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Research Article

House Dust Mite Immunoglobulin G Antibodies in Patients with Allergic Conjunctivitis

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Abstract

Purpose: To examine the role of IgG in perennial allergy, we evaluated the relation between perennial allergic conjunctivitis and serum levels of specific IgG antibodies for HDM.

Methods: A prospective study was conducted in patients with seasonal allergic conjunctivitis (seasonal group, N = 10), patients with perennial allergic conjunctivitis (perennial group, N = 10) and control subjects (control group, N = 10). Serum levels of specific IgE and IgG antibodies for HDM were measured, as well as the total IgE level in tear fluid (total tear IgE), and a skin prick test was performed. Changes of the palpebral/bulbar conjunctiva and limbal or corneal lesions associated with allergic conjunctivitis were assigned a score (0 - 3), and the total conjunctivitis score was calculated (0 - 30).

Results: Serum HDM-specific IgG antibody levels were higher in the perennial group than the seasonal group (3.5 ± 0.5 vs. 1.3 ± 1.1), but there was no significant difference of HDM-specific IgE between the two groups (1.4 ± 0.7 vs. 1.4 ± 0.5). Multivariate analysis demonstrated that the mean wheal diameter in the skin prick test and the total conjunctivitis score were related to the serum level of HDM-specific IgG (odds ratio [OR] = 5.4, $p < 0.01$ and OR = 23.4, $p < 0.01$), but not to the serum level of HDM-specific IgE (OR = 1.4, $p = 0.68$ and OR = 0.4, $p = 0.35$).

Conclusion: The serum HDM IgG antibody level was more closely associated with allergic manifestations than the HDM IgE antibody level. Measurement of specific IgG antibodies may be helpful for detecting allergens responsible for perennial allergic diseases.

Keywords: *Allergic Conjunctivitis; House Dust Mite; Seasonal Allergy; Specific Ige; Specific Igg; Perennial Allergy*

Abbreviations

Ig: Immunoglobulin; HDM: House Dust Mites; ELISA: Enzyme-Linked Immunosorbent Assay; HRP: Horseradish Peroxidase; ANOVA: One-Way Analysis Of Variance; OR: Odd Ratio

Introduction

Immunoglobulin (Ig) E mediates immediate type I hypersensitivity reactions and has an important role in various allergic diseases, such as allergic asthma, allergic rhinitis, food allergies, and atopic dermatitis [1]. Accordingly, measurement of IgE is widely performed

for diagnosis of allergy and measurement of allergen-specific IgE levels is useful for identifying the causes of allergic reactions [2,3]. In contrast, IgG mediates type II and type III hypersensitivity reactions, which usually manifest as chronic inflammation. IgG antibodies increase in response to infection (after the early elevation of IgM), which means that measurement of IgG can be a diagnostic tool for infections and inflammatory diseases. Elevated serum IgG levels are also associated with atopic disorders4 and with food allergies [5-7], and measurement of serum food-specific IgG has been used for diagnosis of allergy to particular foods [8,9]. IgG is the most abundant Ig class in the serum of healthy persons, accounting for 70 - 75% of the total Ig pool [10], while IgE only represents 0.05% of the serum Ig pool [11]. However, the usefulness of measuring serum IgG for assessment of chronic allergic diseases has not been fully evaluated, unlike the extensive investigation of IgE in relation to allergy.

Allergic conjunctivitis, including seasonal, perennial and atopic conjunctivitis, is one of the most common allergic diseases. We subsequently demonstrated that the serum level of cat-specific IgG antibodies was more closely associated with the clinical features of perennial allergic conjunctivitis than the serum level of cat-specific IgE antibodies [12]. This finding suggests that IgG has a role in perennial allergy induced by indoor allergens. The most common indoor allergens are related to house dust mites (HDM), mold, cat and dog dander, and cockroaches, with HDM allergen being the most frequent cause of perennial allergy. Accordingly, measurement of HDM-specific IgG may be useful for diagnosis of perennial allergy, assessment of hyposensitization therapy, and avoidance of HDM allergen for prevention of perennial allergy.

Based on the above, we have investigated the relationship between IgE and IgG responses to indoor allergens in patients with perennial allergy, focusing on the role of HDM allergen in perennial allergic conjunctivitis.

Objective of the Study

The present study was performed with the following objectives: 1) to compare sensitization to HDM allergen between healthy subjects and patients with seasonal or perennial allergic conjunctivitis, 2) to compare the levels of specific IgG and IgE antibodies for HDM allergen with the cutaneous reaction to HDM allergen in patients with perennial allergic conjunctivitis, and 3) to assess the relation between serum levels of specific IgG antibodies for HDM allergen and the clinical features of perennial allergic conjunctivitis.

Materials and Methods

Study design

This prospective, nonrandomized, cross-sectional, consecutive case series study was conducted at Tokyo Women's Medical University Medical Center East Hospital and affiliated hospitals. This study was performed in accordance with the Declaration of Helsinki. Our institutional review board approved the study protocol and informed consent was obtained from each subject.

Subjects

The following three groups of subjects were enrolled (Table 1). 1) The seasonal group was comprised of 10 outpatients with acute seasonal allergic conjunctivitis (5 males and 5 females with a mean age of 29.2 ± 15.3 years; range: 7 - 56 years). The perennial group consisted of 10 patients with perennial allergic conjunctivitis (5 males and 5 females with a mean age of 29.8 ± 14.1 years; range: 7 - 58 years). 3) The age- and sex-matched control group comprised 10 healthy non-smoking subjects with no history of allergic disease (4 males and 6 females with a mean age of 30.5 ± 10.7 years; range: 14 - 46 years). The control group was enrolled from among persons attending the outpatient clinic for eye screening (Table 1).

Diagnosis of allergic conjunctivitis

Allergic conjunctivitis was diagnosed from symptoms (such as ocular itching and tearing) and the findings on slit lamp examination, including conjunctival hyperemia, follicles, and papillae. Diagnosis was performed by a single ophthalmologist (T.M.) according to published guidelines [13].

