

Salivary Immunoglobulin A, E, and G4 Levels Specific to *Dermatophagoides pteronyssinus* in Allergic Rhinitis Patients Treated With Subcutaneous Immunotherapy

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Abstract

Background: Allergen-specific immunotherapy (AIT) is an effective treatment for allergic rhinitis (AR). During the course of AIT, many biomarkers in body fluids change. It is necessary to find effective indicators of AIT.

Objective: To examine levels of salivary immunoglobulin A, E, and G4 (IgA, IgE, and IgG4, respectively) specific to *Dermatophagoides pteronyssinus* (Dp-IgA, Dp-IgE, and Dp-IgG4, respectively) and their changes in AR patients undergoing subcutaneous immunotherapy (SCIT).

Methods: This study included 82 patients with AR sensitized only to Dp and 14 healthy controls. Among patients with AR, 30 patients were not treated with specific immunotherapy (group A), while the remainder (n = 52) received house dust mite SCIT in the up-dosing phase (n = 27; group B) or the maintenance treatment phase (n = 25; group C). Dp-IgA, Dp-IgE, and Dp-IgG4 levels in the saliva were measured using the enzyme-linked immunosorbent assay. Clinical symptoms, concomitant medication, and the Rhinoconjunctivitis Quality of Life Questionnaire score were recorded and correlated with immunoglobulin levels.

Results: Salivary Dp-IgG4 and Dp-IgA levels were significantly lower in AR patients than in healthy controls ($P < .001$ for both), while Dp-IgE levels were significantly higher ($P < .001$). SCIT resulted in sustained increases in Dp-IgG4 and Dp-IgA in the maintenance phase compared to the up-dosing phase ($P < .001$ for both), whereas Dp-IgE only increased in the up-dosing phase ($P = .004$, $P < .0125$). There was no correlation between the different salivary immunoglobulins and clinical scores during SCIT.

Conclusions: This study shows that allergen-specific IgE levels are increased in the saliva of sensitized patients, suggesting that measuring salivary IgE testing should be further considered for the diagnosis of AR. Moreover, allergen-specific IgA and IgG4 in the saliva, which may play protective roles against allergy, may serve as objective indicators for evaluating treatment response to SCIT. However, none of the immunoglobulin reflects subjective symptoms.

Keywords

allergic rhinitis, subcutaneous immunotherapy, immunoglobulin, saliva, quality of life, symptom, clinical scores

Introduction

Allergic rhinitis (AR) is a common allergic disease affecting up to 40% of the population worldwide.¹ The characteristic nasal mucosa inflammation in AR is mediated by immunoglobulin E (IgE) response elicited by exposure to allergens.² AR is associated with

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impairments in the patient's social life, sleep, and work performance,³ and its prevalence is on the rise.⁴

Currently, AR is diagnosed based on the serum levels of allergen-specific IgE. However, the involvement of other allergen-specific antibodies, such as immunoglobulin G4 and A (IgG4 and IgA), during the course of allergic diseases has also been reported.⁵ It has also been reported that salivary allergen-specific immunoglobulins in AR patients differ from those in healthy individuals.⁶ Thus, increasing attention has been paid to the levels of immunoglobulins in the saliva, as they can be monitored easily, noninvasively, rapidly, and safely.

Treatment strategies for AR mainly involve allergen avoidance, pharmacotherapy, and allergen-specific immunotherapy (AIT), the goal of which is to achieve tolerance by exposing the patient to gradually increasing doses of the allergen. However, indicators of a clinical response to AIT therapy are still lacking. In the study, we evaluated levels of salivary IgA, IgE, and IgG4 specific to *Dermatophagoides pteronyssinus* (Dp-IgA, Dp-IgE, and Dp-IgG4, respectively) in AR patients receiving subcutaneous immunotherapy (SCIT) and examined the relationship between these antibodies and subjective clinical scores.

