

IgE and eosinophil cationic protein (ECP) as markers of severity in the diagnosis of atopic keratoconjunctivitis

Tais H Wakamatsu,^{1,2} Yoshiyuki Satake,³ Ayako Igarashi,³ Murat Dogru,^{1,3} Osama M A Ibrahim,^{1,2} Naoko Okada,² Kazumi Fukagawa,^{2,4} Jun Shimazaki,³ Hiroshi Fujishima⁵

¹Johnson & Johnson
Department of Ocular Surface
and Visual Optics, Keio
University School of Medicine,
Tokyo, Japan
²Department of Ophthalmology,
Keio University School of
Medicine, Tokyo, Japan
³Department of Ophthalmology,
Tokyo Dental College, Ichikawa
Hospital, Chiba, Japan
⁴Ryogoku Eye Clinic, Tokyo,
Japan
⁵Department of Ophthalmology,
Tsurumi University School of
Dental Medicine, Kanagawa,
Japan

Correspondence to
Professor Hiroshi Fujishima,
2-1-3 Tsurumi, Tsurumi-ku,
Yokohama, Kanagawa
230-8501, Japan;
hfujijsima6@gmail.com

This work was presented at the
6th International Conference on
the Tear Film and Ocular
Surface: Basic Science and
Clinical Relevance, 22–25
September 2010, Florence,
Italy.

Accepted 8 November 2011

ABSTRACT

Aims To evaluate tear and serum IgE and eosinophil cationic protein (ECP) as severity markers for atopic keratoconjunctivitis (AKC).

Methods Thirty eyes of 30 patients with AKC and 10 eyes of 10 healthy control subjects were examined in this prospective study. All subjects underwent fluorescein staining, conjunctival injection, conjunctival oedema and papillary formation grading. Tear and serum IgE and ECP levels were measured, and correlations between them investigated with reference to the ocular surface clinical parameters.

Results The mean fluorescein scores, conjunctival injection, oedema scores and papillary formation were significantly higher in AKC patients compared to controls ($p < 0.05$). Higher total IgE and ECP levels were detected in AKC tears compared with the control group. Tear ECP levels showed a significant correlation with fluorescein staining, conjunctival injection and oedema scores ($r = 0.70$, 0.62 and 0.62 , respectively). Tear IgE had no correlation with clinical signs. Serum IgE and ECP levels were elevated in AKC patients but did not show any correlation with clinical signs.

Conclusion This study suggests the presence of an eosinophilic response in AKC disease independent of IgE sensitisation. Tear ECP was a useful marker delineating the severity of ocular surface disease in AKC.

INTRODUCTION

Atopic keratoconjunctivitis (AKC) is a chronic atopic disease of the conjunctiva and cornea. The disease occurs in both children and adults and is associated with atopic dermatitis. The male to female ratio varies from 2.4:1 to below 1:1 depending on the report.^{1–3} AKC is the most debilitating of the allergic conjunctival diseases owing to its chronicity and ability to cause loss of vision due to frequent corneal complications.^{1 4 5} Ocular symptoms include intense itching, photophobia, burning and foreign body sensation. The clinical signs are observed as corneal staining, conjunctival injection, oedema and papillae on the upper tarsal conjunctiva. In the most severe cases, ulceration and neovascularisation of the cornea, subepithelial fibrosis of the conjunctiva, fornix shortening and symblepharon are present. Ocular clinical expression includes different levels of severity, from mild injuries to sight-threatening complications.¹

The immunopathogenic mechanism in AKC is complex. The term AKC refers to a collection of hypersensitivity disorders of the lid, conjunctiva and cornea. AKC signs and symptoms can be related to T-cells, neutrophils and eosinophil activation. Involvement of secondary inflammatory cells (particularly cytoplasmic eosinophil granule containing toxic proteins such as eosinophil cationic protein (ECP) and major basic proteins), in addition to the mast cells and chemokines⁶ can lead to more serious corneal damage with tissue remodelling and subepithelial fibrosis.^{7 8}

AKC is not always associated with specific allergic sensitisation. Skin tests and/or specific serum IgE antibody tests show no specific sensitisation in almost 45% of AKC patients, although total serum IgE levels are high and eosinophils are present in both tears and conjunctival scrapings.⁹

Even though IgE serum-specific tests are recommended for aetiological diagnosis, they provide no information about the pathogenesis and severity of the disease. Total serum IgE tests are no longer considered indispensable for diagnosis, because normal values do not exclude an allergic diagnosis, and conversely, high levels of IgE can be observed in numerous pathologies other than atopy.

