

RESEARCH ARTICLE

Specific IgE for wheat in tear fluid of patients with allergic conjunctivitis

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Abstract

Context: Allergy to hydrolyzed wheat protein in facial soap has become a major social issue in Japan. It has been reported that the most frequent early symptoms of allergy to hydrolyzed wheat protein in soap are allergic conjunctivitis and rhinitis, while wheat-dependent exercise-induced anaphylaxis can be induced by long-term use.

Objective: We evaluated the relation between tear fluid levels of specific IgE for wheat and the features of allergic conjunctivitis.

Methods: A prospective, non-randomized, cross-sectional study was conducted in 103 patients with moderate to severe allergic conjunctivitis (allergic group) and 20 age- and sex-matched healthy control subjects (control group). Specific IgE for wheat was measured in tear fluid with an immunochromatography assay, and a skin prick test (SPT) was also performed. Symptoms (sneezing, rhinorrhea, nasal obstruction, ocular itching, and lacrimation) were assessed in each subject along with the activities of daily living (ADL) score and the total ocular symptom score for allergic conjunctivitis. A severity score (0, 1, 2, or 3) was assigned for various changes of the palpebral and bulbar conjunctiva, as well as for limbal and corneal lesions associated with allergic conjunctivitis.

Results: The IgE positive rate and specific IgE score were both higher in the allergic group than in the control group (71.8% versus 40.0% and 1.9 ± 0.7 versus 1.4 ± 0.5). A positive SPT for wheat was also more frequent in the allergic group than in the control group (6.8% versus 0.0%). Within the allergic group, patients with a positive SPT had higher specific IgE scores than patients with a negative SPT (3.3 ± 0.5 versus 1.8 ± 0.6 , $p < 0.001$). In the allergic group, the wheat IgE level in tear fluid was correlated with the severity of allergic conjunctivitis symptoms, including ocular itching ($r = 0.665$), tearing ($r = 0.672$), and the total ocular symptom score ($r = 0.204$). Wheat IgE in tear fluid was also correlated with the severity of rhinitis symptoms, including sneezing ($r = 0.610$), nose blowing ($r = 0.640$), and nasal obstruction ($r = 0.677$). Furthermore, the tear fluid wheat IgE score was correlated with five objective features of allergic conjunctivitis ($p < 0.05$).

Conclusions: These results suggest that wheat allergy may be involved in the development of allergic conjunctivitis.

Keywords

Allergic conjunctivitis, clinical findings, immunochromatography, specific IgE for wheat, tear fluid

History

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Introduction

Wheat is widely used in both foods and non-food products. Among the non-food products, wheat starch is used in a variety of cosmetics and pharmaceuticals. Wheat is the most common cause of food allergy, and it can also produce contact allergy in persons using cosmetics that contain hydrolyzed wheat protein (HWP) and persons with occupational exposure to wheat^{1,2}. Furthermore, wheat is a frequent cause of food-dependent exercise-induced anaphylaxis, which is called “wheat-dependent exercise-induced anaphylaxis (WDEIA)”³. Another type of wheat IgE-induced allergy is

baker's asthma, which is an important occupational disease caused by inhalation of wheat^{4–6}.

In 2011, allergy to HWP in facial soap became a major social issue in Japan. A mail-order facial soap (Cha no Shizuku®; Yuuka Co. Ltd., Fukuoka, Japan) caused at least 535 people to suffer symptoms of wheat allergy, with many of them requiring hospitalization. The soap was first marketed in Japan in 2004 and quickly became one of the most popular brands. However, many of its users only developed soap-induced allergic reactions in 2011 and some now have permanent food allergy to wheat as a result⁷. The manufacturer voluntarily began to recall about 46 million bars of HWP-containing soap, while the Ministry of Health, Labour and Welfare of Japan has warned consumers not to use the soap. It has been reported that the most frequent early symptoms of allergy to HWP in soap are facial contact

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dermatitis and allergic rhinitis^{8–12}. In addition, WDEIA can be induced by long-term use of the soap^{7,13,14}.

Allergic conjunctivitis is one of the most common allergic diseases and it can be caused by various allergens, such as pollen, house-dust mite, and animal dander^{15,16}. It has also been reported that the HWP-containing facial soap can cause allergic conjunctivitis¹⁷, but a relation between wheat and allergic conjunctivitis has not been described previously. Accordingly, the aim of the present study was to investigate the relation between the tear fluid level of specific IgE for wheat and various features of allergic conjunctivitis.

Materials and methods

Study design

This was a prospective, non-randomized, cross-sectional, consecutive case series study conducted at the hospital of the University of Tokyo Graduate School of Medicine. This study was performed in accordance with the Helsinki Declaration of 1975 and its 1983 revision. Our institutional review board gave its approval and informed consent was obtained from each subject.

Subjects

All subjects had a Schirmer 1 test score >15 mm and normal meibomian glands. None of them had used topical or systemic drugs during the 6-week period before enrollment. We excluded patients with dry eyes, patients wearing contact lenses, and patients with a history of cataract surgery, corneal refractive surgery, or infectious conjunctivitis.