Methods

Study Subjects

Patients with AR seeking treatment at the Peking University People's Hospital (PKUPH) and Beijing Hospital of Traditional Chinese Medicine, Beijing, China, between August 2015 and October 2015 were enrolled. The diagnosis of AR was made based on the Allergic Rhinitis and Its Impact on Asthma guideline. All patients were positive only to Dp, as determined by a skin prick test, with serum anti-Dp-IgE levels >0.70 kU/L. Patients with AR were grouped as follows: group A, not treated with SCIT; group B, treated with SCIT (in the up-dosing phase); and group C, treated with SCIT (in the maintenance phase). The control group, recruited from PKUPH, consisted of asymptomatic, nonallergic healthy individuals with no clinical history of allergic diseases and who were seronegative to allergen-specific IgE. The study was approved by the ethics committee of Constitution of PKUPH. All patients provided written informed consent.

SCIT Protocol

SCIT was initiated with a standardized house dust mite (HDM) allergen vaccine (ALK, Denmark) administered on day 1 (100 SQU/mL) and then once a week with gradual increases at fixed intervals. Fifteen weeks later, a maintenance dose (10 000 SQU) was administered every 4 weeks to patients.

Saliva Collection

Saliva samples (1–2 mL) were collected from the oral cavity using the standard Salivette (Sarstedt AG&Co, Numbrecht, Germany) device for approximately 5 minutes. The samples were centrifuged immediately at 3000 rpm for 10 min at 4°C. The supernatants were collected and stored in aliquots at –20°C until used in the assays.

Enzyme-Linked Immunosorbent Assay

Dp-IgE, Dp-IgA, and Dp-IgG4 levels in the saliva were measured by sandwiched Enzyme-linked immunosorbent assay (ELISA). All reagents and samples were brought to room temperature before use. Goat Anti-Human IgA (2050-05), Mouse Anti-Human IgG4 pFc (9190-05), and Mouse Anti-Human IgE Fc (9160-05) (all purchased from Southern Biotech, CA, USA) were used as secondary antibodies. A higher concentration of each immunoglobulin to be tested in our laboratory was selected as the standard, and its concentration was set as 1 U/mL. Ninety-six-well plates (WeiTing Biotechnology Co., Shanghai, China) were coated overnight at 4°C with 100 µL of Dust Mite Der f 2 Recombinant (R01752A, Meridian, TN, USA) at a 1:1000 dilution in carbonate buffer (0.05M, pH9.6). Plates were washed 3 times with phosphate-buffered saline (PBS; pH 7.4). Test wells were incubated with 10 µL of the saliva sample and 10% bovine serum albumin (BSA) in 40 µL of PBS, while control wells were incubated with 10% BSA in 40 µL of PBS alone. Assay standards (diluted in 10% BSA in PBS) ranging in concentration from 31.25 IU/mL to 1000 IU/mL were added to standard wells. Then, 100 µL of secondary antibody (Goat Anti-Human IgA [1:4000 dilution], Mouse Anti-Human IgG4 pFc [1:4000 dilution], and Mouse Anti-Human IgE Fc [1:2000 dilution]) were added and incubated for 1 hour at 37°C. Washes were repeated for 5 times and then 50 µL of Tetramethylbenzidine was added to each well. Plates were incubated for 15 minutes at 37°C with protection from light. Then, the reaction was stopped by 50 µL of 2M H₂SO₄, and absorbance at 450 nm was read within 15 minutes using an ELISA plate reader (Bio-Rad, France). All standards, saliva samples, and controls were tested in duplicate. Dp-IgE, Dp-IgA, and Dp-IgG4 levels in the saliva were quantified by extrapolation against the standard curve.

Assessment of Symptoms and Medication Scores

The symptoms and medications were recorded using a questionnaire. Four nasal symptoms (sneezing, rhinorrhea, congestion, and itching) were assessed, and scores ranged from 0 to 3 (0 = none, 1 = mild, 2 = moderate, and 3 = severe). Medication scores were used to evaluate

the clinical effect in AR patients (1 point for antihistamines, 2 points for nasal corticosteroids, and 3 points for oral corticosteroids).⁷ A Rhinoconjunctivitis Quality of Life Questionnaire (RQLQ) was used to assess patient's quality of life.⁸ In addition, symptom scores, medication scores, and RQLQ scores before SCIT were reviewed in groups B and C.