In each subject, the findings on slit lamp examination were assessed at the first visit as follows. Changes of the palpebral conjunctiva (hyperemia, edema, follicles, papillae, and giant papillae), changes of the bulbar conjunctiva (hyperemia and chemosis), changes of the limbus (Trantas' dots and edema), and corneal involvement were each classified into 4 grades (0 = normal, 1+ = mild, 2+ = moderate, and 3+ = severe) [14-16]. Then the total allergic conjunctivitis score (maximum: 30 points) was calculated as the sum of the scores for the above-mentioned 10 findings [15]. While both eyes were evaluated, only data for the right eye were used in this study.

Measurement of total IgE in tear fluid

Measurement of the total IgE level in tear fluid (total tear IgE) was performed with the Allerwatchâ test according to the manufacturers instructions (Hitachi Chemical Co., Ltd., Tokyo, Japan; and Wakamoto Pharmaceutical Co., Ltd., Tokyo, Japan) [17-26].

Measurement of serum specific IgE for HDM allergens

Specific IgE antibodies for HDM allergen were measured with the ImmunoCAP Rapid immunochromatography assay kit according to the manufacturer's instructions (Phadia Diagnostics, Uppsala, Sweden).

Measurement of serum specific IgG for HDM allergen

Specific IgG antibodies for HDM allergen were measured by enzyme-linked immunosorbent assay (ELISA). Blood was obtained from a fingertip using a Microtainer safety lancet and about 150 ml was applied to each of the three test strips, which were dried and packed into plastic bags for shipping to US BioTek Laboratories (Seattle, WA) where measurement of specific IgG for HDM allergens was performed by ELISA [27]. In brief, whole blood was added to an ELISA plate coated with HDM antigens and excess blood was washed off. Then anti-human IgG antibody conjugated with horseradish peroxidase (HRP) was added to the plate to detect the bound IgG. After washing, tetramethylbenzidine was added to develop a blue color by reacting with HRP. The reaction was stopped by adding the stop solution, causing the color to become yellow. Then the optical density was measured at 450 nm to determine the titer of HDM-specific IgG antibodies [28], and the results were expressed as a class score (0 to 6).

Skin prick test

The skin prick test for HDM allergen was performed with a single-use sterile lancet (Yayoi Corporation, Tokyo, Japan). A drop of HDM allergen solution (1:20 wt/vol; Torii Pharmaceutical Co., Ltd., Tokyo, Japan) was applied to the forearm and introduced into the epidermis by lancet puncture. Physiological saline (isotonic sodium chloride; Otsuka Pharmaceutical Co., Tokyo, Japan) was used as the negative control. Evaluation was done after 15 min and a mean wheal diameter ≥ 3 mm was considered positive.

Statistical analysis

Mean values were compared between two groups with the two-tailed unpaired Student's t-test or among three groups by one-way analysis of variance and Scheffe's multiple comparison test. Frequencies were analyzed with the chi-square test of independence or Fisher's exact probability test. In the case of semiquantitative data, differences between two groups were examined by the two-tailed Mann-Whitney U test, while Kruskal-Wallis one-way analysis of variance (ANOVA) by ranks was employed for three groups. Relations among variables were investigated by calculation of Pearson's correlation coefficients and differences were assessed with Fisher's Z transforma-

tion. Factors associated with the result of the skin prick test or with the total conjunctivitis score were investigated by multivariate logistic regression analysis, with explanatory variables including the score for each feature of allergic conjunctivitis. The sensitivity, specificity, and positive and negative predictive values were calculated with standard formulae. Statistical analysis was performed with SAS System software version 9.1 (SAS Institute Inc., Cary, North Carolina, USA) and significance was accepted at $p < 0.05$.

Results

A positive result for total tear IgE was significantly more frequent in the seasonal group (100.0%) and the perennial group (100.0%) than in the control group (0.0%) ($c^2 = 30.0$, df = 2, $p < 0.001$, Fisher's exact probability test) (Table 1). A positive result for serum specific IgE antibodies to HDM allergen (serum HDM IgE) was also significantly more frequent in the seasonal group (90.0%) and the perennial group (100.0%) than in the control group (0.0%, $c^2 = 26.12$, df = 2, $p < 0.001$, Fisher's exact test) (Table 1). In addition, the positive rate for serum specific IgG antibodies to HDM allergen (serum HDM IgG) was significantly higher in the perennial group (100.0%) than in the control group (0.0%) or the seasonal group (60.0%, $c^2 = 20.36$, df = 2, $p < 0.001$, Fisher's exact test). Table 2 shows the sensitivity, specificity, positive predictive value, and negative predictive value of total tear IgE, serum HDM IgE, and serum HDM IgG in relation to the skin prick test for HDM.

	Control Group	Seasonal Group	Perennial Group	P value
Number of Subjects	10	10	10	-
Gender: Male/Female	4/6	5/5	5/5	*NS
Age (years)	30.5 ± 10.7	29.2 ± 15.3	29.8 ± 14.1	**0.9793
Carpets or rugs at home	3	3	4	*NS
Allergy Tests				
Total tear IgE	0 (0.0%)	10 (100.0%)	10 (100.0%)	*<0.001
Serum HDM IgE	0 (0.0%)	9 (90.0%)	10 (100.0%)	*<0.001
Serum HDM IgG	0 (0.0%)	6 (60.0%)	10 (100.0%)	*<0.001
Skin prick test (HDM)	0 (0.0%)	4 (40.0%)	7 (70.0%)	*0.005

Table 1: *Clinical profile of each group.*
Number of patients (percentage) or mean ± standard deviation (SD).
**Chi-square test of independence or Fisher's exact probability test.*
***One-way analysis of variance and Scheffe's multiple comparison test.*
NS: Not significant. HDM: House Dust Mite.

	Total tear IgE	Serum HDM IgE	Serum HDM IgG
Sensitivity	100.0%	100.0%	100.0%
Specificity	58.8%	64.7%	82.4%
False-positive rate	41.2%	0.0%	0.0%
False-negative rate	0.0%	35.3%	17.6%
Positive predictive value	65.0%	68.4%	81.3%
Negative predictive value	100.0%	100.0%	100.0%
Positive likelihood ratio	2.4	2.8	5.7

Table 2: *Results of immunoglobulin assays.*
Detection of IgE or IgG antibody in relation to positive/negative results of the skin prick test for HDM allergen in all subjects (N = 30).

