

ORIGINAL ARTICLE

Atopy patch test with house dust mite in Chinese patients with atopic dermatitis

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Abstract

Background The atopy patch test (APT) has been defined as an important tool in the diagnosis of cutaneous hypersensitivity caused by house dust mites in atopic dermatitis (AD).

Objective The aim of this study was to evaluate the diagnostic value of the APT to mite allergens by comparing its positive results with those of the skin prick test (SPT) and serum specific IgE test in Chinese AD patients.

Methods The APT, SPT and serum specific IgE test with *Dermatophagoides pteronyssinus* and *Dermatophagoides farina* were performed on a total of 120 patients with atopic dermatitis.

Results Overall, 37.5% of the patients showed a positive APT reaction to mite allergens. A statistically significant association was observed between positive APT results and air-exposed eczema pattern. The positive APT results were not significantly associated with SCORAD scores and accompanied atopic respiratory disorders in AD patients. A higher frequency of APT positive reactions to mite allergens was observed in adolescent and adult patients. There was no significant difference in APT positivity between extrinsic patients (40.7%) and intrinsic patients (27.6%).

Conclusion This study identified house dust mite induced cutaneous hypersensitivity in Chinese patients with AD by APT, especially in air-exposed distribution patterns or adolescent and adult patients. Both intrinsic and extrinsic AD patients showed positive APT reactions to house dust mite.

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Conflicts of interest

None declared

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Introduction

Atopic dermatitis (AD) is a common, chronic skin condition affecting up to 15–30% of children and 2–10% of adults worldwide.¹ It is characterized by epidermal barrier defects that may allow for the development of inflammation due to increased penetration of antigen through the skin and activation of T cells.² Aeroallergens, food allergens and antigens from microorganisms colonized on the surface of skin have been shown to elicit eczematous skin lesions. House dust mites sensitization is the most common sensitization in atopic dermatitis identified by the skin prick test (SPT) or serum specific IgE measurement.³

In 1982, Mitchell *et al.* performed the first patch test with purified mite antigen in atopic dermatitis patients and confirmed that acute eczematous lesions can be induced by the

application of inhalant allergens to the skin.⁴ The atopy patch test (APT), which consists of applying suspected allergens to the skin using a method similar to patch testing in contact dermatitis, was introduced in 1989 as a technique to evaluate cutaneous hypersensitivity to aeroallergens in individuals with AD.⁵ A variety of subsequent studies^{6–10} confirmed the ability of the APT to detect such hypersensitivity in different populations, particularly to house dust mites. However, the utility of the APT has not been documented in Chinese patients with AD. In this study we performed the APT using two species of mite (*Dermatophagoides pteronyssinus*, *D. pter.* and *Dermatophagoides farinae*, *D. far.*) in patients with AD and evaluated the diagnostic value of the APT by comparing the results with those of the SPT and serum specific IgE test in patients with different clinical characteristics.

Methods

Patients

One hundred twenty atopic dermatitis patients (73 males and 47 females) aged 3–63 years (mean $\pm$ SD = 16.1 $\pm$ 10.5) from outpatient dermatology clinics were included in the study. AD was diagnosed using Hanifin and Rajka's Criteria.¹¹ Atopic respiratory disorders (asthma and/or allergic rhinitis) were found in 89 patients (74.2%). The SCORAD index was used to evaluate the clinical severity of AD with a mean score of 28.2 $\pm$ 11.8 (mean $\pm$ SD). Disease severity was classified as mild (objective SCORAD <15), moderate (objective SCORAD 15–40) and severe (objective SCORAD >40). A detailed description of lesions was obtained. Of the 120 patients, 63 (52.5%) had eczematous skin lesions predominantly in the air-exposed areas of the neck, face, scalp, arms and lower legs. Patients were stratified into extrinsic AD (eAD) and intrinsic AD (iAD) categories primarily according to their serum IgE level. eAD was defined as serum total IgE levels $\geq$ 400 kU/L or total IgE $\leq$ 400 kU/L in addition to a positive multiallergen IgE screen result for aeroallergens (Phad) or food allergens (fx5E). Ninety-one patients had eAD (75.8%) and 29 had iAD (24.2%). An atopy patch test and skin prick test with mite allergens were performed on all patients. Oral antihistamines were discontinued for at least 7 days before testing, and systemic corticosteroids or immunosuppressant were discontinued for at least 4 weeks before testing. Topical corticosteroids or UV therapy had not been used in the last 4 weeks at the test sites. Serum samples were taken from each patient and stored at -80°C until used.