Two groups were enrolled in this study (Table 1). The allergic conjunctivitis group consisted of 103 outpatients suffering from perennial allergic conjunctivitis, who were treated at our hospital and its affiliated hospitals (47 males

and 56 females with a mean age of 23.8 ± 17.4 years; range: 5–71 years). We also enrolled an age- and sex-matched control group, comprising 20 healthy non-smoking subjects with no history of allergic diseases and a negative skin prick test (SPT) for wheat (1:20 wt/vol; Torii Pharmaceutical Co., Ltd., Tokyo, Japan) (10 males and 10 females with a mean age of 24.0 ± 5.8 years; range: 18–39 years). The control group was selected from among persons attending our outpatient clinic for eye screening (Table 1). There was no baker or pastry chef in the allergic and control groups.

Definition of allergic rhinitis

Two definitions of allergic rhinitis were assessed¹⁸. Physician-diagnosed allergic rhinitis was defined by an affirmative answer from the patient or caregiver to the question: “Have you ever being diagnosed as having allergic rhinitis by a physician?” Information on the symptoms of allergic rhinitis was obtained by completion of a questionnaire following a positive response to the question: “Have you ever had a problem with sneezing, or with a running or blocked nose, when you did not have a cold or the flu?”

The scores for symptoms of nasal allergy were calculated in accordance with the 2005 Practical Guidelines for the Management of Allergic Rhinitis in Japan¹⁹. Patients assessed each symptom on a five-point scale (0 = absent; 1 = mild; 2 = moderate; 3 = severe, and 4 = very severe), as shown in Table 2. Scores were assigned for sneezing and rhinorrhea (0 = none, 1 = 1–5 episodes, 2 = 6–10 episodes, 3 = 11–20 episodes, and 4 = >20 episodes), as well as for nasal obstruction (0 = none; 1 = slight, no mouth breathing; 2 = moderate, frequent mouth breathing; 3 = severe, very frequent mouth breathing; and 4 = complete, mouth breathing all day).

Activities of daily living score (ADL)

The ADL score was calculated from information provided by patients or family members about daily activities, such as working, housekeeping, studying, reading, playing sport, going out, sleeping, and conversing²⁰. An ADL score ranging from 0 to 4 was assigned for each subject (0 = no problems, 1 = mild symptoms not interfering with ADL, 2 = moderate symptoms interfering somewhat with ADL, 3 = severe symptoms interfering markedly with ADL, and 4 = very severe symptoms preventing ADL) (Table 2).

Definition of allergic conjunctivitis

Allergic conjunctivitis was diagnosed from the findings on slit lamp examination, including conjunctival hyperemia, follicles, and papillae, as well as from symptoms such as ocular itching and tearing. The diagnosis was made by a single ophthalmologist (T.M.) according to published guidelines for the diagnosis and treatment of conjunctivitis²¹.

Symptoms of allergic conjunctivitis were evaluated by using the following scales proposed by Abelson et al.²² for 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 the eyes; and 4 = unbearable,

Table 1. Characteristics of the allergic and control groups.

	Control group	Allergic group	p Value
Number of subjects	20	103	
Age (years)	24.0 ± 5.8	23.8 ± 17.4	NS ^a
Male/female	10/10	47/56	NS ^b
History of facial soap use			
Current use of facial soap	20 (100.0%)	103 (100.0%)	NS ^c
Current use of HWP soap	0 (0.0%)	0 (0.0%)	NS ^c
Prior use of HWP soap	0 (0.0%)	9 (8.7%)	NS ^c
History of food allergy			
Cow's milk			
(current)	0 (0.0%)	0 (0.0%)	NS ^c
(previous)	0 (0.0%)	6 (5.8%)	NS ^c
Egg			
(current)	0 (0.0%)	0 (0.0%)	NS ^c
(previous)	0 (0.0%)	2 (1.9%)	NS ^c
Wheat			
(current)	0 (0.0%)	0 (0.0%)	NS ^c
(previous)	0 (0.0%)	1 (1.0%)	NS ^c
Atopic dermatitis			
(current)	0 (0.0%)	0 (0.0%)	NS ^c
(previous)	0 (0.0%)	4 (3.9%)	NS ^c

Data are shown as the number (%) or mean $\pm$ standard deviation for age. NS, not significant. ^aUnpaired *t*-test. ^b χ^2 test. ^cFisher's exact test.

HWP soap, hydrolysed wheat protein-containing soap (Cha no Shizuku[®]).

Table 2. Symptom scores for allergic rhinitis and activities of daily living score (ADL).

Symptoms	Severity	Score
Sneezing	No symptoms	Number of sneezing episodes per day 0 = 0
	Mild	1 = 1–5
	Moderate	2 = 6–10
	Severe	3 = 11–20
	Most severe	4 > 20
Nose blowing	No symptoms	Number of times nose blown per day 0 = 0
	Mild	1 = 1–5
	Moderate	2 = 6–10
	Severe	3 = 11–20
	Most severe	4 > 20
Nasal obstruction	No symptoms	Frequency of symptom 0 = None
	Mild	1 = Slight (no mouth breathing)
	Moderate	2 = Moderate (frequent mouth breathing)
	Severe	3 = Severe (very frequent mouth breathing)
	Most severe	4 = Complete (mouth breathing all day)
ADL score	No symptoms	Severity of ADL ^a 0 = No problems
	Mild	1 = Mild symptoms not interfering with ADL
	Moderate	2 = Moderate symptoms interfering somewhat with ADL
	Severe	3 = Severe symptoms interfering markedly with ADL
	Very severe	4 = Very severe symptoms preventing ADL

^aInterfering with daily activities such as working, housekeeping, studying, reading, going out, and sleeping.

Table 3. Modified grading system for symptoms of allergic conjunctivitis^a.

