

Comparison of the skin-prick test and Phadia ImmunoCAP as tools to diagnose house-dust mite allergy

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ABSTRACT

Background: When the skin-prick test (SPT) and in vitro test such as ImmunoCAP assay are performed simultaneously, results do not always coincide in some patients. Our objectives, therefore, were (1) to assess differences in allergic test results according to age group and (2) to establish appropriate guidelines for diagnosing mite allergy according to age.

Methods: A total of 692 participants complaining of allergic rhinitis symptoms participated. Patients were divided according to age; the mean age was 32 years (range, 8–76 years). The SPT and ImmunoCAP assays were performed to detect allergies to house-dust mites (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*). The association between age and the result of each allergy test were examined, and a cutoff age for proper application of each test was then estimated.

Results: Three hundred thirty-six patients (48.6%) were allergic to *D. pteronyssinus* and 350 patients (50.6%) were allergic to *D. farinae*. In the case of *D. pteronyssinus*, SPT was proved to be more useful in detecting allergy for subjects <50 years old ($p < 0.0001$). However in case of *D. farinae*, ImmunoCAP was useful for all age groups, but SPT showed decreased ratio of positive result for subjects >30 years old ($p < 0.0001$).

Conclusion: This study was the first to compare results of allergy tests according to age using true allergens. For patients >50 years of age, the ImmunoCAP was found to be the preferred method for detecting allergy to house-dust mites and for patients <30 years old, SPT is the recommended first choice.

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Key words: Allergic rhinitis, allergy diagnosis, *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, IgE, in vitro testing, old age, skin test

The fundamental step in the etiologic diagnosis of allergic rhinitis is identifying the presence of sensitization. A clinical suspicion of allergic rhinitis may be drawn from a personal history of allergic symptoms.¹ Nonetheless, the causative allergen must be identified to confirm the diagnosis of allergic rhinitis. When this condition is suspected, the detection of allergen-specific IgE antibodies can be done by an *in vivo* skin-prick test (SPT) or by a variety of *in vitro* assays.

The SPT has gained popularity as the most practical and valuable test for the detection of allergen-specific IgE among known variable tests. However, there are some drawbacks in using the SPT test. In cases of extensive dermatitis, a history of dermatographism, or anaphylaxis, it may be difficult to perform the SPT. Furthermore, there is poor standardization of the allergenic extracts used for this test with differences in composition and titration.²

The ImmunoCAP system for measuring specific IgE was introduced in 1989, allowing quantitative measurement of IgE antibodies and offering better sensitivity than previous methods.³ Compared with the previous technique, radioallergosorbent test, results from the ImmunoCAP system show good concordance with the *in vivo* SPT.^{2,4,5}

In our clinic, we usually use ImmunoCAP or SPT according to the physician's preference and patient's status. When some patients were tested with both techniques, however, the results were discordant in some patients, especially in older patients. Although it is known that elderly patients show reduced skin reactivity to histamine,⁶ no large-scale studies have compared skin reactivity with true allergens in different age groups, and there have been no reports of discrepancies between the SPT and ImmunoCAP in different age groups. The aim of this study was therefore to evaluate the concordance between these

two techniques according to age group and to identify appropriate tools for detecting causative allergens according to age.

MATERIALS AND METHODS

Study Design and Population

After approval by the Institutional Ethics Committee at Samsung Medical Center, this prospective study enrolled 692 patients suspected of having allergic rhinitis who were consecutive referrals to our clinic between June 2007 and January 2008. Patients ranged in age from 8 to 76 years (median, 32 years), and the majority were male subjects (male/female = 444/248). All patients presented two or more symptoms from the three major symptoms of allergic rhinitis (itching, sneezing, and watery rhinorrhea) lasting >3 months. Some patients that had chronic paranasal sinusitis or nasal septal deviation were also included. We excluded patients who had dermatographism, a history of previous immunotherapy, and those taking any medication that could influence the allergic test results.

Clinical Evaluation

After informed consent was obtained, histories were taken, physical examinations were performed, and all enrolled patients underwent the SPT, the ImmunoCAP test, a complete blood cell count including differential count, an eosinophilic cationic protein (ECP) test, and an assay for total IgE. All tests were conducted on the same day to reduce day-to-day variation.