The total tear IgE level and the serum levels of HDM IgE and HDM IgG were compared among the control, seasonal, and perennial groups (Figure 1-3). There were significant differences of the total tear IgE grade between the control group and the seasonal group or the perennial group (0.0 ± 0.0 vs. 1.8 ± 0.4 or 1.6 ± 0.5, respectively, both $p < 0.001$) (Figure 1), as well as significant differences of the serum HDM IgE grade (0.0 ± 0.0 vs. 1.4 ± 0.7 or 1.4 ± 0.5, respectively, $p < 0.001$) (Figure 2). The serum HDM IgG class showed a significant difference among all three groups (0.0 ± 0.0 vs. 1.3 ± 1.2 vs. 3.5 ± 0.5, respectively, all $p < 0.001$, Kruskal-Wallis one-way analysis of variance) (Figure 3).

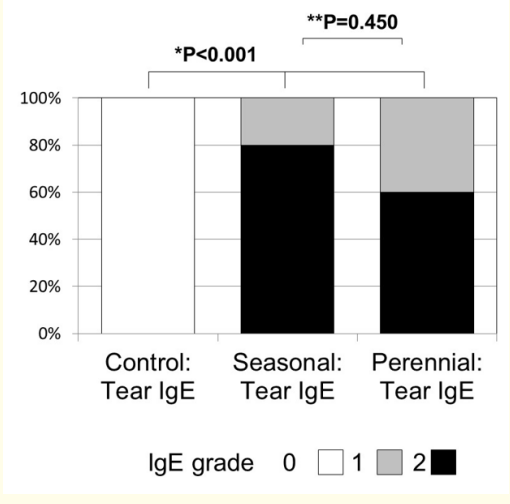

Figure 1: *Comparison of the grade of positivity for total tear immunoglobulin E (IgE) among the control, seasonal, and perennial groups. Total tear IgE was determined by the Allerwatch® test and a grade was assigned as described in Methods. Results were compared among the three groups by Kruskal-Wallis one-way analysis of variance*, while the seasonal and perennial groups were compared by the two-tailed Mann-Whitney U test**.*

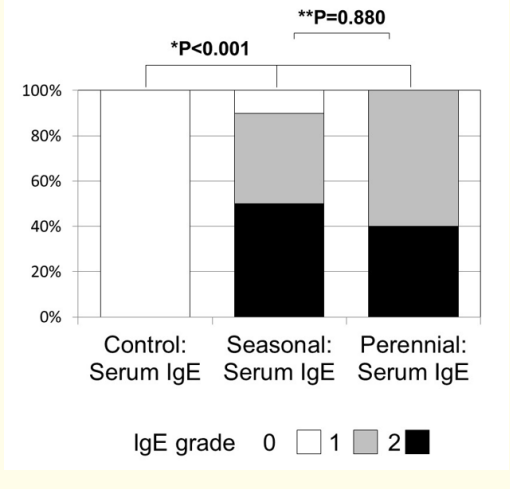

Figure 2: *Comparison of the serum house dust mite (HDM)-specific IgE grade among the control, seasonal, and perennial groups. HDM-specific IgE was determined by the ImmunoCAP Rapid* immunochromatography assay and a grade was assigned as described in Methods. Results were compared among the three groups by Kruskal-Wallis one-way analysis of variance*, while the seasonal and perennial groups were compared by the two-tailed Mann-Whitney U test**.*

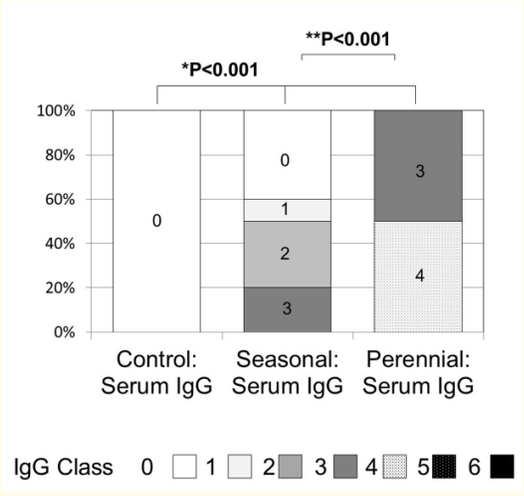

Figure 3: *Comparison of the serum HDM-specific IgG class among the control, seasonal, and perennial groups. HDM-specific IgG was determined by ELISA and the result was converted to a class (0-6). Results were compared among the three groups by Kruskal-Wallis one-way analysis of variance*, while the seasonal and perennial groups were compared by the two-tailed Mann-Whitney U test**.*

When the seasonal and perennial groups were compared, the serum HDM IgG class was significantly higher in the perennial group than in the seasonal group (1.3 ± 1.2 vs. 3.5 ± 0.5, p < 0.001) (Figure 3). However, there was no significant difference of the total tear IgE grade (1.8 ± 0.4 vs. 1.6 ± 0.5, P = 0.450, Figure 1) or the serum HDM IgE grade between the two groups (1.4 ± 0.7 vs. 1.4 ± 0.5, P = 0.880, two-tailed Mann-Whitney U test) (Figure 2).

A positive skin prick test for HDM allergen was more frequent in the seasonal group (40.0%) and the perennial group (70.0%) than in the control group (0.0%) (c^2 = 10.6, df = 2, p = 0.005, Fisher's exact probability test) (Table 1). The mean wheal diameter in the skin prick test was significantly larger in the seasonal group (3.5 ± 1.7 mm) and the perennial group (8.1 ± 3.3 mm) compared with the control group (1.8 ± 1.0 mm, both p < 0.001, Kruskal-Wallis one-way analysis of variance) (Figure 4). Mean wheal diameter was also significantly larger in the perennial group than the seasonal group (p = 0.004, two-tailed unpaired Student's t-test) (Figure 4).