Symptom	Score
Itching	0 = none
	1 = slight (occasional tingling sensation)
	2 = moderate (no need to rub eyes)
	3 = severe (need to rub eyes)
Tearing	4 = unbearable (compulsive desire to rub eyes)
	0 = none
	1 = slight
	2 = moderate (occasional tears)
Total ocular symptom score	3 = severe (tears running down cheeks)
	The number of the following 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

^aAbelson and associates²².

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) (Table 3).

In addition, the total ocular symptom score for allergic conjunctivitis was calculated by assessing nine symptoms (ocular itching, conjunctival injection, discharge, tearing, foreign body sensation, overall discomfort, lid swelling, burning, and photophobia) as follows: score 0 = no symptoms, score 1 = 1–2 symptoms, score 2 = 3–4 symptoms, score 3 = 5–6 symptoms, and score 4 = 7–9 symptoms (Table 3).

The ocular findings on slit lamp examination were also scored at each patient’s first visit. Changes of the palpebral conjunctiva (hyperemia, edema, follicles, papillae, and giant papillae), bulbar conjunctiva (hyperemia and chemosis), and limbus (Trantas’ dots and edema), as well as corneal involvement, were classified into 4 grades (0 = normal, 1+ = mild, 2+ = moderate, and 3+ = severe; Table 4), as described elsewhere^{23–25}. In addition, the total objective score for allergic conjunctivitis (maximum: 30 points) was calculated as the sum of the scores for the above-mentioned 10 findings²⁴. Data were obtained for both eyes, but only data for the right eye were used in the present analysis.

Measurement of specific IgE for wheat in tear fluid

The level of specific IgE in tear fluid has been reported to be a useful diagnostic indicator for allergic conjunctivitis^{26–32}, as along with the total tear fluid IgE level^{33–44}. Measurement of specific IgE was performed with the Immfast Check J2[®] immunochromatography kit (Immfast Check J2[®], Mitsubishi Kagaku Iatron Inc., Tokyo, Japan), which allows direct measurement of IgE antibodies to wheat. The assay was performed according to the manufacturer’s instructions with slight modifications, as described previously^{29–32}. In brief, tear fluid samples were obtained with Schirmer test strips (Schirmer Tear Production Measuring Strips; Showa Yakuhin Kako, Tokyo, Japan), and 5-mm pieces of filter paper soaked with tear fluid were placed into test tubes containing 100 µl of reaction buffer. After mixing the tear fluid sample with the reaction buffer by gentle pipetting for 10 s at room temperature, the mixture was transferred to an Immfast Check J2[®] cassette. Then 200 µl of enzyme substrate, a mixture of 5-bromo-4-chloro-indolylphosphate and nitroblue tetrazolium (BCIP/NBT), was immediately added to the substrate reservoir pad. At 20 min after application of the sample, the color

Table 4. Scoring system for the clinical features of allergic conjunctivitis^a.

Sign	Score
Palpebral conjunctiva	
Hyperemia	0 = None 1 = Dilatation of several vessels 2 = Dilatation of many vessels 3 = Impossible to distinguish individual blood vessels
Edema	0 = None 1 = Slight edema 2 = Diffuse edema 3 = Severe edema with opacity
Follicles	0 = None 1 = 1–9 follicles 2 = 10–19 follicles 3 = 20 or more follicles
Papillae	0 = None 1 = Papillae measuring 0.1–0.2 mm 2 = Papillae measuring 0.3–0.5 mm 3 = Papillae measuring 0.6 mm or larger
Giant papillae (≥ 1 mm)	0 = None 1 = Flat papillae 2 = Elevated papillae covering $<1/2$ of the upper palpebral conjunctiva 3 = Elevated papillae covering $\geq 1/2$ of the upper palpebral conjunctiva
Bulbar conjunctiva	
Hyperemia	0 = None 1 = Dilatation of several vessels 2 = Dilatation of many vessels 3 = Diffuse dilated blood vessels over the entire bulbar conjunctiva
Chemosis	0 = None 1 = Localized edema 2 = Diffuse edema 3 = Bullous edema
Limbus	
Trantas' dots	0 = None 1 = 1–4 dots 2 = 5–8 dots 3 = 9 or more dots
Limbal edema	0 = None 1 = $<1/3$ of the limbal circumference 2 = $1/3$ to $<2/3$ of the limbal circumference 3 = $2/3$ or more of the limbal circumference
Cornea	
Corneal epithelial signs	0 = None 1 = Superficial punctate keratitis 2 = Exfoliative superficial punctate keratitis 3 = Shield ulcer or corneal erosion

^aUchio and associates²⁴.

of the test line was assessed according to the manufacturer's instructions. Phosphate-buffered saline (PBS) was used as a negative control. The intensity of the blue color of the test line depends on the level of specific IgE in the sample. The assay was calibrated against a WHO standard for IgE over the range 0.35–100 kU/L and results were classified into the following four categories according to the manufacturer's instructions: class 1 was no visible test line (0.35–0.69 kU/L), class 2 was a test line weaker than line A (0.70–3.49 kU/L), class 3 was a test line similar to or stronger than line A and