This study was approved by the ethics committee of the Shenzhen Hospital, Peking University, and conducted according to the principles of the Declaration of Helsinki. All patients or their guardians provided their written informed consent before inclusion in the study.

Atopy patch test

For the APT, lyophilized house dust mite extracts (*D. pter.* and *D. far.*; Wolw Pharma[®], Deqing, Zhejiang, China) at 7000 PNU/g in a petrolatum vehicle were applied to clinically uninvolved, un-pretreated upper back skin using Large Finn Chambers[®] on Scanpor (SmartPractice[®], Phoenix, Arizona, U.S.A.) for 48 h. Petrolatum alone served as the negative control. The results were recorded at 48 and 72 h according to ETFAD (European Task Force on Atopic Dermatitis) (i.e. –, negative result; ?, only erythema, questionable; 1+, erythema, infiltration; 2+, erythema, few papules (up to 3); 3+, erythema, papules more than 4; 4+, erythema, many or spreading papules; 5+, erythema, vesicles).¹² Only 72-h readings were incorporated in the analysis. Clear-cut reactions of 1+ or more were regarded as positive.

Skin prick test and serum IgE levels

All patients underwent SPT with mite allergens (*D. pter.* and *D. far.*; Wolw Pharma[®], Deqing, Zhejiang, China) following the recommendations of the European Academy of Allergy and Clinical Immunology.¹³ A positive reaction consisted of a wheal with a mean diameter of at least 3 mm. The negative and positive controls were 0.9% saline and histamine (10 mg/mL) respectively. Serum total IgE levels were measured with an electrochemiluminescence immunoassay system (Roche, Cobas E601, Mannheim, Germany). The multiallergen IgE screen for Phad and fx5E and the specific IgE (sIgE) for *D. pter.* and *D. far.* were assessed using the ImmunoCAP[®] specific IgE system (Phadia, Inc., Uppsala, Sweden). Only levels of 0.70 U/L, or above were regarded as positive.

Statistical analysis

All statistical analyses were carried out with SPSS version 16.0 software (SPSS Inc., Chicago, IL, USA). Continuous variables are shown as means plus or minus standard deviations. To analyse statistically significant associations between categorical variables, the chi-square test or the Fisher exact test was used as appropriate. The agreement of the positive results from different diagnostic tests was assessed by statistical κ calculation: $\kappa < 0.20$, poor; 0.21–0.40, fair; 0.41–0.60, moderate; 0.61–0.80, good/substantial and 0.81–1, very good. For comparison of positive APT results in different age groups, a chi-square trend test (linear-by-linear association) was performed. All calculated *P* values were two-sided, and *P* < 0.05 was considered to be statistically significant.

Results

Forty-five (37.5%) of the 120 screened subjects showed at least one clear-cut positive APT reaction to house dust mite allergens. Twenty-eight (23.3%) were positive to *D. pter.* and 37 (30.8%) to *D. far.* The frequencies of positive reaction in the SPT and sIgE tests were 73.3% (88/120) and 70.0% (84/120) for *D. pter.*, and 76.7% (92/120) and 73.3% (88/120) for *D. far.* The agreement between different tests is detailed in Table 1. The κ values showed statistically significant moderate agreement between the

Table 1 Agreement of positive results between the atopy patch test (APT), skin prick test (SPT) and specific IgE (sIgE)

Allergen	APT vs SPT		APT vs sIgE		SPT vs sIgE	
	κ -value	<i>P</i> -value	κ -value	<i>P</i> -value	κ -value	<i>P</i> -value
<i>D. pter.</i>	0.146	0.008	0.148	0.011	0.754	<0.001
<i>D. far.</i>	0.073	0.218	0.081	0.200	0.778	<0.001

D. pter., *Dermatophagoides pteronyssinus*; *D. far.*, *Dermatophagoides farinae*.

Bold number means that the *P* value is less than the significant level (e.g. *P* < 0.05).