Serum levels of ECP have been used successfully to diagnose and monitor eosinophilic inflammation in asthma and other allergic diseases.^{10 11} ECP is one of several highly basic proteins present in the secretory granule of the eosinophil that is released upon its activation. ECP belongs to the superfamily of RNases and has both cytotoxic and non-cytotoxic effects. ECP has already been found to play an important role in the pathogenesis of allergic conjunctivitis.¹² ECP serum values are increased in AKC and vernal keratoconjunctivitis (VKC) patients and also in other forms of allergic conjunctivitis, but to a lesser extent than in perennial allergic conjunctivitis and seasonal allergic conjunctivitis.¹³ Nevertheless, local tests are far more useful and indicative of the conjunctival inflammatory state, since this disease is often the result of local hyper-reactivity. By measuring ECP levels in the tears of patients with allergic conjunctivitis, one can estimate the degree of inflammation and also assess correlation of the mediators to the severity of the disease.

Although the ECP level seems to be a potent marker that can be used to monitor allergic conjunctivitis and evaluate responsiveness to a specific treatment, which marker is best remains controversial.

Table 1 Scoring tarsal conjunctival clinical signs

Score	Conjunctival injection, oedema and papillary formation
0	None
1	Mild
2	Moderate
3	Severe

The purpose of this study was to evaluate tear and serum IgE and ECP as severity markers for AKC. We measured tears and serum ECP and IgE levels from the same patient and assessed the correlation between these markers and clinical parameters such as conjunctival injection, oedema, corneal damage and conjunctival proliferation.

METHODS

Subjects

Thirty eyes of 30 patients with AKC (19 males and 11 females; mean age 21.2 ± 13.2 years) and 10 eyes of 10 healthy control subjects (two males and eight females; mean age 27.6 ± 7.5 years) were enrolled in this study. The investigation was conducted in two centres: the Department of Ophthalmology of Tokyo Dental College at Ichikawa Hospital, and the International University of Health and Welfare at Mita Hospital. None of the patients had a history of Stevens-Johnson syndrome; chemical, thermal or radiation injury; or infectious conjunctivitis or other unclassified conjunctivitis such as toxic conjunctivitis. Nor had any patients undergone any ocular surgery that would have compromised the ocular surface. The control subjects also had no history of drug or contact lens use that would alter the ocular surface.

Routine ophthalmic examinations consisted of best corrected visual acuity measurements, slit-lamp examination and anterior segment photography. Patients underwent tear sampling and venous peripheral blood collection.

The study protocol was approved by the Ethics Committee of Mita Hospital, International University of Health and Welfare and the Tokyo Dental College Ichikawa Hospital review board. Informed consent for the procedures was obtained from all patients and control subjects. All experiments in this study followed the tenets of the Declaration of Helsinki.

Slit-lamp examination

The three major upper tarsal conjunctival findings of conjunctival injection, oedema and papillary formation were assessed and a clinical severity score¹⁴ was assigned for each sign (table 1).

The ocular surface was initially stained with 2 μ l of 1% fluorescein solution. Fluorescein staining scores, ranging between 0 and 9, were assigned according to the protocol described in a previous study.¹⁵ Clinical signs scores are

displayed in frequency distribution tables for AKC patients (table 2) and control subjects (table 3). The values are arranged as lists ordered by quantity showing the number of times each clinical score appears and also by their respective percentages in parenthesis.