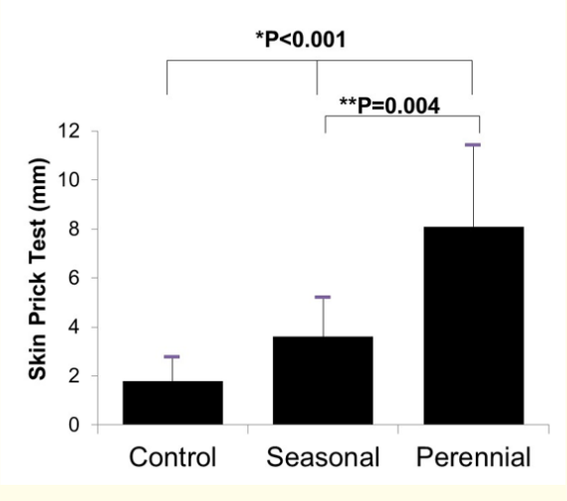

Figure 4: *Comparison of the mean wheal diameter in the skin prick test for HDM allergen among the control, seasonal, and perennial groups. Results were compared among the three groups by Kruskal-Wallis one-way analysis of variance*, while the seasonal and perennial groups were compared by the two-tailed Mann-Whitney U test**.*

The allergy patients were divided into three groups based on the grade of specific HDM IgE (grade 0 in 1 patient, grade 1 in 10 patients, and grade 2 in 9 patients). They were also divided into three groups based on the class of specific HDM IgG (class 0 in 4 patients, class 1 in 1 patient, class 2 in 3 patients, class 3 in 7 patients, class 4 in 5 patients, and no patients in class 5 or 6). When the total conjunctivitis score was compared, it showed a significant difference across the five IgG class groups (p = 0.011), but no significant difference was noted among the three IgE grade groups (p = 0.804, Kruskal-Wallis one-way analysis of variance) (Figure 5). Patients with carpets or rugs at home (n = 7) and those without carpets or rugs (n = 13) showed no significant differences with respect to positivity for total tear IgE (100.0% vs. 100.0%) and the total tear IgE grade (1.6 ± 0.5 vs. 1.9 ± 0.3, p = 0.245), or positivity for serum HDM IgE (92.3% vs. 100.0%, p = 0.650) and the HDM IgE grade (1.5 ± 0.6 vs. 1.3 ± 0.5, p = 0.508) (Fisher's exact probability test and the two-tailed Mann-Whitney U test) (Table 3). In contrast, patients with and without carpets or rugs showed a significant difference of positivity for serum HDM IgG (69.2% vs. 100.0%, p = 0.148) and the HDM IgG class (1.9 ± 1.5 vs. 3.3 ± 0.7, p = 0.017), as well as differences of the skin prick test positive rate and the mean wheal diameter (46.2% vs. 71.4%, p = 0.272 and 3.1 ± 2.1 vs. 8.0 ± 4.2, p = 0.029, respectively, Fisher's exact probability test and the two-tailed Mann-Whitney U test) (Table 3).

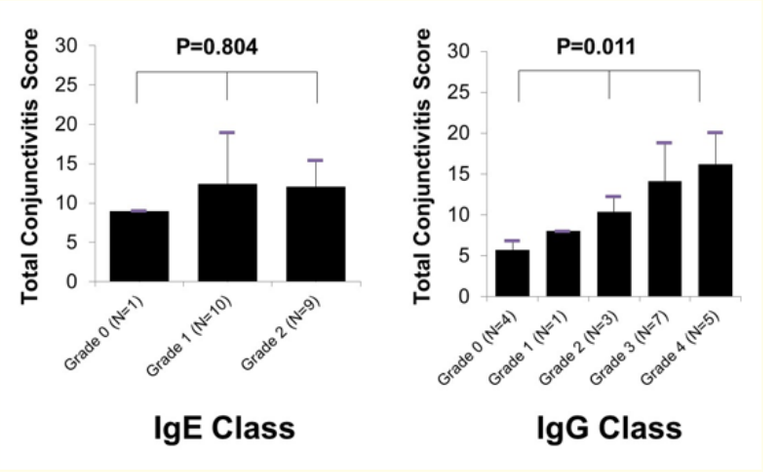

Figure 5: *Comparison of the mean total conjunctivitis score for ocular signs of allergic conjunctivitis among groups with different serum levels of HDM IgE or IgG. Patients were divided into three groups according to the HDM IgE grade or HDM IgG class. Results are expressed as the mean ± SD. Comparison among the groups was performed by Kruskal-Wallis one-way analysis of variance.*

	No carpets or rugs at home	Carpets or rugs at home	P Value
Number of Patients	13/20 (65.0%)	7/20 (35.0%)	-
Total tear IgE			
Positive rate	13/13 (100%)	7/7 (100%)	-
Score (mean ± SD)	1.6 ± 0.5	1.9 ± 0.3	**0.245
Serum HDM IgE			
Positive rate	12/13 (92.3%)	7/7 (100.0%)	*0.650
Score (mean ± SD)	1.5 ± 0.6	1.3 ± 0.5	**0.508
Serum HDM IgG			
Positive rate	9/13 (69.2%)	7/7 (100.0%)	*0.148
Score (mean ± SD)	1.9 ± 1.5	3.3 ± 0.7	**0.017
Skin prick test			
Positive rate	6/13 (46.2%)	5/7 (71.4%)	*0.272
mm (mean ± SD)	3.1 ± 2.1	8.0 ± 4.2	**0.029

Table 3: *Comparison of results between patients with or without carpets or rugs at home.*
Number of patients (percentage) or mean ± standard deviation (SD).
**Fisher's exact probability test. **Two-tailed Mann-Whitney U test.*

The 20 patients with allergic conjunctivitis were divided into an IgG-negative group (n = 4) and an IgG-positive group (n = 16) to compare the clinical features of allergic conjunctivitis between patients who were positive or negative for serum HDM IgG (Table 4). The IgG-positive group had significantly higher scores for the clinical features of allergic conjunctivitis compared with the IgG-negative group, including the scores for palpebral conjunctival hyperemia (1.5 ± 0.5 vs. 2.3 ± 0.7, p = 0.028), palpebral conjunctival edema (1.0 ± 0.0 vs. 1.9 ± 0.8, p < 0.001), bulbar conjunctival hyperemia (1.5 ± 0.5 vs. 2.3 ± 0.4, p = 0.037), and limbal edema (0.0 ± 0.0 vs. 0.3 ± 0.6, p = 0.028). The IgG-positive group also had a significantly higher total conjunctivitis score (7.0 ± 2.1 vs. 12.1 ± 4.0, p = 0.007, two-tailed unpaired Student's t-test) (Table 4).