Table 2 Association of positivity of different diagnostic tests with eczema pattern

Test	<i>D. pter.</i> No. (%)			<i>D. far.</i> No. (%)		
	Air-exp (n = 63)	Non Air-exp (n = 57)	P-value	Air-exp (n = 63)	Non Air-exp (n = 57)	P-value
APT	24 (38.1)	4 (7.0)	<0.001	32 (50.8)	5 (8.8)	<0.001
SPT	48 (76.2)	40 (70.2)	0.457	48 (76.2)	44 (77.2)	0.897
sIgE	46 (73.0)	38 (66.7)	0.386	47 (74.6)	41 (71.9)	0.741

Air-exp, air-exposed pattern; APT, atopy patch test; *D. pter.*, *Dermatophagoides pteronyssinus*; *D. far.*, *Dermatophagoides farinae*; SPT, skin prick test. Bold number means that the *P* value is less than the significant level (e.g. *P* < 0.05).

SPT and sIgE (*P* < 0.001), while there was poor agreement between the APT and SPT or sIgE.

The positive results of different diagnostic tests in patients with an air-exposed pattern of AD were compared to patients with truncal AD lesions (Table 2). Positive APT results (at least one positive reaction to any mite allergen) were observed in 60.3% (38/63) of patients with an air-exposed eczema distribution pattern. The chi-square analysis showed a significant (*P* < 0.001) association between positive APT results and an air-exposed eczema pattern. No significant association between an air-exposed eczema distribution pattern and positive SPT or sIgE results was observed (*P* > 0.05).

Eighty-nine of 120 AD patients also had respiratory atopic disorders. In terms of APT positivity, no significant difference was seen between the respiratory and non-respiratory atopic group (37.1% vs. 38.7%), whereas statistically significant associations between respiratory allergy and positive SPT and sIgE (*P* < 0.01) results were observed (for SPT 84.3% vs. 58.1% and for sIgE 80.9% vs. 51.6% in the respiratory and non-respiratory atopic group respectively).

Thirty-three patients were assessed as mild AD according to objective SCORAD scores. Eighty-one were moderate and only six were severe patients. No significant association was seen between objective SCORAD scores and APT, SPT or sIgE results (data not shown).

The association between positivity of different diagnostic tests and age group were analysed and detailed in Table 3. The positive reaction of APT to house dust mites was more common in adolescence and adult patients, and the positive rate increased significantly with increasing age (*P* < 0.05). There was no significant

increase in the frequency of positive SPT or sIgE results with increasing age.

A comparison of clinical features and APT positivity between iAD and eAD patients is shown in Table 4. There was no significant difference in positive results of APT to house dust mites between iAD and eAD patients.

Discussion

The role of house dust mite aeroallergens in the initiation and continuation of atopic dermatitis has been proposed for some time. It has been confirmed that epicutaneous application of mite allergens can elicit eczematous skin reactions.^{14–16} Although there is no commercially available test kit, the APT has been established as a research method to evaluate the clinical relevance of mite allergens with eczematous lesions in AD patients in many studies.^{5–7,17–19} This is the first study presenting the results of the APT to mite allergens (*D. pter.* and *D. far.*) in Chinese patients with AD. The frequency of positive reactions to at least one mite allergen was 37.5%. In other studies, positivity rates of the APT to house dust mite in AD patients ranged from 39% to 69%.^{6–10,20} This difference might be due to differences in patient selection and APT methodology, such as mite species, concentration and vehicle of allergen, skin condition, test site and reading time.^{17,21}

Our population exhibited much higher positive rates for IgE-related allergy tests including SPT and serum specific IgE (77.5% and 73.3% respectively) than the APT. Agreement analysis of the different diagnostic tests demonstrated poor correlation of APT results with SPT or sIgE, whereas positive SPT results showed statistically significant good agreement with

Table 3 Association of positivity of different diagnostic tests with age

Test	Age Groups No. (%)			
	3–12 years (n = 58)	13–17 years (n = 12)	≥18 years (n = 50)	P-value
APT	16(27.6)	6(50.0)	23(46.0)	0.047
SPT	43(74.1)	11(91.7)	39(78.0)	0.613
sIgE	44(75.9)	9(75.0)	35(70.0)	0.496

APT, atopy patch test; SPT, skin prick test.

Bold number means that the *P* value is less than the significant level (e.g. *P* < 0.05).