Severity of disease based on clinical scores

To evaluate IgE and ECP values at different levels of atopic disease, we divided disease severity into two groups (i) based on the conjunctiva clinical scores: 0–1 (none and mild clinical signs) and 2–3 (moderate and severe clinical signs) and (ii) based on the cornea clinical scores: <4 (none and mild) and ≥ 4 (moderate and severe). IgE and ECP values in tears were compared with severity scores for corneal fluorescein staining, conjunctival injection, oedema grades and papillary formation.

Tear and serum ELISA assay

Tear samples were collected before the vital staining tests. With the use of a capillary micropipette, tears were gently collected from the external canthus, placed in Eppendorf tubes and centrifuged at 10 000 rpm for 5 min at 4°C. The supernatants were then stored at –30°C until assayed. Peripheral blood was collected from 10 patients and 10 healthy control subjects.

Subsequently, the appropriate dilution was established and the concentrations of tears and serum ECP (MBL, Tokyo, Japan) and total IgE (Mabtech, Nacka Strand, Sweden) were measured by ELISA according to the manufacturer's instructions. IgE and ECP values were expressed as mean $\pm$ SEM.

Statistical analysis

Data were processed using InStat 3.0 (GraphPad, San Diego, California, USA). The Mann–Whitney test was used to compare the parameters between AKC patients and normal controls and also to analyse the difference between tear and serum IgE and ECP concentrations. Spearman rank correlation analysis was used to determine the correlation between clinical parameters with IgE and ECP concentrations for both tear and serum data. A probability level of <0.05 was considered statistically significant.

RESULTS

Conjunctival and corneal findings

Detailed summaries of the four evaluated components comprising conjunctival injection, oedema and proliferation and corneal fluorescein staining scores in AKC patients and healthy control subjects are shown in table 2 and table 3, respectively.

Among the 30 eyes from AKC patients examined, 17 eyes (56%) presented with superficial punctate keratopathy. Conjunctival injection was present in 26 eyes (87%),

Table 2 Vital staining scores and conjunctival status grades in patients with atopic keratoconjunctivitis (30 eyes)

Score	0	1	2	3	4	5	6	7	8	9
Fluorescein staining scores (0–9 points)	13 (44%)	5 (17%)	0 (0%)	0 (0%)	1 (3%)	0 (0%)	1 (3%)	0 (0%)	0 (0%)	10 (33%)
Score	0			1			2			3
Conjunctival injection (0–3)	4 (13%)			7 (23%)			10 (34%)			9 (30%)
Conjunctival oedema (0–3)	5 (17%)			10 (33%)			7 (23%)			8 (27%)
Papillary formation (0–3)	7 (23%)			1 (3%)			2 (7%)			20 (67%)

Table 3 Vital staining scores and conjunctival status grades in healthy control subjects (10 eyes)

Score	0	1	2	3	4	5	6	7	8	9
Fluorescein staining scores (0–9 points)	9 (90%)	1 (10%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Score	0			1		2		3		
Conjunctival injection (0–3)	8 (80%)			2 (20%)		0 (0%)		0 (0%)		
Conjunctival oedema (0–3)	10 (100%)			0 (0%)		0 (0%)		0 (0%)		
Papillary formation (0–3)	10 (100%)			0 (0%)		0 (0%)		0 (0%)		

conjunctival oedema was present in 25 eyes (83%) and papillary formation was present in 23 eyes (77%).

Among the 10 eyes from control subjects examined, one eye (10%) presented with superficial punctate keratopathy and conjunctival injection was present in two eyes (20%). Conjunctival oedema and papillary formation were not present in the eyes of control subjects. Corneal and conjunctival complications were more frequent and more severe in AKC patients compared to healthy control subjects.

ELISA for measuring tear and serum IgE and ECP levels

Tear ECP levels were significantly higher in the AKC group compared to the control subjects, as shown in table 4.

Tear ECP levels in patients with moderate and severe (2–3) conjunctival injection scores (2029.20 ± 748.08 ng/ml) and conjunctival oedema scores of 2–3 (2436.20 ± 919.97 ng/ml) were significantly higher ($p < 0.05$) compared to patients with zero to mild (0–1) conjunctival injection scores (363.57 ± 172.88 ng/ml) and conjunctival oedema scores of 0–1 (400.79 ± 157.41 ng/ml) (figure 1A,B).