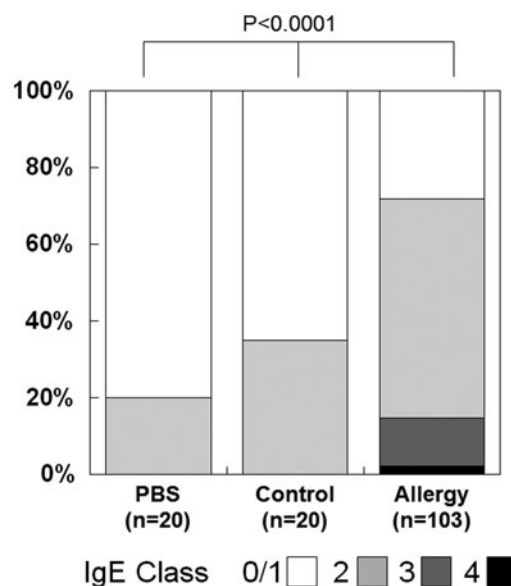


Figure 1. Comparison of the grade of positivity for specific immunoglobulin E (IgE) to wheat in negative control phosphate-buffered saline (PBS) samples and tear fluid samples from the control and allergic groups. Positivity for IgE antibodies directed against wheat was determined by the Immufast Check J2[®] test and the specific IgE score was graded as described in Methods section. Results were compared among the three groups by the Kruskal–Wallis test.

weaker than line B (3.50–17.49 kU/L), and class 4 was a test line similar to or stronger than line B (≥ 17.50 kU/L).

Statistical analysis

Mean values were compared with the unpaired *t*-test. Frequencies were analyzed by using the χ^2 test or Fisher's exact test. Comparison of semi-quantitative measurements between two groups was done by the two-tailed Mann–Whitney *U* test, while Kruskal–Wallis one-way analysis of variance and Scheffe' multiple comparison test were employed for comparison among four groups. Relations among variables were investigated by calculating Pearson's correlation coefficients. Factors associated with the wheat-specific tear IgE class were investigated by multivariate logistic regression analysis, with explanatory variables including the scores for clinical features of allergic conjunctivitis. The level of significance was set at $p < 0.05$. Statistical analysis was performed with SAS System 9.1 software (SAS Institute Inc., Cary, NC).

Results

Positivity for specific antibody to wheat in tear fluid was significantly more frequent in the allergic group (74/103, 71.8%) than in the control group (7/20, 35.0%) or the negative control samples (PBS without tear fluid, 4/20, 20.0%, $\chi^2 = 24.4$, $df = 2$, $p < 0.0001$, Fisher's exact test) (Figure 1). The grade of specific wheat IgE in tear fluid was also higher in the allergic group (1.88 ± 0.69) than in the control group (1.35 ± 0.48) or in negative control PBS (1.20 ± 0.40 , Kruskal–Wallis one-way analysis of variance) (Figure 1). There was significant difference in the grade of specific wheat IgE in tear fluid between in the allergic and control groups

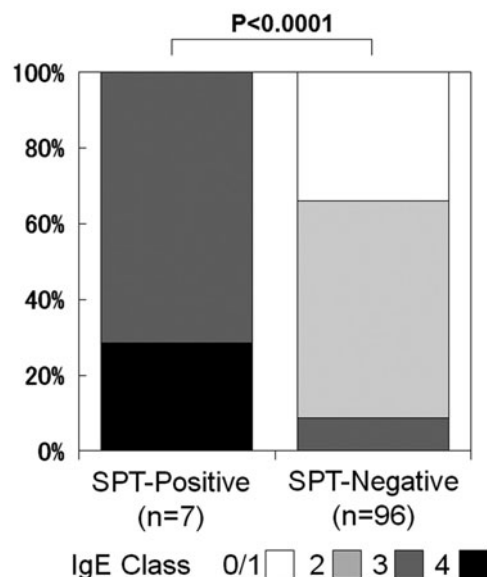


Figure 2. Comparison of specific wheat IgE scores in tear fluid between patients with a positive wheat skin prick test (SPT) ($n=7$) and patients with a negative SPT ($n=96$). The two groups were compared by the two-tailed Mann-Whitney U test.

($p=0.0031$, two-tailed Mann-Whitney U test, Figure 1). To compare the tear fluid levels of specific wheat IgE between patients who had a positive or negative result for the SPT, the allergic group was divided into an SPT-positive group ($n=7$) and an SPT-negative group ($n=96$). One patient of the seven patients in the SPT-positive group, had a previous history of wheat food allergy (1/7, 14.3%), but no patient had clinical allergic condition such as urticarial or exercise-induced anaphylaxis. The SPT-positive group had a significantly higher class of specific wheat IgE in tear fluid (3.3 ± 0.5) compared with the SPT-negative group (1.8 ± 0.6 , $p<0.0001$, unpaired t -test; $p<0.0001$, two-tailed Mann-Whitney U test, Figure 2).

Symptom scores for rhinitis and conjunctivitis are shown in Figures 3 and 4. The scores of the allergic group were higher for all symptoms than those of the control group (all $p<0.05$, Mann-Whitney U test, Figure 3). Patients were divided into four groups based on the class of specific IgE for wheat (group 1 = class 1, group 2 = class 2, group 3 = class 3, and group 4 = class 4). There were significant differences of all the symptom scores among these four groups based on the class of wheat IgE (all $p<0.0001$, one-way analysis of variance and Scheffe's multiple comparison test, Figure 4).

The allergic group had significantly higher scores for seven of the clinical features of allergic conjunctivitis (hyperemia, edema, follicles, papillae, and giant papillae of the palpebral conjunctiva, as well as hyperemia and chemosis of the bulbar conjunctiva) compared with the control group (all $p<0.01$, two-tailed Mann-Whitney U test) (Figure 5). The total objective score for allergic conjunctivitis was also significantly higher in the allergic group (10.6 ± 4.4) than in the control group (0.0 ± 0.0) ($p<0.0001$, unpaired t -test; $p<0.0001$, two-tailed Mann-Whitney U test).