Skin-Prick Tests

SPTs were performed following manufacturer's guidelines for *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*. No patients had taken antihistamines of any type or any other medication that could influence the result of skin tests for 7 days before the prick test. Allergen extracts (Allergy Therapeutics, Ltd., ATL, Bencard, England), along with the appropriate negative (normal saline) and positive (1 mg/mL histamine) controls, were applied on the upper back using the prick technique. The wheal that developed after pricking was considered to be positive if it was 3 mm greater than the negative control at 15 minutes after pricking.

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Table 1 Demographic data of patients in each age group

	Age Group (yr)						Total	
	≤13	14~18	19~30	31~40	41~50	≥51		
Numbers	93	102	171	98	108	120	692	
Sex (M/F)	60/33	75/27	109/62	67/31	65/43	68/52	248/444	$p = 0.139$
Total IgE	301.6	447.7	312.3	203.9	312.6	319.4	316.7	$p = 0.142$
Eosinophil (%) [*]	4.50	4.16	3.80	4.38	4.93	4.20	4.27	$p = 0.098$
ECP#	29.8	30.6	27.6	38.2	30.4	29.3	30.7	$p = 0.380$

^{*}From peripheral blood sample.

ECP = eosinophil cationic protein.

Table 2 Comparisons of test results to *Dermatophagoides pteronyssinus* in each age group

	Age Group (yr)						Total
	≤13	14~18	19~30	31~40	41~50	≥51 [*]	
SPT (+)#	52	74	105	41	31	19	322
ImmunoCAP (+)§	51	65	86	33	24	20	279
Allergic¶	56	76	105	41	33	25	336
Nonallergic	37	26	66	57	75	95	356
Total	93	102	171	98	108	120	692

^{*}Cutoff age; patients who were <50 yr old showed significantly higher positive response in SPT ($p < 0.001$).

#Positive result in SPT irrespective of ImmunoCAP.

§Positive result in ImmunoCAP irrespective of SPT.

¶Patients showing typical allergic symptoms and positive result in allergic test (SPT or ImmunoCAP).

SPT = skin-prick test.

ImmunoCAP Assay

Blood samples were allowed to clot, and after centrifugation, serum samples were stored immediately at -20°C until testing. Assays were performed simultaneously according to the manufacturer's standard protocol (Pharmacia Diagnostics AB, Uppsala, Sweden). We measured the specific IgE of two major inhaled allergens: *D. pteronyssinus* and *D. farinae*. Results were classified from 0 to 4, with class 2 and above judged positive. To reduce day-to-day bias, we performed a calibration using the standard ImmunoCAP-specific IgE control each day according to the manufacturer's guidelines. Total IgE and ECP were also measured by the ImmunoCAP assay.

Outcome Measures

The ratio of positive results were compared between SPT and ImmunoCAP for each allergen. To assess the impact of age on the result of specific IgE tests, we divided patients into six groups according to age: <13 (age of last grade of elementary school), <18 (age of the last degree of high school), <30, <40, <50, and >50 years. Among the 51 inhalant allergens tested in the SPT, we selected two (*D. pteronyssinus* and *D. farinae*) to compare with the ImmunoCAP test. We then compared the distribution of positive results for each test according to age.

Statistical Analysis

If at least one of the two tests was positive, we regarded the patient as having allergic rhinitis. We used one-way analysis of variance (ANOVA) to verify the equal distribution of total IgE, the proportion of eosinophils, and ECP in each age group. The ratio of positive reaction in each tests and the distribution of sexes in each age group were tested with the chi-square independence test.

Statistical analyses were done using SPSS software (Version 12.0; Statistical Package for Social Science, Chicago, IL). After confirmation of differences according to age, we calculated a cutoff age for use as a diagnostic method according to age using the method of least significant p value. To reduce bias, we included only patients with

allergies to both *D. pteronyssinus* and *D. farinae*. Statistical analyses were done with SAS software (Version 12.0, Statistical Analysis System Institute, Inc., Cary, NC) with the assistance of the Biostatistics Unit of the Samsung Biomedical Research Institute.

RESULTS

Characteristics of Enrolled Patients

Table 1 summarizes the demographic data including the number of patients in each age group, distribution of sexes, and allergic marker data (total IgE^{*}, eosinophil[†], and ECP[‡]). There were no statistical differences in the distribution of sexes between age groups ($p = 0.139$), and each allergic marker showed an even distribution according to age ($p = 0.142^*$, $p = 0.098^{\dagger}$, and $p = 0.380^{\ddagger}$).