	IgG-negative	IgG-positive	P value
Number of Patients	4	16	-
Palpebral conjunctiva			
Hyperemia	1.5 ± 0.5	2.3 ± 0.7	0.028
Edema	1.0 ± 0.0	1.9 ± 0.8	<0.001
Follicles	1.3 ± 0.4	1.6 ± 0.8	0.181
Papillae	0.8 ± 0.4	1.3 ± 0.7	0.056
Giant papillae	0.0 ± 0.0	0.1 ± 0.5	0.167
Bulbar conjunctiva			
Hyperemia	1.5 ± 0.5	2.3 ± 0.4	0.037
Chemosis	0.8 ± 0.8	1.8 ± 0.8	0.062
Limbus			
Trantas' dots	0.3 ± 0.4	0.5 ± 0.7	0.224
Edema	0.0 ± 0.0	0.3 ± 0.6	0.028
Cornea			
Epithelial damage	0.0 ± 0.0	0.1 ± 0.2	0.166
Total Conjunctivitis Score	7.0 ± 2.1	12.1 ± 4.0	0.007

Table 4: *Scores for allergic conjunctivitis in IgG-negative and IgG-positive patients. The two-tailed unpaired t-test was used to compare the clinical features of allergic conjunctivitis between patients who were positive and negative for serum HDM IgG. Ocular findings were assessed by slit lamp examination on a scale of 0 - 3 (0 = normal, 3 = severe) and the total conjunctivitis score was calculated as the sum of the individual scores (0 - 30 points).*

According to two-tailed Pearson's correlation coefficient analysis, the mean skin prick test wheal diameter for HDM allergen in all 30 subjects showed a significant positive correlation with the total tear IgE grade (r = 0.44, p = 0.008), the serum HDM IgE grade (r = 0.45, p = 0.006), and the serum HDM IgG class (r = 0.78, p < 0.001) (Table 5). The correlation between the mean wheal diameter and serum HDM IgG was significantly stronger than that for serum HDM IgE (p = 0.037, Fisher's z statistics). In addition, multivariate analysis demonstrated that the mean wheal diameter in the skin prick test was associated with serum HDM IgG (but not HDM IgE) in all 30 subjects (odds ratio [OR] = 5.4, p < 0.001) (Table 5). In all of the patients with allergic conjunctivitis (n = 20), as well as in the seasonal group (n = 10) and the perennial group (n = 10), the mean wheal diameter in the skin prick test was strongly correlated with the serum HDM IgG class (r = 0.68, p < 0.001; r = 0.74, p = 0.006; and r = 0.80, p = 0.003, respectively), but was not correlated with the total tear IgE grade (r = -0.18, p = 0.784; r = 0.03, p = 0.466; and r = -0.10, p = 0.606, respectively) or the serum HDM IgE grade (r = 0.00, p = 0.492; r = 0.33, p = 0.173; and r = -0.21, p = 0.718, respectively, two-tailed Pearson's correlation coefficient analysis) (Table 5).

	Correlation Coefficient		Multivariate analysis		
Variable	**R**	**P value**	**OR**	**(95% CI)**	**P value**
All Subjects (N = 30)					
Total tear IgE	0.44	0.008	0.7	(0.1 - 3.7)	0.633
Serum HDM IgE	0.45	0.006	1.4	(0.2 - 8.7)	0.681
Serum HDM IgG	0.78	< 0.001	5.4	(2.8 - 10.6)	<0.001
Seasonal and Perennial Groups (N = 20)					
Total tear IgE	-0.18	0.784	0.5	(0.0 - 11.8)	0.633
Serum HDM IgE	0.00	0.492	1.3	(0.1 - 14.5)	0.681
Serum HDM IgG	0.68	< 0.001	5.2	(2.0 - 13.7)	<0.001
Seasonal Group (N = 10)					
Total tear IgE	0.03	0.466	1.6	(0.2 - 16.4)	0.607
Serum HDM IgE	0.33	0.173	2.4	(0.6 - 9.2)	0.161
Serum HDM IgG	0.74	0.006	3.0	(1.4 - 6.2)	0.011
Perennial Group (N = 10)					
Total tear IgE	-0.10	0.606	0.3	(0.0 - 8.6)	0.393
Serum HDM IgE	-0.21	0.718	0.2	(0.0 - 5.0)	0.242
Serum HDM IgG	0.80	0.003	76.9	(6.4 - 926.9)	0.005

Table 5: *Correlations between skin prick test results and immunoglobulins, as well as the results of multivariate analysis. R=Two-tailed Pearson's correlation coefficients were calculated for the association between the mean wheal diameter in the skin prick test for HDM allergen and each immunoglobulin grade/class. OR: Odds Ratio; CI: Confidence Interval.*

In all 30 subjects, two-tailed Pearson's correlation coefficient analysis showed that the total conjunctivitis score was correlated with the total tear IgE grade ($r = 0.75$, $p < 0.001$), as well as with the serum HDM IgE grade ($r = 0.66$, $p < 0.001$) and serum HDM IgG class ($r = 0.87$, $p < 0.001$) (Table 6). Multivariate analysis revealed that the total conjunctivitis score was strongly correlated with total tear IgE (OR = 23.3, $p = 0.002$) and with serum HDM IgG (OR = 23.4, $p < 0.001$) (Table 6). Multivariate logistic regression analysis was used to assess the relation between the total conjunctivitis score and serum HDM IgG or IgE in the patients with allergic conjunctivitis. This analysis showed that an increase of the serum HDM IgG class score by 1 point resulted in an increase of the total conjunctivitis score by 3.00 points (OR = 20.1, $p < 0.001$, Table 6 and figure 6). In contrast, an increase of the HDM IgE grade score by 1 point was associated with a decrease of the total conjunctivitis score by 1.98 points (OR = 0.1, $p = 0.207$) (Table 6 and figure 6).

