, and informed consent was obtained from each patient and each healthy volunteer.

### METHODS

Tears were sampled by the modified Schirmer method 1 using Schirmer filter paper (Schirmer Tear Production Measuring Strips, Showa Yakuhin, Tokyo, Japan). The total IgE in tears was determined by ENTEBE IgE IC (Hepatica, Mataram, Indonesia). Based on the product information circulars of ENTEBE IgE IC, a 7-mm length of filter paper with tear sample was immersed in 100  $\mu$ l of reaction buffer solution in a test tube, and eluted for 10 minutes at room temperature. Then the test strip of immunochromatography, ENTEBE IgE IC, was immersed in the same reaction buffer solution for 20 minutes for reactive labeling. The results were determined by visual observation (Fig. 1). In the test strip, IgE was indicated by a red colored line (test line) that developed within 20 minutes. A second red colored line indicated the result in the control (control line). Thus, two colored lines on the test strip indicated the presence of total IgE in tears. In the absence of total IgE in tears, only one colored line is developed.

In addition, the intensity of each red line was

**Table 2** Summary of detection rate of total IgE in tears

|                                        | Control | AC        | AKC     | VKC       |
|----------------------------------------|---------|-----------|---------|-----------|
| No. of patients                        | 7       | 18        | 8       | 15        |
| Positive case<br>(No. of patients [%]) | 0 (0)   | 16 (88.9) | 8 (100) | 14 (93.3) |
| Negative case<br>(No. of patients)     | 7       | 2         | 0       | 1         |
| Sensitivity                            |         | 92.7%     |         |           |
| Specificity                            |         | 100%      |         |           |

VKC, vernal keratoconjunctivitis; AKC, atopic keratoconjunctivitis; AC, allergic conjunctivitis.

**Table 3** Clinical details of the patients with allergic conjunctivitis

| Subgroup | Clinical detail                                        | No. of patients | Positive ratio<br>of Entebe IgE |      | IgE index<br>(Mean $\pm$ SD) |      |
|----------|--------------------------------------------------------|-----------------|---------------------------------|------|------------------------------|------|
| 1        | SAC in out of season or PAC<br>without ocular symptoms | 6               | 5/6                             | ] NS | 2.5 $\pm$ 1.7                | ] NS |
| 2        | PAC with ocular symptoms                               | 12              | 11/12                           |      | 7.6 $\pm$ 11.6               |      |

SAC, seasonal allergic conjunctivitis; PAC, perennial allergic conjunctivitis.

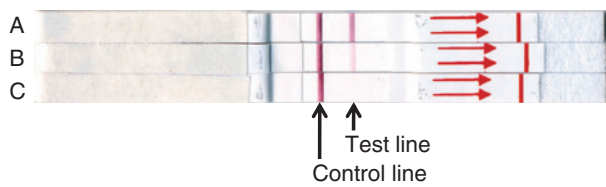


**Fig. 1** Results of immunochromatography in a test strip. Photograph of a test strip indicating the positive total IgE in tears shows both control and test lines turning red (**A**: strong, **B**: weak). On the other hand, photograph of a test strip indicating negative total IgE shows only a red control line (**C**).

determined semiquantitatively by peak % reflectance, using an immunochromatoreader, Immunochromato Reader C10066 (Hamamatsu Photonics, Iwata, Japan). Then the IgE index was calculated by the following formula: (Intensity of red line for total IgE/Intensity of red line for the control)  $\times$  100. Furthermore, the relationship between IgE indices in tears and total IgE levels in serum, or between IgE indices and the clinical scores were examined. The subjects examining a correlation between IgE indices in tears and total IgE levels in serum were the ACD patients that satisfied the following requirements; IgE index in tears is positive, and both IgE index and total IgE in serum are examined at the same time. The subjects comprised 19 patients who consisted of 11 patients with VKC, 5 patients with AKC, and 3 patients with AC. The subjects examining a correlation between IgE indices in tears and clinical scores of ACD comprised 35 of ACD patients with positive results of IgE indices, as shown in Figure 4. The clinical scores

of ACD were performed according to the criteria of clinical score described in the guidelines of the Japanese ophthalmology society.

Differences in the proportions of positive to negative findings by immunochromatography between the ACD group and the control group were evaluated using the Fisher exact test. IgE indices among the patient groups with ACD were analyzed by the Kruskal-Wallis H-test. The relationship between IgE indices in tears and total IgE levels in serum or between IgE indices and the clinical scores of ACD was examined statistically using Spearman's correlation coefficients. *P*-values less than 0.05 were determined to indicate statistical difference.

