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ORIGINAL ARTICLE

Comparison and Evaluation of Several *Dermatophagoides Pteronyssinus* Allergen Extracts for Skin Prick Test[△]

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Objective To evaluate the significance of several *Dermatophagoides pteronyssinus* allergen extracts for skin prick test (SPT) in patients allergic to *Dermatophagoides pteronyssinus*.

Methods Two hundred and nineteen patients enrolled in Peking Union Medical College Hospital underwent SPT and serum specific IgE assay to detect the *Dermatophagoides pteronyssinus* allergen. Three kinds of house dust mite allergen extracts were used for SPT, including the *Dermatophagoides pteronyssinus* extract prepared by our laboratory (group A), standardized *Dermatophagoides pteronyssinus* extract (group B), and mixed extracts of *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* (group C). Human serum specific IgE result was regarded as the reference standard for diagnosis of *Dermatophagoides pteronyssinus* allergy. The receiver operating characteristic (ROC) curve was used to evaluate the diagnostic performance of SPT with the extracts of three groups.

Results SPT results showed that the median wheal diameter of group A, group B, and group C was 0.43, 0.35, and 0.28 cm, respectively, with significant difference among three groups ($P < 0.05$). The difference was significant between group A and B ($P < 0.01$) as well as group A and C ($P < 0.01$), but not between group B and C ($P > 0.05$). There was no local urticaria or systemic allergic reactions following the procedure of SPT. Local reaction was observed in 5 patients and delayed reaction was in 2 patients of group A. As for group B and C, local reaction occurred in 3 cases and delayed reaction in 2 cases in each group. The area under ROC curve of SPT with extract in group A, group B, and group C was 0.765, 0.801, and 0.782, respectively. Based on the detection results of serum specific IgE, the sensitivity of SPT in diagnosis of *Dermatophagoides pteronyssinus* allergy with extract of group A, group B, and group C was 92.4%, 87.0%, and 81.5%, and the specificity was 60.6%, 73.2%, and 74.8%, respectively.

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Conclusion The *Dermatophagoides pteronyssinus* extract for SPT prepared by our laboratory offers good sensitivity and specificity comparable to commercially available allergen extracts, and it may be an appropriate candidate for clinical screening and diagnosis of *Dermatophagoides pteronyssinus* allergy.

ALLERGIC diseases, with an increasing trend of global incidence, are mostly type I allergic diseases resulting from inhalation of house dust mites, pollen, dander, or other allergens.¹ The major diagnosis methods of allergic diseases include skin tests, serum specific IgE (sIgE) detection, and provocation test. Since the skin test possesses simple, quick, inexpensive, and highly sensitive characteristics, it has long been one of the most essential methods for diagnosis of type I allergic diseases in spite of the rapid development of science and technology over the past century.^{2,3} At present, the most commonly used skin tests are skin prick test (SPT) and intradermal skin test. Both methods are practical and safe, with good reproducibility and reliability. SPT is more specific than intradermal skin test, and it allows the detection of IgE-mediated sensitivity with simple device and low risk of causing an allergic reaction.^{2,3}

House dust mite is the most common indoor allergen that can trigger allergic rhinitis, allergic asthma, dermatitis, urticaria, and other allergic diseases. The study of house dust mite allergy has been a hotspot of clinical allergy research.^{1,2,4-6} *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* are two major species of house dust mite.

The receiver operating characteristic (ROC) curve is a graphic representation of the relation existing between the sensitivity and the specificity of a test. It allows description and comparison of the accuracy of diagnostic tests, offering a simple visual assessment of the overall diagnostic performance of a test. In this present study, ROC curve was employed to evaluate the diagnostic value of SPT with various allergen extracts on *Dermatophagoides pteronyssinus* allergy.

PATIENTS AND METHODS

Patients

From March to August 2005, 219 patients admitted to Department of Allergy of Peking Union Medical College (PUMC) Hospital were enrolled. Some of them had typical history and symptoms of house dust mite allergy, including rhinitis, asthma, urticaria, and dermatitis, the others were not allergic to house dust mite and served as controls. Totally, 91 males and 128 females were included, with an average age of 35.27 ± 15.77 (range, 4-75) years. Their

diagnosis included 53 cases of simple rhinitis, 50 cases of simple asthma, 26 cases of combined rhinitis and asthma, 40 cases of urticaria, 22 cases of dermatitis, and 28 cases of other allergic diseases. Patients taking oral short-acting antihistamine drug within 3 days or long-acting antihistamine drug within 3 weeks prior to the admission were excluded. Informed consent was obtained from all the patients. This trial was approved by the Ethics Committee of PUMC Hospital.