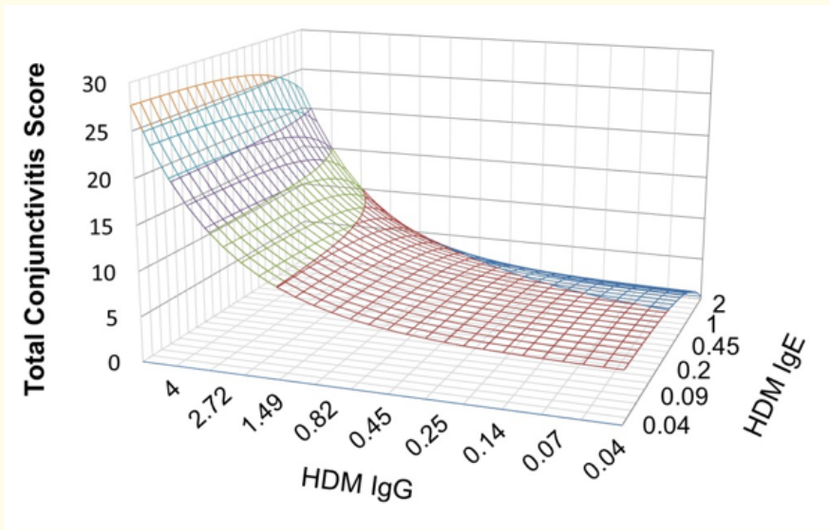

Figure 6: *Predicted probability of each total conjunctivitis score for ocular changes in relation to HDM IgE and HDM IgG. Probabilities were calculated by multivariate logistic regression analysis.*

	Correlation Coefficient		Multivariate analysis		
Variable	**R**	**P value**	**OR**	**(95% CI)**	**P value**
All Subjects (N = 30)					
Total tear IgE	0.75	<0.001	23.3	(3.6 - 150.5)	0.002
Serum HDM IgE	0.66	<0.001	0.4	(0.0 - 3.4)	0.351
Serum HDM IgG	0.87	<0.001	23.4	(8.4 - 64.8)	<0.001
Seasonal and Perennial Groups (N = 20)					
Total tear IgE	0.10	0.686	3.5	(0.1 - 155.4)	0.499
Serum HDM IgE	0.05	0.826	0.1	(0.0 - 3.3)	0.207
Serum HDM IgG	0.74	<0.001	20.1	(5.5 - 73.3)	<0.001
Seasonal Group (N = 10)					
Total tear IgE	0.14	0.708	22.3	(0.1 - 6008.8)	0.223
Serum HDM IgE	0.22	0.535	0.9	(0.0 - 48.8)	0.990
Serum HDM IgG	0.85	0.002	19.6	(3.9 - 97.8)	0.004
Perennial Group (N = 10)					
Total tear IgE	0.23	0.526	0.6	(0.0 - 604.9)	0.855
Serum HDM IgE	-0.43	0.222	0.1	(0.0 - 87.2)	0.465
Serum HDM IgG	0.78	0.007	73.5	(1.3 - 4053.5)	0.039

Table 6: *Correlations between the total conjunctivitis score and immunoglobulins, as well as the results of multivariate analysis. R=Two-tailed Pearson's correlation coefficients were calculated for the association between the total conjunctivitis score for allergic conjunctivitis and the results of the other allergy tests. OR: Odds Ratio; CI: Confidence Interval.*

Discussion

This study showed that the serum level of HDM-specific IgG antibodies (HDM IgG class) and the serum HDM IgG positive rate were higher in patients with perennial allergic conjunctivitis than in healthy controls or patients with seasonal allergic conjunctivitis. In addition, the HDM IgG class was more strongly correlated with the skin test response to HDM allergen and with the severity of ocular signs of allergic conjunctivitis (total conjunctivitis score) than the serum level of HDM-specific IgE antibodies (HDM IgE grade). Furthermore, multivariate analysis demonstrated that the HDM IgG class was the most important determinant of the severity of allergic conjunctivitis and the skin test response to HDM allergen in both groups of patients with allergic conjunctivitis (seasonal and perennial). These results suggest that specific IgG antibodies targeting culprit allergens are more closely involved in the development of perennial allergic conjunctivitis than specific IgE antibodies.

The skin prick test for HDM allergen was positive in 55.0% (11/20) of our study population with allergic conjunctivitis. Park., *et al.* reported that HDM was the most common perennial allergen (55.6%) detected by the skin prick test in Korean patients with allergic rhinitis [29], and the skin prick test for HDM was positive in 63.0% (613/977) of patients with chronic rhinitis from Hong Kong [30], the present findings were consistent with these other reports about Asian patients.

In this study, we compared total tear IgE, serum HDM IgE, serum HDM IgG, and the skin prick test for HDM between patients with seasonal and perennial allergic conjunctivitis. We found no significant differences of total tear IgE (Figure 1) and serum HDM IgE (Figure 2) between these two groups. However, the serum HDM IgG class was higher in the perennial group than the seasonal group (Figure 3), and the mean wheal diameter in the skin prick test for HDM was larger in the perennial group (p = 0.004, Figure 4). In addition, the mean

wheal diameter in the skin prick test was correlated with the serum HDM IgG class in the perennial group (r = 0.80, Table 5). Furthermore, the total conjunctivitis score for ocular signs of allergic conjunctivitis was not significantly correlated with serum HDM IgE in the perennial group (r = -0.43), but was strongly correlated with serum HDM IgG (r = 0.78, Table 6). These results suggest that indoor allergens such as HDM allergen are associated with the development of perennial allergic conjunctivitis. Small allergen particles such as animal dander and HDM can remain in the environment for a long time, floating in the air or contaminating bedclothes and mattresses. Therefore, indoor allergens like HDM could trigger ongoing allergic reactions in patients with perennial allergic conjunctivitis. These findings also support our previous observations about serum HDM IgE levels in the autumn season [3].