## RESULTS

### DETECTION RATE OF TOTAL IgE IN TEARS

The positive detection ratio of total IgE in tears by ENTEBE IgE IC is shown in Table 2. The positive detection ratio in the ACD groups was significantly higher than that in the control group ( $P < 0.001$ ). There were two groups in the AC groups depending on ocular symptoms; SAC and PAC subgroups without ocular symptoms, and PAC with ocular symptoms. However, the positive ratio and IgE indices between the two subgroups showed no statistical difference, as shown in Table 3. In the control group, there was no positive sample for total IgE. Therefore, sensitivity was 92.7% and specificity was 100% for this new method to determine total IgE in tears rapidly by using the immunochromatography assay.

### IgE INDEX IN TEARS

The results of determining IgE indices for each group are shown in Figure 2. IgE indices were  $27.5 \pm$

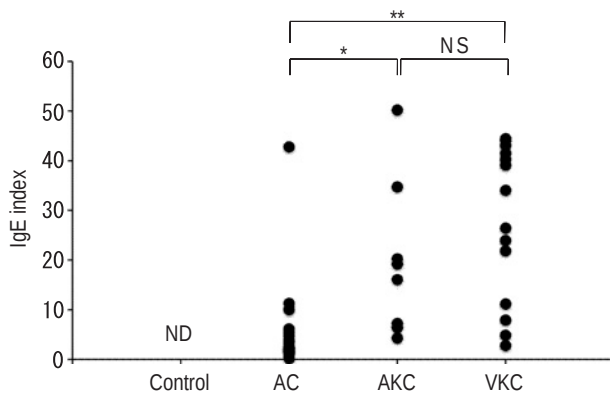
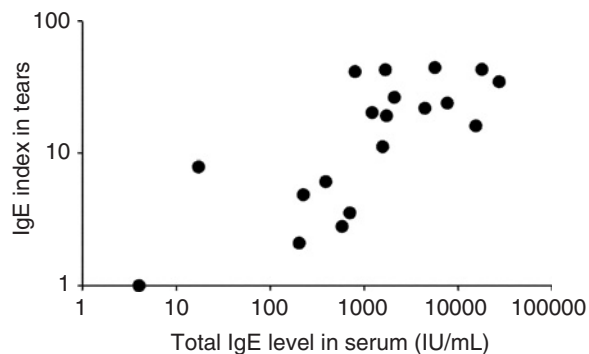


**Fig. 2** IgE index in each allergic conjunctivitis disease (ACD) subgroup and control group. IgE indices for atopic keratoconjunctivitis (AKC) and vernal keratoconjunctivitis (VKC) groups are significantly higher than that for allergic conjunctivitis (AC) group (\* $P < 0.05$ , \*\* $P < 0.001$ , respectively). NS, not significant; ND, not detected.



| $n = 19$         | IgE index       | Total IgE in serum (IU/mL) |
|------------------|-----------------|----------------------------|
| Mean ( $\pm$ SD) | $19.6 \pm 15.5$ | $4712.0 \pm 7482.2$        |
| Range            | 0-44.5          | 4-27319                    |

**Fig. 3** Relationship between IgE indices in tears and IgE level in serum. IgE indices in tears showed a significant correlation with clinical scores of ACD ( $P < 0.001$ ,  $r = 0.76$ ).

15.6 (mean  $\pm$  SD) for the VKC group,  $19.8 \pm 15.8$  for the AKC group, and  $4.0 \pm 3.1$  for the AC group. IgE indices for both the VKC group ( $P < 0.001$ ) and the AKC group ( $P < 0.05$ ) were significantly higher than those for the AC group.

As shown in Figure 3, IgE indices in tears showed a significant correlation with total IgE levels in serum ( $P < 0.001$ ,  $r = 0.76$ ). Also, IgE indices in tears showed a significant correlation with clinical scores of ACD, as shown in Figure 4 ( $P < 0.001$ ,  $r = 0.57$ ).