Furthermore, there was a significant difference of the total objective score among the four groups based on the class of wheat IgE (8.9 ± 3.2 versus 10.8 ± 4.73 versus 12.7 ± 4.9

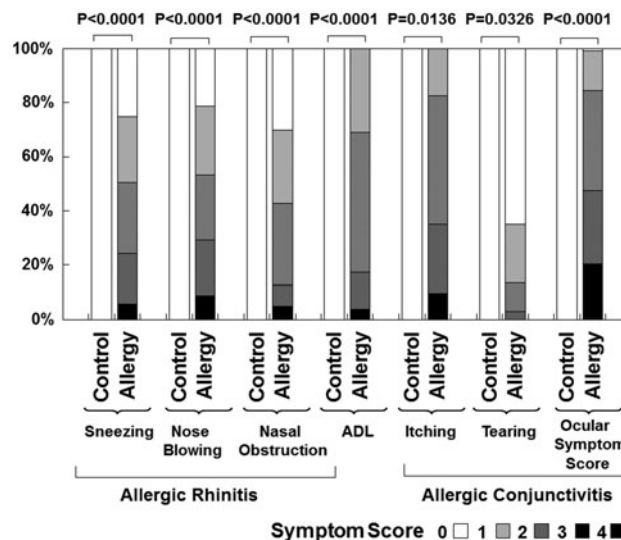


Figure 3. Comparison of the symptom scores for allergic rhinitis, symptom scores for allergic conjunctivitis, and activities of daily living (ADL) score between the control group ($n=20$) and the allergic group ($n=103$). The two groups were compared by the two-tailed Mann-Whitney U test.

versus 19.0 ± 1.0 , $p=0.0034$), as shown by one-way analysis of variance and Scheffe's multiple comparison test (Figure 6).

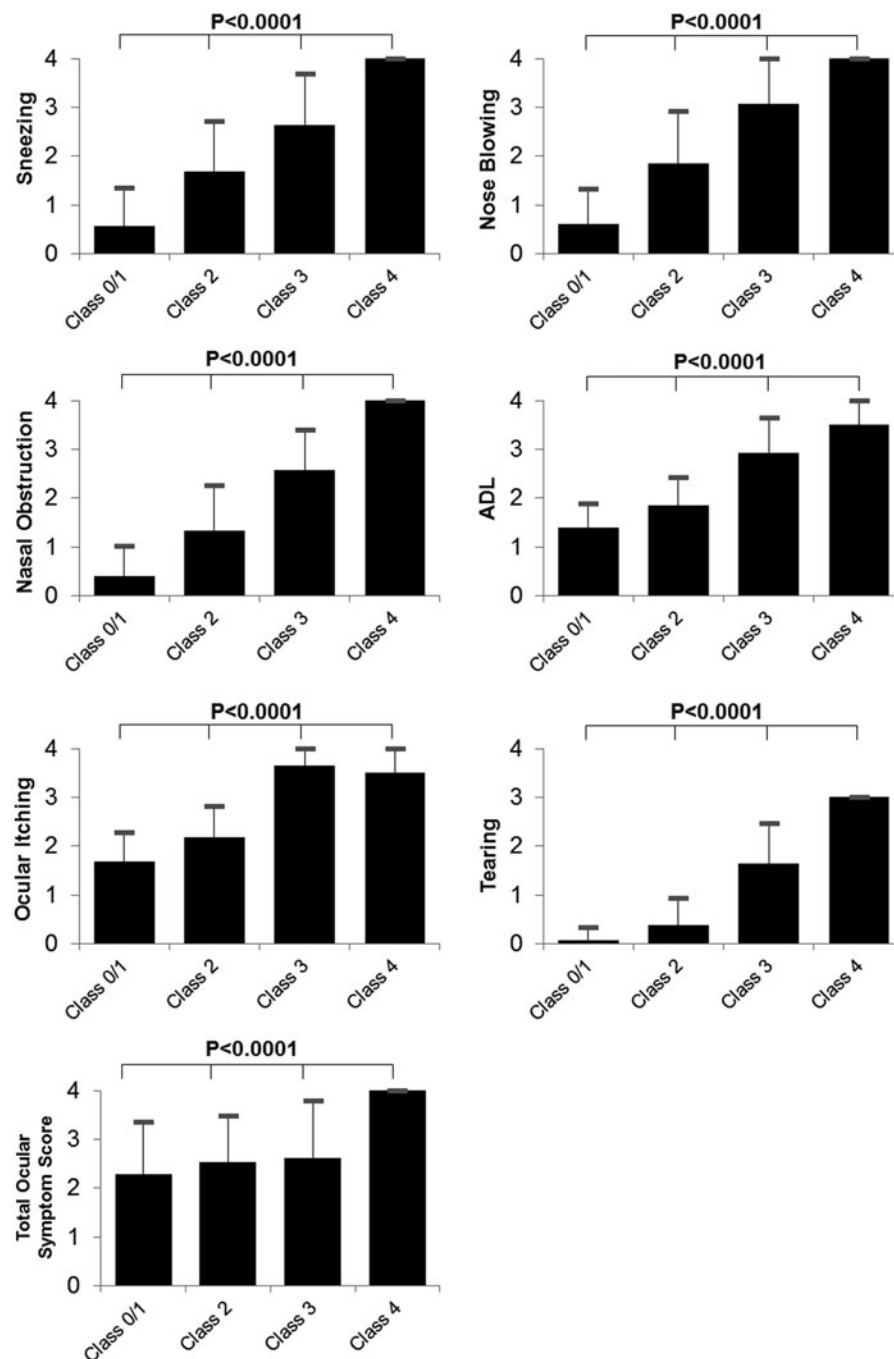
Tear fluid levels of specific IgE for wheat were strongly correlated with the severity of symptoms of allergic conjunctivitis, including ocular itching ($r=0.665$, $p<0.0001$), tearing ($r=0.672$, $p<0.0001$), and the ocular symptom score ($r=0.204$, $p=0.0390$), and were also correlated with the severity of rhinitis symptoms, including sneezing ($r=0.610$, $p<0.0001$), nose blowing ($r=0.640$, $p<0.0001$), and nasal obstruction ($r=0.677$, $p<0.0001$, Pearson's correlation coefficient analysis) (Table 5). In addition, multivariate analysis demonstrated that the class of specific wheat IgE in tear fluid was associated with ocular itching (OR = 1.174, $p=0.0449$) and tearing (OR = 1.274, $p=0.0009$), although there was no significant relation between the class of specific wheat IgE and any of the symptoms of allergic rhinitis (Table 5).