Tear ECP levels in patients with corneal damage scores ≥ 4 (2737.34 ± 1168.00 ng/ml) were significantly higher ($p < 0.02$) compared to patients with scores of < 4 (759.03 ± 414.00 ng/ml) as shown in figure 1D.

Higher total tear IgE levels were detected in AKC tears when compared with the control group, as shown in table 4. Tear IgE levels in patients with conjunctival injection scores of 0–1 (1284.30 ± 469.27 ng/ml) and conjunctival oedema scores of 0–1 (971.02 ± 338.26 ng/ml) were not significantly different compared with patients with conjunctival injection scores of 2–3 (772.65 ± 231.42 ng/ml) and conjunctival oedema scores of 2–3 (949.46 ± 311.15 ng/ml) (figure 1A,B). Tear IgE levels in patients with corneal scores of < 4 (963.15 ± 368.67 ng/ml) were not significantly different compared with tear IgE levels in patients with corneal damage scores ≥ 4 (954.41 ± 290.78 ng/ml), as shown in figure 1D.

There were no significant differences in IgE and ECP levels in the two groups with different papillary formation scores, as shown in figure 1C.

Tear ECP levels showed a significant correlation ($p < 0.0002$) with cornea fluorescein staining scores as well as both conjunctival injection and oedema ($r = 0.70$, 0.62 and 0.62 , respectively). However, tear IgE showed no correlation with any clinical signs, as demonstrated in figure 2.

Table 4 Tear IgE and ECP levels

	AKC group	Control group	p Value
IgE (ng/ml)	960.24 ± 225.80	1.52 ± 0.27	< 0.0001
ECP (ng/ml)	1418.47 ± 496.00	9.80 ± 6.20	< 0.0001

AKC, atopic keratoconjunctivitis; ECP, eosinophil cationic protein.

Serum IgE (6580.44 ± 2607.30 ng/ml) and ECP (14.69 ± 11.25 ng/ml) levels showed no correlation with measured clinical signs.

DISCUSSION

Our results showed that tear ECP levels were higher in AKC patients compared with normal subjects and that they correlated with corneal staining, conjunctival injection and oedema scores. Similar correlation results were not observed between tear IgE levels and all clinical signs. These results suggest that the presence of an overall eosinophilic response in AKC disease is independent of IgE sensitisation.

No significant differences in IgE and ECP levels were observed between the two groups with different papillary formation scores. Papillary formation in AKC is thought to occur in relation to tissue remodelling. However, the most relevant factor for the exacerbation of AKC seems to be inflammation rather than papillary formation (fibrosis).

The allergic response in AKC disease is based on distinct pathophysiological abnormalities involving different factors, including environmental allergens, climate and genetic predisposition.¹⁶ The immunoglobulin E (IgE) response to HLA genes and environmental allergen exposure does not completely explain the severity and clinical course of AKC.^{17 18} This disease is probably also related to a marked T helper type 2 (Th2) profile, resulting in a typical allergic inflammation with polyclonal IgE activation and mast cell, basophil and eosinophil prevalence. Conjunctival hyper-reactivity is another hallmark of AKC and is certainly influenced by the degree of eosinophilic inflammation. The influx of these cells is fundamental to acute and chronic allergic inflammation and produces profound changes in the conjunctival mucosa.

An accurate eye examination is essential for the differential diagnosis of different clinical allergic entities and for distinguishing conjunctival (perennial allergic conjunctivitis and seasonal allergic conjunctivitis) disease from forms with possible involvement of the cornea, such as VKC and AKC. The diagnosis of specific allergy in allergic eye diseases does not differ from that of other allergic diseases. Systemic assays are of little diagnostic value but can be used to monitor the progress of the patient: serum total IgE levels can be either normal or high, serum ECP is a marker for systemic eosinophil activation, and blood cell count also shows increased numbers of eosinophils.^{13 19 20} Nevertheless, local tests are more useful and indicate the conjunctival inflammatory state as a result of local hyper-reactivity. Since assessment of the type and degree of allergic inflammation is important for distinguishing between different forms of allergic eye disease and for adequate management of individual cases, standard tests should be more widely used in current practice.