Extracts and controls for SPT

The *Dermatophagoides pteronyssinus* allergen extract (group A) was prepared by our laboratory with the following procedures. Pure *Dermatophagoides pteronyssinus*, with its morphology identified by professor Arlian (Department of Biological Sciences, Wright State University, Ohio, USA), was cultured at 20-25°C with 70%-75% relative humidity using unsealed plastic bottles. When the proportion of *Dermatophagoides pteronyssinus* in the medium reached 80%, saturated saline flotation method was used to collect the mite bodies. After degreased by acetone and dried, the collection was extracted using Coca's solution (pH 8.2) with a ratio of 1:20 (W/V) for 48 hours, and glycerol (1:1) was added to prepare the *Dermatophagoides pteronyssinus* extract. Then the extract was dialyzed with a 0.22 µm filter and stored for use.

Standardized *Dermatophagoides pteronyssinus* extract (group B) and mixed extracts of *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* (group C) manufactured by the ALK Corporation (Denmark) were donated by the Hong Kong Office of ALK Corporation.

Histamine dihydrochloride (10 g/L) was used as positive control and normal saline was as negative control. Both of them were provided by the Hong Kong Office of the ALK Corporation.

Skin prick needle

Single stylus plastic prick needle was developed by Tsinghua University and manufactured by Dongtai Global Medical Devices Factory (Jiangsu, China).

SPT procedure

SPT was performed on the skin of volar forearm using allergen extracts of three groups along with positive and negative controls. The arm was first cleaned, then a drop of

allergen extract or control solution was placed onto a marked area of skin, with a distance of 2-3 cm between two drops. Using a sterile prick needle, a small prick through the drop was made, which allowed a small amount of allergen to enter the skin. Each sample was pricked with a separate needle. Two to three minutes later, the residue fluid on the skin should be carefully wiped off to avoid mixing the adjacent samples. The reaction was measured after 15 minutes by copying the wheal reaction with transparent adhesive tape onto a record sheet for later comparison. All the patients were followed up for 1 more hour to observe whether they would suffer from local itch and swelling, local rash, generalized rash, anaphylactic shock, and other adverse effects. They would be followed up the next day for delayed reaction.

Evaluation of SPT results

The wheal diameters were measured. The size of the wheal was calculated by the following formula: $\text{size} = (D+d)/2$, where D = maximum vertical diameter of wheal, and d = minimal diameter of wheal. Allergen eliciting a wheal size greater than 3 mm was considered positive. The evaluation of wheal diameters was completed by two researchers independently.

Serum sIgE assay

After SPT was completed, 2-4 ml of venous blood was taken from each patient. Following centrifugation, serum was obtained to determine the level of sIgE of *Dermatophagoides pteronyssinus* using the UniCAP 100 system (Pharmacia Company, Sweden) with the method described previously.⁷ The level of serum sIgE < 0.35 KU_A/L was considered negative and ≥ 0.35 KU_A/L was considered positive. Serum sIgE determination was achieved by two experienced investigators who were blinded to the disease history and clinical diagnosis of all patients.

Statistical analysis

All the data of age, gender, diagnosis, SPT result, and serum sIgE result were input into Excel by two investigators independently, followed by checking of all data by another investigator. Statistical analysis was performed using SPSS 10.0 software. The Kruskal-Wallis test was used for comparison of wheal diameter among three groups and the Wilcoxon rank-sum test was used for comparison of wheal diameter between every two groups. The area under the ROC curve of SPT was calculated to evaluate the diagnostic sensitivity and specificity of allergen extracts using sIgE results as the reference standard. Sensitivity = number of patients with positive SPT and sIgE results/number of patients with positive sIgE result $\times 100\%$. Specificity = num-

ber of patients with negative SPT and sIgE results/number of patients with negative sIgE result $\times 100\%$.

RESULTS

SPT results

The median wheal diameter of group A, B, and C was 0.43 (range, 0-1.85), 0.35 (range, 0-1.88), and 0.28 (range, 0-1.80) cm, respectively, with significant difference among three groups ($\chi^2 = 38.63$, $P < 0.05$). The difference was significant between group A and B ($Z = 5.461$, $P < 0.01$) as well as group A and C ($Z = 4.887$, $P < 0.01$), but not between group B and C ($Z = 0.443$, $P = 0.658$).

Adverse effects of SPT

Within 1 hour following SPT, local reaction was observed in 5 cases of group A. The wheal of them had a quickly raising edge with extending pseudopodia, which usually expanded to reach an extremely large size and normally subsided within 2 hours. Delayed reaction occurred in 2 cases of group A, presented with continued red, swelling, itch, and pain of local skin lasting for 1 day after the disappearance of wheal. As for group B and C, local reaction occurred in 3 cases and delayed reaction in 2 cases in each group. All the subjects had no local rash, generalized rash, runny nose, sneezing, dry cough, asthma, anaphylactic shock, or other adverse reactions.