With regard to the clinical findings, positivity for specific wheat IgE in tear fluid was correlated with five signs of allergic conjunctivitis (palpebral conjunctival edema: $r=0.289$, $p=0.0033$; follicles: $r=0.359$, $p=0.0002$; giant papillae: $r=0.197$, $p=0.0488$; bulbar conjunctival hyperemia: $r=0.221$, $p=0.0267$; and bulbar conjunctival edema: $r=0.218$, $p=0.0289$) and with the total objective score ($r=0.255$, $p=0.0099$) (Table 6). Multivariate analysis revealed that the class of specific wheat IgE in tear fluid was strongly associated with palpebral conjunctival follicles, odds ratio (OR) = 1.411, $p=0.0051$ (Table 6).

Discussion

The present study demonstrated that the positive rate for specific wheat IgE in tear fluid and the specific IgE score were higher in the allergic group than in the control group. In the allergic group, the specific wheat IgE score in tear fluid was correlated with the severity of some of the symptoms of allergic conjunctivitis. Multivariate analysis also

Figure 4. Comparison of the mean symptom scores for allergic rhinitis, activities of daily living (ADL), and allergic conjunctivitis among four groups based on the tear fluid wheat IgE classes. Patients were divided into four groups according to the specific wheat IgE class in tear fluid as described in Methods. Data are expressed as the mean $\pm$ SD. Comparisons among the four groups were performed by one-way analysis of variance and Scheffe's multiple comparison test. NS = not significant.



demonstrated that certain palpebral conjunctival changes were associated with the class of specific wheat IgE in tear fluid. These results suggest that allergy to wheat may be involved in the development of allergic conjunctivitis.

The prevalence of allergic conjunctivitis associated with a positive SPT for wheat was 6.8% (7/103) in our study population. Previous studies demonstrated that the SPT for wheat was positive in 3.3% (2/60)⁴⁵ and 17.7% (75/423)⁴⁶ of children with gastrointestinal symptoms that were suspected to be food allergy-related^{45,46}. Additionally, Varjonen et al. reported that more than 10% of patients with suspected contact allergy to wheat-containing cosmetics have a positive SPT for wheat or wheat germ⁴⁷. The positive rate of the SPT for wheat in the present study was similar to or lower than the rates reported previously (3.3–17.7%).

This lower positive SPT rate may have been obtained because our allergic group consisted entirely of patients without food allergy or atopic dermatitis. Interestingly, the tear fluid level of wheat IgE was higher in the SPT-positive group than the SPT-negative group. None of the seven patients with a positive SPT for wheat showed evidence of food allergy, but they may have had an allergic predisposition.

The positive rate of specific IgE for wheat in tear fluid was 71.8% in the allergic group. We previously investigated the positive rates of IgE for other major allergens using the same kit and found rates of 86.5–98.3% for cedar pollen, 51.7–96.2% for *Dermatophagoides pteronyssinus*, and 19.0–25.5% for cat epithelium in the tear fluid of patients with allergic conjunctivitis^{29,30}. Thus, the positive rate for wheat IgE in tear fluid was relatively high compared with the rates