In this study, the increase in eosinophil activation demonstrated by the release of significant levels of ECP in the tears of

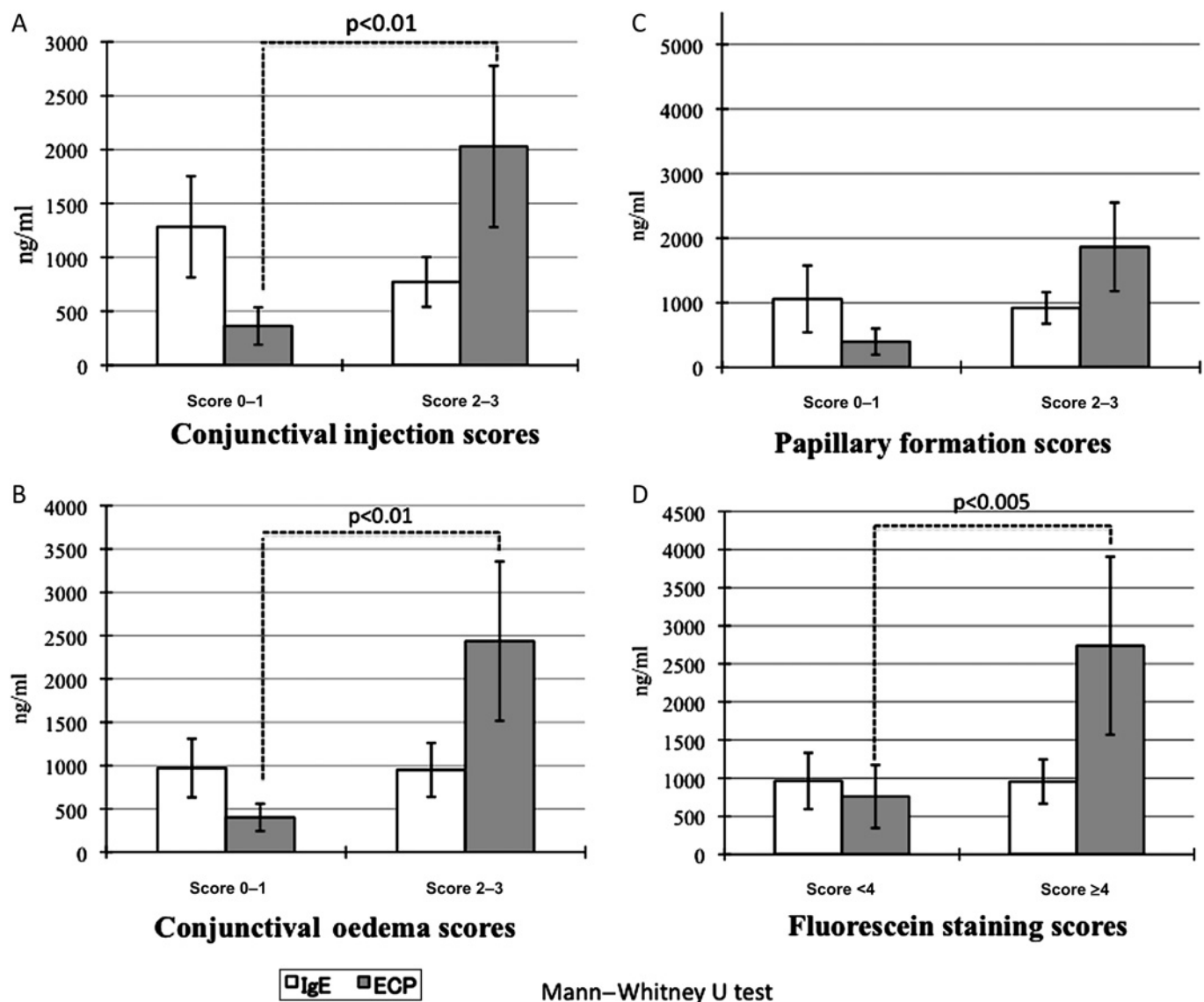


Figure 1 Tear IgE and eosinophil cationic protein (ECP) levels according to the severity of atopic keratoconjunctivitis (AKC) disease. (A, B) Note that the tear ECP level in patients with conjunctival injection and oedema scores of 2–3 was significantly higher compared to patients with conjunctival injection and oedema scores of 0–1; conversely, tear IgE levels did not statistically change according to the severity of the clinical scores. (C) There were no statistically significant changes in IgE and ECP levels in the two both groups with different papillary formation scores. (D) Note