Data Analysis

Statistical analyses were performed using SPSS version 17.0 (IBM SPSS, Armonk, NY, USA). Baseline data were analyzed using a 1-way analysis of variance (ANOVA) and the least significant difference post hoc test. Differences in salivary Dp-IgA, Dp-IgE, and Dp-IgG4 levels among the 4 groups were compared using a 1-way ANOVA and Tamhane's T2 post hoc test. Data that did not conform to a normal distribution were evaluated using Spearman's rank correlation, while data that followed a normal distribution were evaluated by Pearson's correlation to evaluate association between different immunoglobulin species and clinical scores; $P < .05$ was taken as the cutoff for statistical significance. Bonferroni correction was used for multiple comparisons. Adjusted $P < .0125$ (0.05/4) for ANOVA and

$P < .0167$ (0.05/3) for correlation analysis were considered statistically significant.

Results

Subjects

In total, 82 AR patients were enrolled in the test group (30 patients assigned to group A, 27 patients in group B, and 25 patients in group C), and 14 healthy subjects were assigned to the control group. There were no statistically significant differences in baseline demographic characteristics when comparing patients and controls (Table 1). The age range was 7 to 69 years in group A, 6 to 65 years in group B, 4 to 63 years in group C, and 17 to 70 years in the control group. The duration of SCIT ranged from 28 to 105 days in group B and 122 to 457 days in group C.

Difference in Salivary Allergen-Specific Immunoglobulin Among the Groups

The concentrations of salivary Dp-IgA, Dp-IgE, and Dp-IgG4 are shown in Table 2 and Figure 1. Compared to levels in the controls, salivary IgA and

Table 1. Clinical Characteristics of the Study Participants (Mean $\pm$ SD).

Characteristic	Groups			
	Group A (n = 30)	Group B (n = 27)	Group C (n = 25)	Healthy Controls (n = 14)
Age (mean $\pm$ SD)	34.20 $\pm$ 16.81	30.07 $\pm$ 18.73	37.04 $\pm$ 17.83	38.93 $\pm$ 17.43
Sex (male/female)	14/16	11/16	10/15	6/8
Asthma (yes/no)	1/29	1/26	1/24	N/A
SCIT times (days)	N/A	65.93 $\pm$ 28.30	237.28 $\pm$ 97.11	N/A
Symptom scores	6.53 $\pm$ 2.29	7.70 $\pm$ 1.88	7.44 $\pm$ 2.90	N/A
Medication scores	1.63 $\pm$ 1.45	2.22 $\pm$ 1.65	1.56 $\pm$ 1.26	N/A
RQLQ scores	49.37 $\pm$ 28.51	46.07 $\pm$ 25.07	47.12 $\pm$ 30.07	N/A

Abbreviations: AR, allergic rhinitis; Group A, untreated patients with AR; Group B, AR patients treated with SCIT in the up-dosing phase; Group C, AR patients treated with SCIT in the maintenance phase; RQLQ, Rhinoconjunctivitis Quality of Life Questionnaire; SCIT, subcutaneous immunotherapy; SD, standard deviation.

Table 2. Concentration of Salivary Dp-IgA, IgE, and IgG4 in Patients and Controls (Mean $\pm$ Standard Deviation).

Ig (IU/mL)	Groups			
	Group A (n = 30)	Group B (n = 27)	Group C (n = 25)	Healthy Controls (n = 14)
IgA	181.45 $\pm$ 77.30	309.48 $\pm$ 34.73	389.34 $\pm$ 68.05	287.50 $\pm$ 18.54
IgE	301.57 $\pm$ 62.68	352.65 $\pm$ 42.88	346.23 $\pm$ 72.98	224.93 $\pm$ 10.94
IgG4	140.37 $\pm$ 69.87	253.95 $\pm$ 41.35	340.28 $\pm$ 69.33	324.10 $\pm$ 18.32

Abbreviations: Dp, *Dermatophagoides pteronyssinus*; Ig, immunoglobulin; IgA, immunoglobulin A; IgE, immunoglobulin E; IgG4, immunoglobulin G4.