ROC curve of SPT with different extracts

Based on the results of serum sIgE assay, the area under the ROC curve of SPT with allergen extracts from group A, B, and C was 0.765, 0.801, and 0.782, respectively, which were all significantly higher than the area under the ROC curve of reference line ($P < 0.05$, Fig. 1).

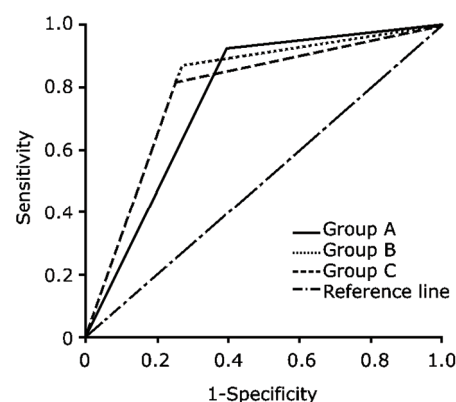


Figure 1. Receiver operating characteristic (ROC) curve of skin prick test for *Dermatophagoides pteronyssinus* allergy with extracts from group A, group B, and group C

Sensitivity and specificity of SPT with different extracts

Results of SPT and serum sIgE assay for *Dermatophagoides pteronyssinus* allergen are shown in Table 1. Based on the results of sIgE assay, the sensitivity of SPT with allergen extract of group A, group B, and group C was 92.4%, 87.0%, and 81.5%, and the specificity was 60.6%, 73.2%, and 74.8%, respectively.

Table 1. Results of skin prick test and serum specific IgE assay for *Dermatophagoides pteronyssinus* allergen

Group	Skin prick test	Serum specific IgE		
		Positive	Negative	Total
Group A	Positive	85	50	135
	Negative	7	77	84
	Total	92	127	219
Group B	Positive	80	34	114
	Negative	12	93	105
	Total	92	127	219
Group C	Positive	75	32	107
	Negative	17	95	112
	Total	92	127	219

DISCUSSION

The rationale for skin test is a series of reactions triggered by introducing harmless micro-allergen into the subcutaneous tissues. The allergen may recognize and bind sIgE antibody on surface of mast cells, causing subsequent serial activation of enzymes, degranulation of mast cells, and release of histamine and other inflammatory mediators into the tissues. This leads to the sequence of vascular dilatation and increase in their permeability, resulting in the appearance of papules and flush reaction.² Clinically, the cause of the type I allergic disease can be diagnosed based on the analysis of the reaction.

Methods for skin test are multiple, among which intracutaneous test and SPT are commonly used.² In intracutaneous test, the allergen solution is injected into dermis, with a relatively high risk to cause a systemic reaction. While in SPT, most of the allergen solution enters epidermis, and only a very small amount (about 1/10 000 of that of intracutaneous test) enters the body, which is quite safe for recipients. SPT causes little or slight pain that can be well tolerated even by pediatric patients. And the test is quick, convenient, and allows the detection of multiple allergens at once. If SPT shows positive result, the intracutaneous test is thus unnecessary. If it shows negative result, along with a history indicating a high degree of

clinical suspicion, the intracutaneous test should be accordingly performed.

Many devices are available for performance of SPT.⁸ Some devices have a single stylus with one or several points, whereas others have multiple heads and allow several tests to be accomplished with one application. Moreover, material of the devices may be steel or plastics. Therefore, the degree of skin trauma created by these devices varies and the criteria for indicating a positive response to tests using various devices are different. The needle used in this present study allows the performer to achieve reproducible and accurate skin test results, and the criteria for interpreting the results of SPT are in accordance with the criteria recommended by ALK Corporation.

In the present study, we used ROC curve to analyze the consistency of diagnosis by SPT and sIgE of *Dermatophagoides pteronyssinus*. Results showed that the area under the ROC curve of group A, B, and C was 0.765, 0.801, and 0.782, respectively, and significantly higher than that of the reference line. It revealed that the *Dermatophagoides pteronyssinus* extract prepared by our laboratory shows satisfactory diagnostic value comparable to commercially available allergen extracts and sIgE assay, allowing effective diagnosis of *Dermatophagoides pteronyssinus* allergy. SPT using such allergen extract can be carried out on all age groups, on those with poor economic conditions, and under such circumstance as short of *in vitro* diagnostic device.