that the tear ECP level in patients with corneal damage scores ≥ 4 was significantly higher compared to patients with corneal damage scores of <4 , while tear IgE levels did not show a significant difference.

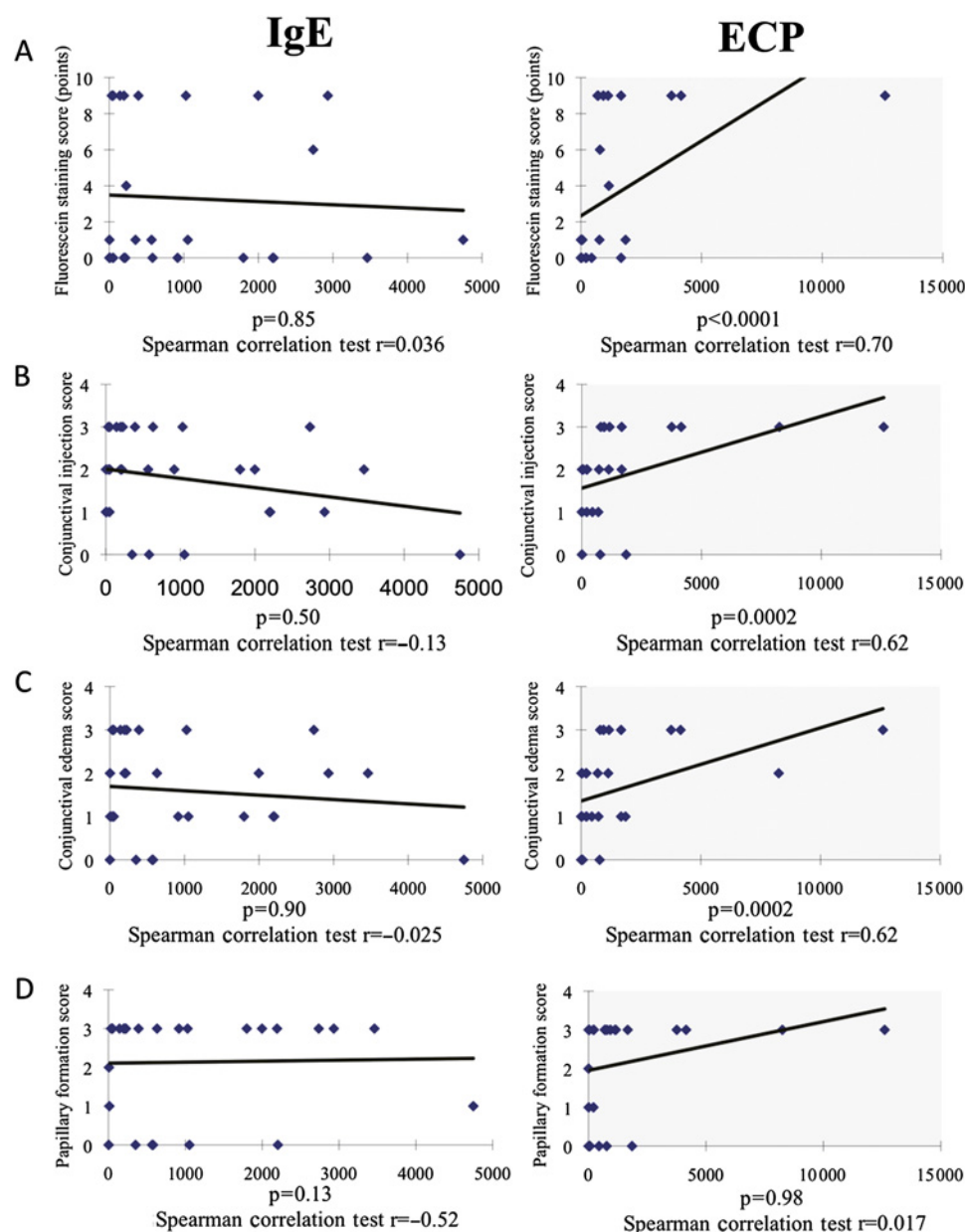
AKC patients compared with controls confirms previous reports of subjects with VKC presenting higher levels of eosinophil-derived basic proteins in tears and tissues.²¹ The subjects with corneal epithelial damage consistently exhibited the highest tear ECP measurements compared with normal subjects. In these cases, eosinophil products such as major basic protein and ECP may cause cellular disaggregation, epithelial desquamation and toxicity leading to corneal epitheliopathy and ulceration.^{22 23}