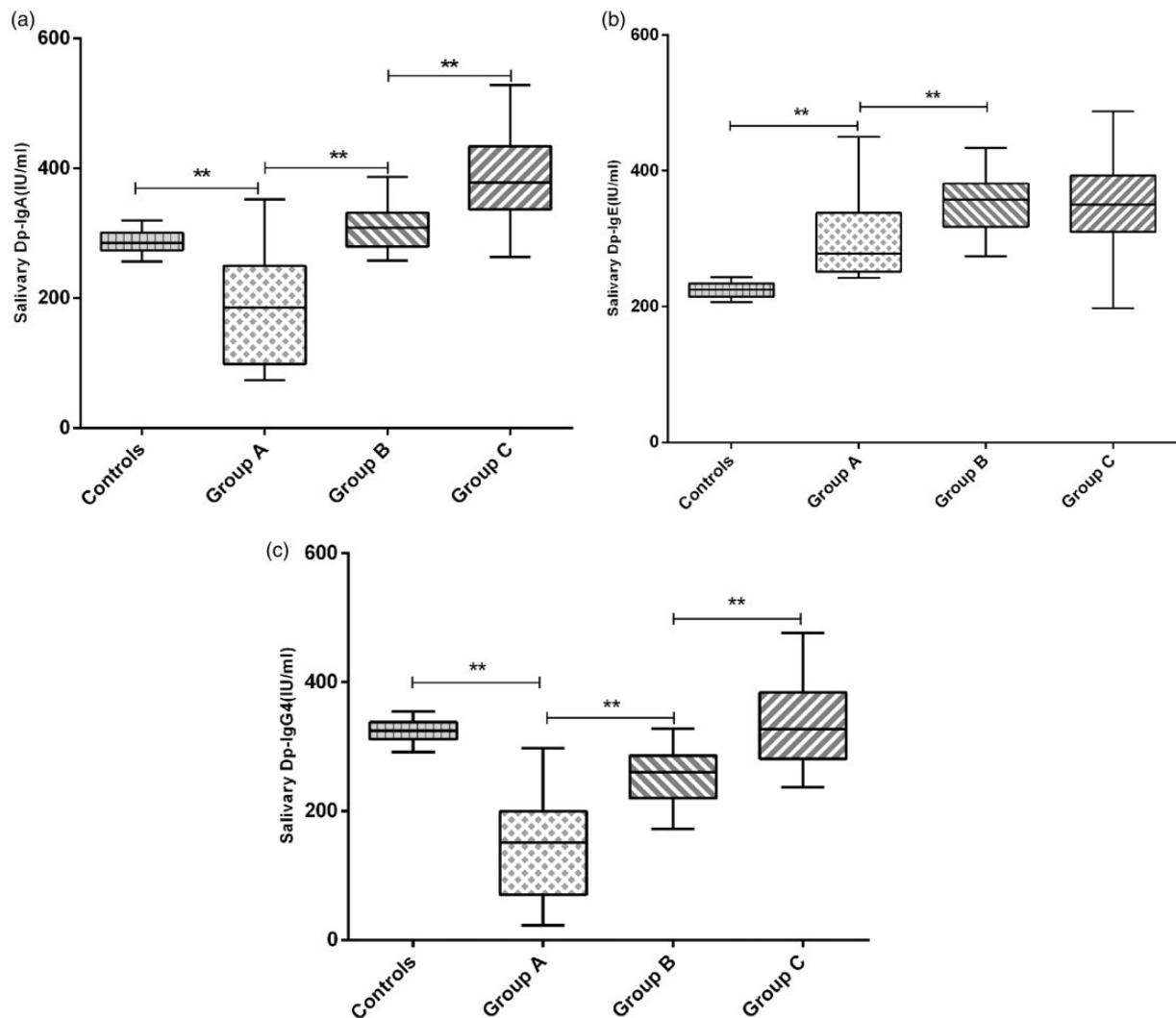


Figure 1. Concentrations of salivary Dp-IgA (A), Dp-IgE (B), and Dp-IgG4 (C) in groups A to C and the controls. Data are expressed as the median and 25th and 75th percentiles. $**P < .01$. Healthy control, group A, group B, and group C. Dp, *Dermatophagoides pteronyssinus*; IgA, immunoglobulin A; IgE, immunoglobulin E; IgG4, immunoglobulin G4.

IgG4 levels were significantly lower in AR patients who did not undergo SCIT ($P < .001$ for both), whereas the salivary IgE levels were significantly higher ($P < .001$). Dp-IgA, Dp-IgE, and Dp-IgG4 levels were significantly higher in patients in the up-dosing phase compared to patients not treated with SCIT ($P < .001$, $P = .004$, $P < .001$). Compared to values in patients in the up-dosing phase, a steady increase in the Dp-IgA and Dp-IgG4 levels were observed in the patients in the maintenance phase ($P < .001$ for both). However, Dp-IgE levels were similar in both groups ($P = 1.000$).

Correlation Between Salivary Allergen-Specific Immunoglobulins and Clinical Scores

Positive correlations between Dp-IgA levels and RQLQ scores ($r = .560$, $P = .001$) and between Dp-IgE levels

and symptom scores ($r = .444$, $P = .014$) were found in group A (Table 3). However, there were no correlations between immunoglobulin levels and the clinical scores in SCIT-treated patients.

Discussion

Saliva is a representative human body fluid: 30% of blood proteins are also present in the saliva.⁹ Changes in salivary proteins in response to various factors and systemic states often accurately reflect changes that occur in the blood. Therefore, analyses of proteins in saliva can aid disease diagnosis and treatment monitoring. Moreover, saliva samples can be collected safely, inexpensively, and noninvasively,¹⁰ encouraging its wider use in disease diagnosis and treatment monitoring.