When we investigated the clinical significance of serum HDM IgG and its relationship with HDM IgE, we found that HDM IgG had a higher specificity for the diagnosis of HDM antigen-induced allergic conjunctivitis than HDM IgE (Table 2). The skin prick test response to HDM allergen was more strongly correlated with the serum HDM IgG class than with the HDM IgE grade (Table 5). Patients who were positive for HDM IgG had significantly higher scores for ocular signs of allergic conjunctivitis compared with IgG-negative patients, including the scores for palpebral conjunctival hyperemia and edema, bulbar conjunctival hyperemia/chemosis, and limbal edema, as well as a significantly higher total conjunctivitis score (Table 4). Furthermore, the total conjunctivitis score was more strongly correlated with serum HDM IgG than with HDM IgE in the perennial group (Table 6). These results suggested that measuring specific IgG may be more useful than measuring specific IgE for diagnosis of indoor allergen-induced perennial allergic conjunctivitis and for assessment of its severity. Specific IgE mediates type 1 hypersensitivity and is primarily responsible for diseases related to immediate allergic reactions [31]. Type I hypersensitivity is accompanied by a shift of the T helper (Th) 1/Th2 balance toward a Th2-dominant state. In contrast to IgE, specific IgG is involved in protective immunity, although it also mediates type II and type III hypersensitivity reactions [32]. Production of specific IgG is regulated by Th1 cells after class switching and is responsible for the secondary immune response. Thus, our findings could be explained by a shift of the Th1/Th2 balance toward a Th1-dominant state. Chronic or delayed-type hypersensitivity reactions are mediated by Th1 cells. Therefore, it is possible that a mild Th1 response to indoor allergens may induce continuous IgG production in patients with chronic perennial allergy rather than a Th2 response associated with IgE. Accordingly, current knowledge about the Th1/Th2 pathways involved in perennial allergy and our results suggest that specific IgG is more closely associated with the development of perennial allergy than specific IgE.

In the present study, we compared sensitization to HDM between patients with and without carpets or rugs at home. There was no significant difference in the prevalence of carpet or rug use between the seasonal group (30.0%) and the perennial group (40.0%, Table 1). Interestingly, there were no significant differences of the total tear IgE and serum HDM IgE grades between patients with or without carpets/rugs at home (Table 3). In contrast, the serum HDM IgG class was higher and the response to HDM in the skin prick test was stronger in patients with carpets/rugs at home than in patients without carpets/rugs (Table 3). The efficacy of avoiding exposure to HDM has recently been debated [33-35]. Although its usefulness is not supported by controlled clinical trials or observational studies, avoidance of exposure to HDM is still widely recommended to improve allergy symptoms. Sebõk., *et al.* performed a cohort study and reported that carpet use in early life as well as indoor pets was associated with an increased risk of atopic dermatitis during childhood [36]. Miyake., *et al.* reported that HDM allergen levels in the bedroom and mold in the kitchen during pregnancy or early life were linked to an increased risk of probable atopic dermatitis in childhood [37]. Also, Chen., *et al.* reported that childhood asthma was associated with early exposure to environmental factors, such as carpets (OR = 2.36) and pets (OR = 2.11) [38]. Mihrshahi., *et al.* surveyed the home environment of 616 families and found that 68% of their beds, 65% of their bedroom floors and 56% of their living room floors had HDM concentrations above 10 µg / gram of fine dust [39]. Hence, it has been suggested that reducing indoor HDM levels may reduce symptoms of allergic reactions. Air conditioning may also reduce the growth of HDM by lowering humidity.

We also examined the relationship between serum HDM IgG and the scores for ocular signs of allergic conjunctivitis. Patients who were positive for serum HDM IgG had higher total conjunctivitis scores and higher scores for several individual features of allergic conjunctivitis compared with IgG-negative patients (Table 4). The serum HDM IgG class was also significantly correlated with the total conjunctivitis score (Table 6). These results indicate that the serum level of HDM-specific IgG antibody reflects the severity of perennial allergic conjunc-

tivitis. Allergens can be divided into indoor and outdoor allergens according to their source. Indoor allergens are more closely related to perennial allergic conjunctivitis, while outdoor allergens like pollens are more frequently associated with seasonal allergic conjunctivitis [39]. HDM represent a major indoor allergen. Continuous exposure to indoor allergens such as HDM may cause chronic proliferative changes of the conjunctiva that persist throughout the year [40,41]. On the other hand, seasonal allergic conjunctivitis is related to a wide variety of outdoor allergens such as pollens that cause acute ocular inflammation, but rarely produce the proliferative changes associated with chronic inflammation [41]. Accordingly, seasonal allergic conjunctivitis is associated with an increase of IgE for outdoor allergens, while serum IgG levels for indoor allergens are increased in patients with perennial allergic conjunctivitis.

Conclusion

In conclusion, serum HDM IgG was more closely associated with the clinical features of perennial allergic conjunctivitis than serum HDM IgE, suggesting that specific IgG antibodies may cause symptoms of perennial allergic conjunctivitis related to indoor allergens such as HDM. Measurement of specific IgG levels may be useful for evaluating the effectiveness of countermeasures for perennial allergic diseases, such as avoiding exposure to HDM or allergen-specific immunotherapy.

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Conflict of Interest

All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

Ethical Approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This article does not contain any studies with animals performed by any of the authors.

Informed Consent

Informed consent was obtained from all individual participants included in the study.

Bibliography

1. Gould HJ., *et al*. "The biology of IGE and the basis of allergic disease". *Annual Review of Immunology* 21 (2003): 579-628.
2. Mimura T., *et al*. "Correlations between allergen-specific IgE serum levels in patients with allergic conjunctivitis in spring". *Ocular Immunology and Inflammation* 12 (2004): 45-51.
3. Mimura T., *et al*. "Allergens in Japanese patients with allergic conjunctivitis in autumn". *Eye* 19 (2005): 995-999.
4. Custovic A., *et al*. "Allergen-specific IgG antibody levels modify the relationship between allergen-specific IgE and wheezing in childhood". *The Journal of Allergy and Clinical Immunology* 127 (2011): 1480-1485.

5. Zimmer A., *et al*. "Food allergen-specific serum IgG and IgE before and after elimination diets in allergic dogs". *Veterinary Immunology and Immunopathology* 144 (2011): 442-447.

6. Bethlehem S., *et al*. "Patch testing and allergen-specific serum IgE and IgG antibodies in the diagnosis of canine adverse food reactions". *Veterinary Immunology and Immunopathology* 145 (2012): 582-589.

7. Sverremark-Ekström E., *et al*. "Peanut sensitization during the first 5 yr of life is associated with elevated levels of peanut-specific IgG". *Pediatric Allergy and Immunology* 23 (2012): 224-229.

8. Zuo XL., *et al*. "Alterations of food antigen-specific serum immunoglobulins G and E antibodies in patients with irritable bowel syndrome and functional dyspepsia". *Clinical and Experimental Allergy* 37 (2007): 823-830.