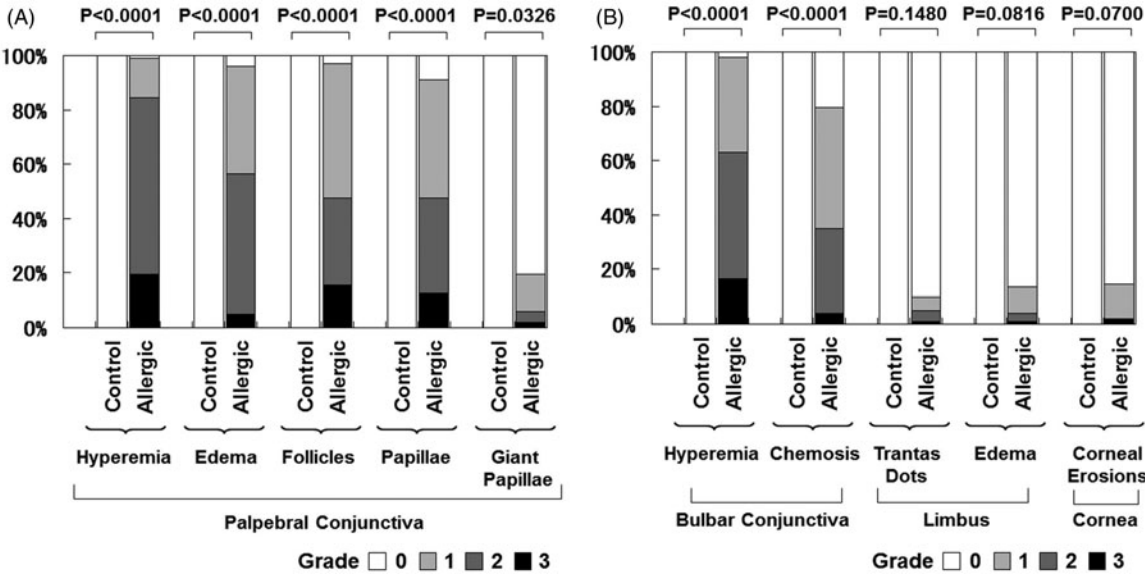


Figure 5. Comparison of the scores for allergic changes of the palpebral conjunctiva (A), the bulbar conjunctiva, limbus, and cornea (B) between the control group ($n=20$) and the allergic group ($n=103$). The scores for each finding were assigned as described in Methods section and Table 4. Comparisons between the two groups were performed by the two-tailed Mann–Whitney U test.

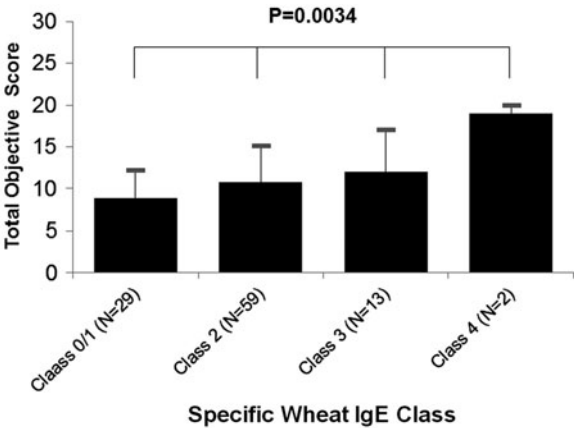


Figure 6. Comparison of the mean total objective score for allergic conjunctivitis among four groups with different classes of wheat IgE in tear fluid. Patients were divided into four groups according to the specific wheat IgE class in tear fluid as described in Methods section. Results are expressed as the mean $\pm$ SD. Comparisons among the four groups were done by one-way analysis of variance and Scheffe’s multiple comparison test. NS = not significant.

for major allergens associated with allergic conjunctivitis. The finding that specific IgE for wheat is increased in the tear fluid of patients with allergic conjunctivitis gives rise to several speculations. First, specific wheat IgE may continuously enter the tear fluid at a low level from the conjunctival vessels. This speculation is supported by our finding of a 35% positive rate for specific wheat IgE in tear fluid even among healthy subjects. As for other major allergens using the same kit, the positive rates of IgE in tear fluid were 2.0–2.3% for cedar pollen, 2.0–2.3% for *D. pteronyssinus*, and 0.0% for cat epithelium in the tear fluid of healthy subjects^{29,30}. The second possibility is that false-positive results are frequent in the allergic group when immunochromatography is performed. Control PBS samples without any specific IgE gave a false-positive rate of 20% with this assay. Third, allergic patients may be sensitive to multiple allergens

Table 5. Correlations between specific wheat IgE in tear fluid and various allergic symptom scores in the allergic group ($n=103$), as well as multivariate ORs and 95% CIs.

Variable	Correlation coefficient		Multivariate analysis		
	R	p Value	OR	(95% CI)	p Value
Sneezing	0.610	<0.0001	1.054	(−0.08 to 0.19)	0.4336
Nose blowing	0.640	<0.0001	1.057	(−0.08 to 0.19)	0.4282
Nasal obstruction	0.677	<0.0001	1.106	(−0.06 to 0.26)	0.2082
ADL score	0.640	<0.0001	1.155	(−0.02 to 0.31)	0.0935
Ocular itching	0.665	<0.0001	1.174	(0.00 to 0.32)	0.0449
Tearing	0.672	<0.0001	1.274	(0.10 to 0.38)	0.0009
Total ocular symptom score	0.204	0.0390	0.991	(−0.10 to 0.08)	0.8402

R=Two-tailed Pearson’s correlation coefficients were calculated to assess the association between the score for specific wheat IgE in tear fluid and various allergic symptoms.
OR, odds ratio; CI, confidence interval; ADL, activities of daily living.

including wheat. Wheat is one of the most common staple foods and is found in a wide range of foods and beverages, including bread, cereals, cakes, pasta, and beer, which are ingested frequently. Even in people without food allergy, the levels of specific IgE for wheat may increase in the serum and tear fluid without causing symptoms if they ingest or come into contact with wheat-containing foods or other products. To confirm this speculation, we would need to compare specific wheat IgE levels in tear fluid with those in serum.