## DISCUSSION

It is well known that ACD shows nonspecific ocular findings in the clinical examination. Although the detection of eosinophils in conjunctiva or tears is useful

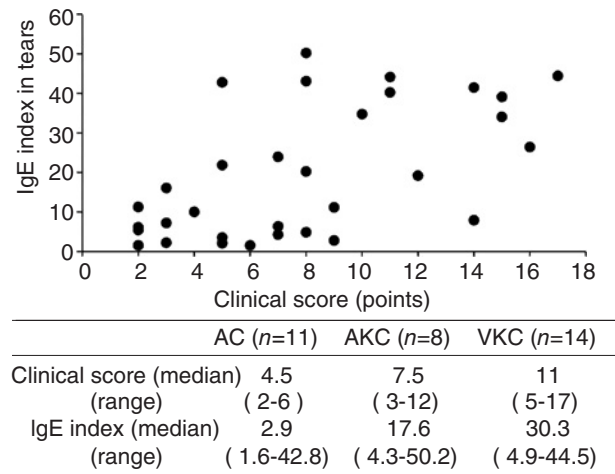


**Fig. 4** Relationship between IgE indices in tears and clinical scores of ACD. IgE indices in tears showed a significant correlation with total IgE levels in serum ( $P < 0.001$ ,  $r = 0.57$ ). AC, allergic conjunctivitis; AKC, atopic keratoconjunctivitis; VKC, vernal keratoconjunctivitis.

for definitive diagnostic findings for ACD, the detection rate of eosinophils in a smear of conjunctival scratch is low.<sup>6</sup> Therefore, a noninvasive and rapid detection method for total IgE in tears is expected to improve the definitive diagnosis of ACD. In this study, the sensitivity of ACD using ENTEBE IgE IC was 92.7% as determined by a visibility test.

IgE plays a pivotal role in the pathogenesis of type I allergy.<sup>2</sup> IgE binds mast cells through Fc $\epsilon$ RI and induces the release of chemical mediators such as histamine and leukotriens for mast cells by allergen stimulation. In the representative allergic disorders such as bronchial asthma and atopic dermatitis, serum IgE levels increase. Local production of IgE in conjunctival tissues or lacrimal glands is controversial.<sup>7,8</sup> However, both total IgE and allergen-specific IgE in the tears of patients with ACD seem to increase depending on the clinical severity of ACD.<sup>9-11</sup> Therefore, the determination of total IgE in tears is useful not only for pathophysiological study but also for a more rapid clinical diagnosis of ACD.<sup>12,13</sup>

The sampling volume of tears is limited and it has been difficult to determine the IgE in tears by clinical laboratory testing. The recent advances in microanalysis technology, including immunochromatography, now enables us to obtain a highly sensitive analysis with a minimum volume of sample. This immunochromatographic assay for total IgE in tears requires both a qualified anti-human IgE antibody and a stable tear sampling method for an improvement in the sensitivity. The lowest detection limit in the currently used method with ENTEBE IgE IC, was 0.44 IU/ml in our preliminary study. Minimum tear volume when using ENTEBE IgE IC was 7  $\mu$ l. There are two major methods for sampling of tears: the micro-

capillary tube method<sup>9</sup> and the filter paper method.<sup>12,14,15</sup> In our measurement of the total IgE in tears, we obtained tears using a filter paper; 1 mm wet length of filter paper indicated 1 µl of tears. In this study, tears sampled using filter paper were eluted by 100 µl of reaction buffer solution, and this elution solution was used directly for immunochromatography to avoid the dilution of the tear sample as much as possible. This treatment of tear samples enabled us to obtain an improvement in the sensitivity and positive ratio of the ACD diagnostic rate. However, a disadvantageous point of our ENTEBE IgE IC kit is that examinations are not able to be performed for the patients with an insufficient volume of tears such as patients with dry eye.

A previous study using the enzyme immunoassay (EIA) method showed a severe increase of IgE in tears of patients with VKC, but a mild increase in patients with PAC.<sup>9</sup> A study using the paper radio immunosorbent test (PRIST) reported that the positive rate of total IgE in tears was 13 of 23 in patients with ACD, and their concentrations ranged from the lowest detection limit (5 IU/ml) to 76 IU/ml.<sup>16</sup> The detection rate of total IgE in tears by PRIST was lower than the results of our study. This is thought to indicate a lower sensitivity for total IgE. The method with lower sensitivity causes a higher false negative rate in the diagnosis of PAC. In France, a diagnosis kit for ACD by examining total IgE in tears using the immunochromatographic method is available as the Lacrytest®. Regarding with the Lacrytest, Sirbikate *et al.*<sup>13</sup> reported a sensitivity and specificity of 91.7% and 98.5%, respectively. And, it was reported that a positive conformity ratio between the clinical diagnosis and this examination for Japanese patients with ACD was 76% in the Lacrytest. In contrast, the diagnosis rate of our Entebe IgE IC kit was 96%. It is impossible to compare the results of both clinical studies in Japan, because the subjects are different respectively. Our results are in full agreement with those of other immunochromatographic tests. Therefore, we believe the immunochromatographic assay is a suitable technique as a rapid clinical diagnostic test of tears for ACD patients.