9. Volpi N and Maccari F. "Serum IgG responses to food antigens in the italian population evaluated by highly sensitive and specific ELISA test". *Journal of Immunoassay and Immunochemistry* 30 (2009): 51-69.

10. Junqueira Luiz C and Jose Carneiro. Basic Histology. McGraw-Hill (2003).

11. Winter WE., *et al*. "Immunoglobulin E: importance in parasitic infections and hypersensitivity responses". *Archives of Pathology and Laboratory Medicine* 124 (2000): 1382-1385.

12. Miyama A., *et al*. "Specific IgG for cat allergens in patients with allergic conjunctivitis". *International Ophthalmology* 35 (2015): 575-586.

13. Takamura E., *et al*. "Japanese Society of Allergology. Japanese guideline for allergic conjunctival diseases". *Allergology International* 60 (2011): 191-203.

14. Japanese Ocular Allergology Study Group. "Guidelines for the clinical management of allergic conjunctival diseases. The Japanese Ophthalmological Society". *Nippon Ganka Gakkai Zasshi* 110 (2006): 103-140.

15. Uchio E., *et al*. "Demographic aspects of allergic ocular diseases and evaluation of new criteria for clinical assessment of ocular allergy". *Archive for Clinical and Experimental Ophthalmology* 246 (2008): 291-296.

16. Ohashi Y., *et al*. "A randomized, placebo-controlled clinical trial of tacrolimus ophthalmic suspension 0.1% in severe allergic conjunctivitis". *Journal of Ocular Pharmacology and Therapeutics* 26 (2010): 165-174.

17. Sirbikaite L ans Ehlers N. "The use of Lacrytest in ocular allergy". *Acta Ophthalmologica Scandinavica* 85 (2007): 117.

18. Monzón S., *et al*. "Conjunctivitis and Total IgE in Lacrimal Fluid: Lacrytest Screening". *Journal of Allergy* (2009): 1-6.

19. Inada N., *et al*. "Clinical evaluation of total IgE in tears of patients with allergic conjunctivitis disease using a novel application of the immunochromatography method". *Allergology International* 58 (2009): 585-589.

20. Mimura FT., *et al*. "Rapid immunochromatography of total tear immunoglobulin E in allergic conjunctivitis with Allerwatch". *The Journal of Investigational Allergology and Clinical Immunology* 20 (2010): 627-628.

21. Mimura T., *et al*. "Immunochromatographic assay for measurement of total IgE in tears, nasal mucus, and saliva of patients with allergic rhinoconjunctivitis". *Journal of Asthma* 47 (2010): 1153-1160.

22. Mimura T., *et al*. "Relationship between total tear and serum IgE in allergic conjunctivitis". *International Archives of Allergy and Immunology* 154 (2011): 349-352.

23. Mimura T., *et al*. "Relation between total tear IgE and specific serum IgE in seasonal allergic conjunctivitis". *Cornea* 30 (2011): 790-795.

24. Mimura T., *et al*. "Relation between total tear IgE and severity of acute seasonal allergic conjunctivitis". *Current Eye Research* 37 (2012): 864-870.

25. Mimura T. "Bilateral eyelid erythema associated with false eyelash glue". *Cutaneous and Ocular Toxicology* 32 (2013): 89-90.

26. Mimura T., *et al*. "Relationship between total tear IgE and specific serum IgE in autumnal allergic conjunctivitis". *Cornea* 32 (2013): 14-19.

27. Suen RM and Gordon S. "The clinical relevance of IgG food allergy testing through ELISA (Enzyme-linked immunosorbent assay". US BioTek Laboratories© (2003): 1-8.

28. Hodsdon W and Zwickey H. "NMJ original research: reproducibility and reliability of two food allergy testing methods". *Natural Medicine Journal* 2.3 (2010): 8-13.

29. Park HJ., *et al*. "Changes in allergen sensitization over the last 30 years in Korea respiratory allergic patients: a single-center". *Allergy, Asthma and Immunology Research* 6.5 (2014): 434-443.

30. Yuen AP., *et al*. "The skin prick test results of 977 patients suffering from chronic rhinitis in Hong Kong". *Hong Kong Medical Journal* 13 (2007): 131-136.

31. Gould HJ., *et al*. "The biology of IGE and the basis of allergic disease". *Annual Review of Immunology* 21 (2003): 579-628.

32. Akdis CA and Blaser K. "Bypassing IgE and targeting T cells for specific immunotherapy of allergy". *Trends in Immunology* 22 (2001): 175-178.

33. Gøtzsche PC and Johansen HK. "House dust mite control measures for asthma: systematic review". *Allergy* 63 (2008): 646-659.

34. Brozek JL., *et al*. "Global Allergy and Asthma European Network; Grading of Recommendations Assessment, Development and Evaluation Working Group. Allergic Rhinitis and its Impact on Asthma (ARIA) guidelines: 2010 revision". *The Journal of Allergy and Clinical Immunology* 126 (2010): 466-476.

35. Calderón MA., *et al*. "House Dust Mite Respiratory Allergy: An Overview of Current Therapeutic Strategies". *The Journal of Allergy and Clinical Immunology: In Practice* 3 (2015): 843-855.

36. Sebõk B., *et al*. "Familiar and environmental factors influencing atopic dermatitis in the childhood". *Journal of the European Academy of Dermatology and Venereology* 20 (2006): 418-422.

37. Miyake Y., *et al*. "Home environment and suspected atopic eczema in Japanese infants: the Osaka Maternal and Child Health Study". *Pediatric Allergy and Immunology* 18 (2007): 425-432.

38. Chen YC., *et al*. "Early-life indoor environmental exposures increase the risk of childhood asthma". *International Journal of Hygiene and Environmental Health* 215 (2011): 19-25.

39. Mihrshahi S., *et al*. "Predictors of high house dust mite allergen concentrations in residential homes in Sydney". *Allergy* 57 (2002): 137-142.

40. Mimura T., *et al*. "Specific tear IgE in patients with moderate-to-severe autumnal allergic conjunctivitis". *International Archives of Allergy and Immunology* 156 (2011): 381-386.

41. Mimura T., *et al*. "Specific IgE in tear fluid and features of allergic conjunctivitis". *Current Eye Research* 38 (2013): 917-925.

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