Dermatophagoides pteronyssinus. Of 692 patients who complained of symptoms representative of allergic rhinitis, 363 (48.6%) showed a positive response in the SPT or ImmunoCAP or both (Table 2). We observed that the rate of positive responses decreased significantly with an increase in patient age ($R = 0.334$; $p < 0.001$; Fig. 1). The prevalence of positive results was highest in the 14- to 18-year age group, at 74.5%. SPT showed a higher positive rate than ImmunoCAP in young patients and the cutoff age for a significant p value was 50 years old ($p < 0.001$).

Dermatophagoides farinae. In the same study group, 350 (50.6%) patients showed a positive response in the SPT or ImmunoCAP or both (Table 3). As the results of *D. pteronyssinus*, the positive responses decreased significantly with an increase in patient age ($R = 0.285$; $p < 0.0001$; Fig. 2) and the prevalence of positive results was highest in the 14- to 18-year age group (75.5%). The proportion of ImmunoCAP-positive patients in each allergic rhinitis age group was similar ($p = 0.195$); however, the proportion of SPT-positive patients decreased significantly in the older age group. The cutoff age for a significant p value was 30 years old ($p < 0.001$).

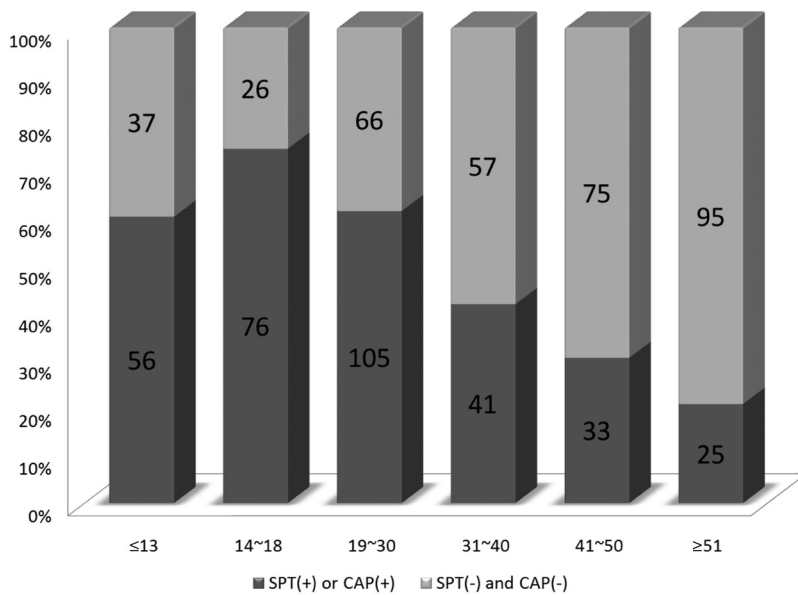


Figure 1. The proportion of patients who were allergic to *Dermatophagoides pteronyssinus* was decreased significantly with increment of age.

Table 3 Comparisons of test results to *Dermatophagoides farinae* in each age group

	Age Group (yr)						Total
	≤13	14~18	19~30	31~40	41~50	≥51	
SPT (+)#	58	74	94	34	27	16	303
ImmunoCAP (+)§	54	69	91	41	36	33	325
Allergic¶	60	77	97	43	40	33	350
Nonallergic	33	25	74	55	68	87	342
Total	93	102	171	98	108	120	692

*Cutoff age; patients who were >31 yr old showed significantly lower positive response in SPT ($p < 0.001$).

#Positive result in SPT irrespective of ImmunoCAP.

§Positive result in ImmunoCAP irrespective of SPT.

¶Patients showing typical allergic symptoms and positive result in allergic test (SPT or ImmunoCAP).

SPT = skin-prick test.