Ricci *et al*⁹ evaluated two *in vitro* tests, UniCAP system (Pharmacia) and ADVIA Centaur immunoassay system (Bayer Diagnostics), for the house dust mite allergies and results showed that the UniCAP system had sensitivity of 91% and specificity of 62%, the ADVIA Centaur immunoassay had sensitivity of 86% and specificity of 64%. SPT with *Dermatophagoides pteronyssinus* allergen extract prepared by our laboratory showed sensitivity of 92.4% and specificity of 60.6%. As for the extract of *Dermatophagoides pteronyssinus* and mixed extracts of *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* manufactured by ALK Corporation, the sensitivity and specificity were 87.0% and 73.2%, 81.5% and 74.8%, respectively. Compared with the UniCAP system, SPT with our self-made allergen extract achieved higher sensitivity and comparable specificity. Other studies showed that the SPT results and results of the *in vitro* tests, including the radioallergosorbent test (RAST), modified RAST, and the Pharmacia CAP System, correlated well with each other, while skin tests remained the most sensitive and specific test available.^{3,10-12}

The maximum and minimum wheal diameter of three

groups in this present study had no significant difference. The mean wheal diameter of group A was significantly larger than that in group B and C. Results of SPT with all the three extracts generally agreed with the serum sIgE results.

The adverse effects of SPT involve local skin reaction, delayed reaction, generalized rash, rhinitis, asthma, anaphylactic shock, and other systemic reactions.¹³ Local reaction was observed in 5 cases and delayed reaction in 2 cases of group A. As for group B and C, local reaction occurred in 3 cases and delayed reaction in 2 cases in each group. Light local reaction normally subsides within 2 hours and need no treatment. More prolonged or severe reaction may be treated with an ice pack to contract the small blood vessels and reduce the absorption of allergens. Occasional patients will experience a more severe reaction showing red and itchy skin, and then 1:1 000 adrenaline should be pricked at various points around the local flush. If systemic urticaria, laryngeal edema, bronchial spasm, or anaphylactic shock occurred in 15-20 minutes after the prick, some emergency measures should be immediately taken. For adult patients, tourniquet should be bonded at the upper arm proximal to the prick position, and 0.3-0.5 ml of 1:1 000 adrenaline should be injected at the contralateral upper arm. For pediatric patients, 0.01 ml/kg of 1:1 000 adrenaline should be injected with a maximal dose of 0.3 ml.

In conclusion, SPT with the *Dermatophagoides pteronyssinus* allergen extract prepared by our laboratory offers good sensitivity and specificity comparable to commercially available allergen extracts in diagnosis of *Dermatophagoides pteronyssinus* allergy. It may be an appropriate candidate for diagnosis of *Dermatophagoides pteronyssinus* allergy eliciting allergic asthma, allergic rhinitis, atopic dermatitis, and other allergic diseases.

REFERENCES

1. Pucci S, Incorvaia C. Allergy as an organ and a systemic disease. Clin Exp Immunol 2008; 153:S1-2.
2. Grammer LC, Greenberger PA. Patterson's allergic diseases. 6th ed. Philadelphia: Lippincott Williams & Wilkins; 2002 .p.132-44.
3. Tripathi A, Patterson R. Clinical interpretation of skin test results. Immunol Allergy Clin North Am 2001; 21:291-300.
4. Diette GB, McCormack MC, Hansel NN, et al. Environmental issues in managing asthma. Respir Care 2008; 53: 602-15.
5. Ferguson BJ. Environmental controls of allergies. Otolaryngol Clin North Am 2008; 41:411-7.
6. Gavino AC, Needham GR, High WA. Atopic dermatitis, patch testing, and house dust mites: a brief review. Dermatitis 2008; 19:121-8.
7. Zhang HY, Wang LL, Rong GS. Evaluation of CAP System in assay of sIgE. Chin J Microbiol Immunol 1996; 16:S6-12.
8. Oppenheimer JJ. Device for epicutaneous skin testing. Immunol Allergy Clin North Am 2001; 21:263-72.
9. Ricci G, Capelli M, Miniero R, et al. A comparison of different allergometric tests, skin prick test, Pharmacia UniCAP and ADVIA Centaur, for diagnosis of allergic diseases in children. Allergy 2003; 58:38-45.
10. Kam KL, Hsieh KH. Comparison of three *in vitro* assays for serum IgE with skin testing in asthmatic children. Ann Allergy 1994; 73:329-36.
11. Kelso JM, Sodhi N, Gosselin VA, et al. Diagnostic performance characteristics of the standard Phadebas RAST, modified RAST and Pharmacia CAP system versus skin testing. Ann Allergy 1991; 67:511-4.
12. Williams PB, Dolen WK, Koepke JW, et al. Comparison of skin testing and three *in vitro* assays for specific IgE in the clinical evaluation of immediate hypersensitivity. Ann Allergy 1992; 68:35-45.
13. Tripathi A, Patterson R. Clinical interpretation of skin testing results. Immunol Allergy Clin North Am 2001; 21:291-300.