During the last decade, several groups have reported the occurrence of HWP-related allergy in persons using HWP-containing facial soap^{7,13,48,49}. Nakamura et al. reported that 10/10 patients with wheat allergy accompanied by skin and rhinoconjunctival sensitization to HWP in facial soap were positive for IgE to both wheat and gluten, while 2/10 patients were positive for IgE to ω -5 gliadin⁴⁸. Chinuki et al. reported that 21/30 patients (70.0%) with WDEIA due to HWP-containing facial soap (HWP-WDEIA) were positive

Table 6. Correlations between specific wheat IgE in tear fluid and various clinical features of allergic conjunctivitis in the allergic group ($n = 103$), as well as multivariate ORs and 95% CIs.

Variable	Correlation coefficient		Multivariate analysis		
	R	p Value	OR	(95% CI)	p Value
Palpebral conjunctiva					
Hyperemia	0.148	0.1410	0.931	(−0.38 to 0.24)	0.6497
Edema	0.289	0.0033	1.249	(−0.07 to 0.51)	0.1303
Follicles	0.359	0.0002	1.411	(0.11 to 0.58)	0.0051
Papillae	0.120	0.2319	0.853	(−0.37 to 0.05)	0.1403
Giant papillae	0.197	0.0488	1.139	(−0.14 to 0.40)	0.3449
Bulbar conjunctiva					
Hyperemia	0.221	0.0267	1.078	(−0.19 to 0.34)	0.5739
Edema	0.218	0.0289	0.964	(−0.30 to 0.23)	0.7836
Limbus					
Trantas' dots	0.054	0.5895	1.389	(−0.25 to 0.91)	0.2627
Swelling	0.007	0.9417	0.633	(−1.07 to 0.15)	0.1410
Cornea					
Epithelial damage	−0.021	0.8330	0.860	(−0.45 to 0.15)	0.3203
Total objective Score	0.255	0.0099	—	—	—

R = Two-tailed Pearson's correlation coefficients were calculated to assess the association between the score for specific wheat IgE in tear fluid and various features of allergic conjunctivitis; OR, odds ratio; CI, confidence interval.

for wheat IgE, while 23/30 patients (76.6%) were positive for IgE to gluten, and 2/30 patients (6.6%) were positive for IgE to ω -5 gliadin⁷. Elevated levels of specific IgE for wheat are also associated with atopic dermatitis^{50–52}. Mehl et al. reported that 82% of atopic children with food allergy were positive for wheat IgE in their serum⁵². Wheat-containing foods, such as bread, pasta, cereals, and noodles, are a common part of the diet, so wheat proteins are absorbed from the intestinal lumen and enter the circulation. Additionally, many cosmetics contain wheat, including facial soaps, creams, lotions, and hairsprays. It is generally considered that the wheat allergens in cosmetics can penetrate the skin and can also enter the body via the rhinoconjunctival route, because these allergens are readily dissolved in aqueous solutions or cosmetic liquids^{7,53}. Molecules derived from wheat enter the venous blood after being absorbed through the skin. Our previous study demonstrated that giant papillae and conjunctival edema were most strongly associated with the total tear fluid IgE class⁴², while Donshik and associates reported that tear fluid IgE levels were increased in the more severely symptomatic eyes of patients with giant papillary conjunctivitis⁵⁴. Studies using transferrin as a marker for the entry of serum proteins into tear fluid have shown that leakage from conjunctival vessels is responsible for increased tear fluid levels of IgE⁵⁴. We previously reported that the total tear fluid level of IgE was correlated with the total serum IgE level in patients with allergic conjunctivitis⁴⁰. An increase of vascular permeability in the palpebral conjunctiva may contribute to local production of IgE that is released into the tears. Our findings suggest the possibility that specific wheat IgE in serum may enter the tear fluid from the conjunctival vessels rather than conjunctival contact allergy to wheat promoting the local production of specific wheat IgE.

In the present study, positivity for specific wheat IgE in tear fluid was correlated with both the objective and subjective scores for allergic conjunctivitis (Tables 5 and 6).

These results suggest that specific wheat IgE levels tear fluid reflect the severity of allergic conjunctivitis. Additionally, the severity of rhinitis symptoms increased and the effect on ADL tended to become greater as the specific wheat IgE level in tear fluid increased (Figure 4). The nasal and oral cavities communicate with the lacrimal sac and tears flows into the nasal cavity/oral cavity via the nasolacrimal duct. Thus, the scores for allergic rhinitis symptoms may be influenced by allergens in tear fluid that are transported through the nasolacrimal duct.

However, with respect to the question of whether wheat is actually a cause of allergic conjunctivitis, this study cannot provide a definite answer because there have been no previous reports about wheat and allergic conjunctivitis. It is also still unclear how patients are sensitized to wheat. Our previous study demonstrated the classes for both *D. pteronyssinus* and cat epithelium/dander IgE in tear fluid were correlated with the severity of allergic conjunctivitis in patients with allergic conjunctivitis³². On the other hand, no correlation was found between the class of cedar pollen IgE and any of the features of allergic conjunctivitis³². It is possible that specific IgE to wheat may be cross-reactive to other major allergens. Therefore, further studies are needed to investigate these issues by performing challenge tests such as the conjunctival allergen provocation test. Measurement of specific wheat IgE in tear fluid after washing the face with wheat-containing soap may also help to solve this question. Furthermore, we need to compare the levels of specific IgE for wheat in tear fluid and serum samples.

Conclusions

In conclusion, this study demonstrated that the tear fluid level of specific wheat IgE was closely associated with the clinical features of allergic rhinitis and allergic conjunctivitis. Although our results suggest that wheat may have a role in the development of allergic conjunctivitis, we need to

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determine whether specific wheat IgE in tear fluid is produced locally or systemically.

Declaration of interest

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