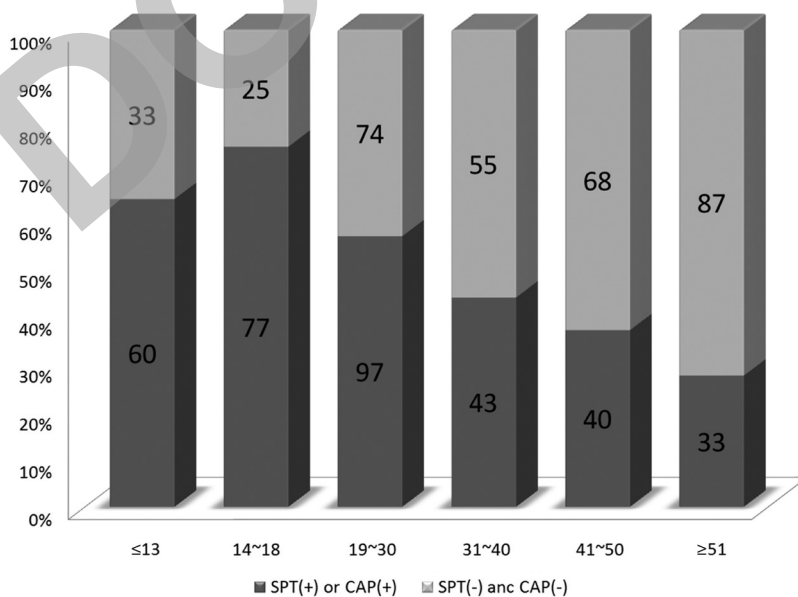


Figure 2. The proportion of patients who were allergic to *Dermatophagoides farinae* was decreased significantly with increment of age.

DISCUSSION

Consistent with a previous study comparing histamine reactivity in skin according to age,⁶ we found that fewer positives were obtained with the SPT than ImmunoCAP test in age groups of >50 years. On the other side, SPT showed a more positive ratio in the young age group of <30 years. In groups between 31 and 50 years, *D. pteronyssinus* and *D. farinae* showed opposite results.

We chose only two species of allergen, *D. pteronyssinus* and *D. farinae*, and excluded other allergens such as cat, dog, tree mixture, and weed mixture allergens for statistical convenience. Patients showing allergic responses only to these allergens were rare. House-dust mites are the most common causative allergen in allergic rhinitis, and most allergic rhinitis patients show positive reaction to house-dust mites.^{7,8} The number of patients showing positive responses only for other allergens with negative responses to *D. pteronyssinus* and *D. farinae* was small, so we selected mites for analysis.

D. pteronyssinus and *D. farinae* are the most common inhalant allergens in Korea, and the SPT has been widely used as the standard *in vivo* method for detecting specific IgE. The SPT, however, takes more time than the ImmunoCAP assay and the results can be affected by a technician's skill and experience. Moreover, the titer of standard allergens and the status of the skin being tested can pose additional variables. The ImmunoCAP assay, in contrast, is done under standardized techniques, and the results are unaffected by the patient's history of previous medication. In this study, we present evidence that if a patient complaining of typical allergic rhinitis symptoms is >50 years old, the ImmunoCAP test can be more useful than SPT for detecting allergy to house-dust mites. But in patients who are <30 years of age, SPT should be chosen as the first test. In this study, multiple possible confounding variables were well controlled with sufficiently large sample sizes for each age group. Sex can influence allergic status; one report found that depending on the allergen tested, male subjects were more likely than female subjects to have positive test responses.⁹ Accordingly, sex should be controlled as a variable when studying the prevalence of atopy. In our study population, there were no significant differences in the male-to-female ratio between age groups.

Some studies have reported that total IgE decreases with age.^{10–12} Our study, however, showed no significant association between age and total IgE. Although this result contradicts previous work, there are some data to support our conclusions,^{13–15} and our results may be the result of taking confounding factors into account.

The peak incidence of atopy to mites in our study was in the 14- to 18-year age group. This was consistent with previous studies that described the highest rate of atopy in the 20- to 34-year age group,¹⁶ and in the <40-year age group.¹⁷ Most previous studies focused on the prevalence and symptomatic severity of allergic disease according to age,^{10,11,18} but this is the first report to describe the variation of allergic tests according to age and to present significant cutoff ages for selecting tools for the detection of mite allergies.

This study has some limitations. We did not confirm identification of specific IgEs using methods such as immunoblotting, immunoCAP inhibition,² or the provocation method.¹⁹ Nonetheless, the sample size in this study is larger than others, so using multiple methods for each patient was challenging. Consistent with our results, other studies have reported that ImmunoCAP has equal clinical efficiency to the SPT in the diagnosis of allergy to house-dust mites.^{2,5,19} In addition, we recruited patients with typical symptoms of allergic rhinitis, so a diagnosis of allergic rhinitis without confirmative methods in this population is appropriate.

CONCLUSIONS

The results of SPT and ImmunoCAP test for the diagnosis of allergy to house-dust mites showed a significant difference in the distribution of positive results according to age. The ImmunoCAP test showed

significantly greater usefulness in patients who were >50 years of age and the SPT was more useful in patients who were <30 years old.

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