Table 3. Correlation Between Specific Immunoglobulins in Saliva and Clinical Scores.

Items	Groups					
	Group A (n = 30)		Group B (n = 27)		Group C (n = 25)	
	r	P	r	P	r	P
Dp-IgA vs symptom score	-.043	.821	.125	.423	-.279	.638
Dp-IgA vs medication score	.194	.305	-.063	.755	-.183	.382
Dp-IgA vs RQLQ score	.560	.001**	-.183	.362	-.133	.526
Dp-IgG4 vs symptom score	.042	.824	-.360	.065	-.048	.819
Dp-IgG4 vs medication score	.107	.573	.034	.866	-.044	.836
Dp-IgG4 vs RQLQ score	.303	.104	.027	.894	.111	.597
Dp-IgE vs symptom score	.444	.014*	.031	.841	-.077	.081
Dp-IgE vs medication score	-.173	.361	.269	.175	.368	.070
Dp-IgE vs RQLQ score	-.142	.454	-.079	.686	-.192	.358

Abbreviations: Dp, *Dermatophagoides pteronyssinus*; IgA, immunoglobulin A; IgE, immunoglobulin E; IgG4, immunoglobulin G4; RQLQ, Rhinoconjunctivitis Quality of Life Questionnaire.

* $P < (0.05/3)$; ** $P < (0.01/3)$.

Salivary IgA, which is locally produced, forms a first-line mucosal defense by neutralizing active antigen. Patients with deficiencies in salivary IgA production are susceptible to respiratory infections.¹¹ Salivary IgA levels are notably lower in children with allergies,¹² and both saliva and serum IgA levels are lower in children with AR in response to HDMs compared to nonallergic children. Studies also showed that children with lower salivary IgA levels are more likely to develop allergic diseases.^{12,13} These findings indicate a protective role of IgA in allergy. The mechanisms of protection may include IgA-mediated blockade of allergen entry through binding to macromolecules on the allergens,¹⁴ and inhibition of dendritic cells activation¹⁵ and antigen presentation.¹⁶ Therefore, IgA deficiency is associated with an increase in allergic diseases.