Pucci *et al* evaluated 110 children with tarsal, bulbar or mixed VKC and reported that serum ECP levels were significantly higher in VKC patients compared with controls.²⁴ Nevertheless, allergy testing was more frequently positive when local, conjunctival methods, such as tear specific IgE or conjunctival allergen challenges, were used to identify a site-specific hypersensitivity. The sensitivity of eosinophil detection in conjunctival scraping and tear fluid has been low, but eosinophil

products are detectable in 2 μ l of tears in most cases of allergic conjunctivitis even during the remission phases of these diseases.

The measurement of tear ECP in AKC patients may be considered as an extra and objective tool for more accurate monitoring of eosinophilic activation than IgE. In addition to the clinical scoring system, it may also be a useful method of assessing local inflammation. Determination of this marker in tears may have particular use in clinical trials to assess the inhibitory properties of pharmacological or immunotherapeutic agents and correlation of these biochemical effects with clinical signs and symptoms. Clinical trials have been performed using an immunochromatoreader to determine semiquantitative measurements of IgE in allergic conjunctivitis patient tears.²⁵ However, the use of a more precise and consistent marker like tear ECP may be more accurate for monitoring eosinophilic activation in this atopic eye disease group.

Figure 2 Correlation of tear IgE and eosinophil cationic protein (ECP) levels with corneal epithelial damage, conjunctival injection, oedema and papillary proliferation. (A) Tear ECP levels showed a significant correlation with corneal epithelial damage. (B) There was a significant positive correlation between ECP levels and conjunctival injection. (C) Only ECP tear concentrations were positively correlated with conjunctival oedema. (D) Tear ECP levels did not show a significant correlation with papillary proliferation.



In conclusion, we have demonstrated that tear ECP, in contrast to tear and serum IgE and serum ECP, was a useful marker to delineate the severity of ocular surface disease in AKC. It may have particular use in clinical trials and treatment follow-up.

Acknowledgements We are grateful to Samantha K Ward for her correction of the English in the manuscript.

Competing interests None.

Ethics approval The study protocol was approved by the Ethics Committee of Mita Hospital, International University of Health and Welfare and the Tokyo Dental College Ichikawa Hospital review board.

Contributors THW conceived and designed the study, monitored data collection, wrote the statistical analysis plan, analysed the data, and drafted and revised the paper. YS conceived and designed the study, monitored data collection, analysed the data and revised the paper. AI designed the study, monitored data collection, cleaned and analysed the data, and drafted the article. MD conceived and designed the study, analysed the data and revised the paper. OMAI designed the study, monitored data collection, analysed the data and participated in drafting the paper. NO designed the study, monitored data collection, cleaned and analysed the data, and drafted the article. KF conceived and designed the study, analysed the data and drafted the article. JS conceived and designed the study, provided administrative technical and

logistic support and revised the article. HF conceived and designed the study, monitored data collection, cleaned and analysed the data and revised the paper. He is guarantor.

Provenance and peer review Not commissioned; externally peer reviewed.

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Br J Ophthalmol published online January 23, 2012

doi: 10.1136/bjophthalmol-2011-300143

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