In terms of total IgE in tears, we defined a new index, the IgE index, and examined its efficacy. The IgE index for VKC was significantly higher than that for AC and it agreed with the previous reports.<sup>9</sup> Furthermore, using the immunochromatoreader, we could verify positive/negative determination in cases showing a vague visibility line on ENTEBE IgE IC. In this study, the IgE indices in tears had a positive correlation between both total IgE levels in serum and clinical scores of ACD. We can estimate the severity of the atopic condition as the background of the ACD by measuring IgE indices in tears objectively.

In this study, total IgE in tears was positive in patients with asymptomatic AC, as shown in Table 3.

Further study is needed to determine whether this is due to a false positive finding in the methodology itself or to the subclinical expression of IgE in the asymptomatic stage of AC.

Our study confirmed that ENTEBE IgE IC is a useful tool to diagnose ACD. Semiquantitative determination of IgE in tears using the immunochromatoreader can contribute to a better understanding of the pathophysiology of ACD by providing rapid, beneficial information for diagnosis and for developing a treatment plan for the patients with ACD.

## REFERENCES

1. Friedlander MH. Conjunctivitis of allergic origin: clinical presentation and differential diagnosis. *Surv Ophthalmol* 1993;**38** (Suppl):105-14.
2. Ishizaka T. IgE and mechanisms of IgE-mediated hypersensitivity. *Ann Allergy* 1982;**48**:313-9.
3. Kinet JP, Blank U, Brini A *et al.* The high-affinity receptor for immunoglobulin E: a target for therapy of allergic diseases. *Int Arch Allergy Appl Immunol* 1991;**94**:51-5.
4. Leonardi A. In-vivo diagnostic measurements of ocular inflammation. *Curr Opin Allergy Clin Immunol* 2005;**5**:464-72.
5. Japanese Ocular Allergology Study Group. [Guidelines for the clinical management of allergic conjunctival disease]. *Nippon Ganka Gakkai Zasshi [J Jpn Ophthalmol Soc]* 2006;**110**:101-40 (in Japanese with English abstract).
6. Abelson MB, Madiwale N, Weston JH. Conjunctival eosinophils in allergic ocular disease. *Arch Ophthalmol* 1983;**101**:555-6.
7. Ballow M, Mendelson L. Specific immunoglobulin E antibodies in tear secretions of patients with vernal conjunctivitis. *J Allergy Clin Immunol* 1980;**66**:112-8.
8. el-Asrar AM, Tabbara KF, Geboes K, Missotten L, Desmet V. An immunohistochemical study of topical cyclosporine in vernal keratoconjunctivitis. *Am J Ophthalmol* 1996;**121**:156-61.
9. Nomura K, Etsuko T. Tear IgE concentrations in allergic conjunctivitis. *Eye* 1998;**12**:296-8.
10. Kitazawa M, Shoji J, Inada N, Sawa M, Kato H. [Clinical evaluation of measurement method for antigen-specific IgE in tears of patients with allergic conjunctival disease]. *Nippon Ganka Gakkai Zasshi [J Jpn Ophthalmol Soc]* 2003;**107**:578-82 (in Japanese with English abstract).
11. Shoji J, Kato H, Kitazawa M, Inada N, Sawa M. Evaluation of staphylococcal enterotoxin-specific IgE antibody in tears in allergic keratoconjunctival disorders. *Jpn J Ophthalmol* 2003;**47**:609-11.
12. Didierlaurent A, Bloch-Michel E, Couret MN *et al.* Tear IgE detected by a new method: Stallerdiag-IgE. *Ocul Immunol Inflamm* 1994;**2**:93-9.
13. Sirbikate L, Ehlers N. The use of Lacrytest® in ocular allergy. *Acta Ophthalmol Scand* 2007;**85**:117.
14. Liotet S, Warnet VN, Arrata M. Lacrimal immunoglobulin E and allergic conjunctivitis. *Ophthalmologica* 1983;**186**:31-4.
15. Baudouin C, Bourcier T, Brignole F *et al.* Correlation between tear IgE levels and HLA-DR expression by conjunctival cells in allergic and nonallergic chronic conjunctivitis. *Graefes Arch Clin Exp Ophthalmol* 2000;**238**:900-4.
16. Kari O *et al.* Allergic conjunctivitis, total and specific IgE in tear fluid. *Ophthalmologica* 1985;**63**:97-9.