In this study, we found distinct decreases in salivary allergen-specific IgA levels in AR patients, which were increased in response to SCIT. These findings suggest that SCIT induces mucosal IgA production. However, salivary allergen-specific IgA levels were found to be positively correlated with the RQLQ score only in patients with AR who had not received immunotherapy.

Secretory IgE is gaining attention in AR, especially in patients with local AR.¹⁷ The site of salivary IgE production is unknown, but a previous study showed that immunoglobulins such as IgA and IgE were produced locally.¹⁸ In contradiction to these findings, other studies have found that salivary IgE is produced neither locally in the mucosa nor passively transferred from serum.¹⁹ In one study, allergen-specific IgE levels in the serum were initially high during the first 7 weeks of AIT and then subsequently declined.²⁰ Mimura et al. reported a significant positive correlation between the allergen-specific IgE levels in saliva and allergy symptoms in AR patients,⁶ while in another study, allergen-specific IgE

were not detectable in the saliva.¹⁸ Regardless of the site of its production, salivary-specific IgE reflects the symptoms of AR, thus serving as an objective index of the disease and a simple diagnostic marker.

Previous studies have demonstrated a protective role for IgG4, which is produced by a longtime natural allergen exposure or a specific allergen immunotherapy.^{21,22} The positive correlation between serum and salivary IgG4 levels to allergens reflects that IgG4 was likely passively transferred rather than locally produced.¹⁹ AIT is the only disease-modifying treatment for allergic disease recommended by the World Health Organization.² Treatment responsiveness to AIT usually coincides with a significant increase in allergen-specific IgG4 levels in the serum.²³ Specific IgG4 has been shown to have blocking activities by competing with IgE for allergen binding to IgE receptor.²⁴⁻²⁶ During the course of AIT, allergen-specific IgG4 levels continue to increase.²⁷ The magnitude of increase in allergen-specific IgG4 concentrations is dependent on the concentration of the allergenic extract used for immunotherapy²⁸ and on the duration of treatment,²⁹ which can be linked to the accumulated dose being administered. In the current study, we found the salivary-specific IgG4 also continued to increase, and during the maintenance phase of immunotherapy, the concentration of Dp-specific IgG4 was similar to that of the healthy controls. This may be due to the short duration of immunotherapy in our study subjects. Tahamiler et al. reported a negative correlation between clinical scores and IgG4 and suggested that IgG4 was an effective indicator of treatment response to AIT.³⁰ However, other studies have found no correlation between serum IgG4 levels and symptoms but have reported a correlation between allergen-specific IgG4 levels in the serum and the dose of antigen administered to patients during AIT. In this setting, IgG4

serves as a dose index.³¹ The absence of a correlation between salivary immunoglobulins and clinical symptoms during AIT in our study can perhaps be attributed to differences in individual tolerance during the course of AIT, which would result in differences in subjective symptoms.

Our study had several limitations. First, all patients were only positive to Dp, and patient reactivity to multiple allergens was not included, which may have caused selection bias. Second, this patient cohort was comparatively small and may have had recall bias because the symptoms and medications of those who underwent SCIT were reviewed before treatment. Finally, our study focused only on specific immunoglobulins in the saliva and did not consider specific immunoglobulins in serum.

Conclusion

The results of this study suggest that allergen-specific IgA and IgG4 in the saliva may protect against allergic disease and may serve as objective indicators for evaluating treatment response to SCIT. However, they do not reflect the subjective symptoms of patients with AR. Our results also suggest that levels of allergen-specific IgE in the saliva should be further evaluated as a noninvasive method for the diagnosis of AR.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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