Table 4 Comparison of iAD and eAD

Medical features	iAD (n = 29)	eAD (n = 91)	P-value
Female (%)	15 (51.7)	32 (35.2)	0.112
Age (mean ± SD)	19.2 ± 14.2	15.2 ± 8.9	0.069
tIgE (kU/L, mean ± SD)	53.1 ± 44.7	522.5 ± 577.4	<0.0001
SCORAD (mean ± SD)	29.1 ± 11.7	27.9 ± 11.8	0.644
APT positivity (%)	8 (27.6)	37 (40.7)	0.205

APT, atopy patch test; eAD, extrinsic atopic dermatitis; iAD, intrinsic atopic dermatitis.

Bold number means that the *P* value is less than the significant level (e.g. *P* < 0.05).

those of sIgE, which might be due to different underlying mechanisms discussed below. The SPT and sIgE strictly assess type-I IgE-mediated allergic responses to a protein. The APT essentially assesses the ability of the protein to induce type-IV T-cell-mediated eczematous skin inflammation. Early studies^{22,23} demonstrated allergen-specific IgE-binding Langerhans cells and so-called inflammatory dendritic epidermal cells (IDECs) in freshly induced APT lesions, which might explain the association of positive APT reaction with sIgE for certain allergens observed in some other studies.^{6,7} However, recent data suggested that the inherent proteolytic enzyme activity of mite allergens and activation of proteinase-activated receptors-2 (PAR-2) may be relevant to skin inflammation as well.^{24,25} Therefore, the inflammation caused by the APT reaction is composed of IgE-mediated allergic responses and inherent mites enzymatic activity, which resulted in a more complex condition, such as the APT reaction to the dust mite observed in intrinsic AD patients (negative IgE tests).²⁶ In an European multicenter study including 314 AD patients, positive APT reactions without SPT or sIgE positive reaction to mite allergens were seen in 9.9% of the patients.⁷ In another study comparing extrinsic and intrinsic AD patients, the rate of positive APT was 47.4% and 66.6% respectively.²⁷ In our study, positive APT reactions were found in 27.6% (8/29) of iAD and 40.7% (37/91) of eAD patients. These observations provide substantial confirmation of a non-IgE-mediated mechanism underlying the APT reaction in AD patients. In addition, a positive APT reaction to mite allergens was observed in 8–14% of healthy volunteers without symptoms or history of eczema, hay fever and asthma,^{28–31} which may also be due to the non-IgE-mediated mechanism. Irritant APT reactions have been observed in some studies, especially for food or aeroallergens.^{17,19} Darsow *et al.* reported less irritant reactions when using smaller concentrations of allergens.⁶ Moreover, whole-body extracts of mites contain not only protein antigens but also lipid antigens and exhibit a proteolytic enzyme activity.²⁴ Therefore, the irritant effects linked to the proteolytic activity of mite allergens should not be underrated, because this seems to account for a consistent number of positive responses.¹⁵

It has been reported that AD patients with an air-exposed pattern have significantly higher rates of positive APT results compared to patients with non-air-exposed pattern.³² In agreement with other studies,^{8,32} our observation showed a statistical association between the APT results and an air-exposed pattern of eczema. The results of the SPT and sIgE did not show any association with the eczema distribution pattern. With regard to AD severity, both the APT and IgE-related diagnostic tests (SPT/sIgE) showed no significant correlation with objective SCORAD in our study population, which may be explained by the fact that APT results only reflected the status or degree of skin hypersensitivity to mite allergens. Patient selection may also have an impact. Severe patients were more likely to be excluded than

mild and moderate patients because of increase in the risks and complications of APT in severe patients.

Although the APT is commonly used to evaluate allergen-induced delayed hypersensitivity reaction via skin, several previous studies revealed positive APT reactions in asthma and allergic rhinitis patients.^{29,33,34} In this study we analysed the association of different diagnostic tests of mite allergens with accompanying respiratory disorders in AD patients. The results showed that positivity of both the SPT and sIgE to mite allergens was strongly correlated with AD patients with respiratory disorders, whereas no correlation was found between positive APT results and respiratory disorders. Another study in a large population of 465 children demonstrated that in subjects with asthma or rhinitis, a positive APT reaction to dust mite allergens was strongly associated with the presence of current or past AD, while SPT and sIgE were significantly more frequently positive in patients with only atopic respiratory disorders.²⁰ Therefore, positive APT reactions might reflect the cutaneous hypersensitivity to mite allergens not only in pure AD patients but also in AD patients with respiratory symptoms.

In addition, we compared the positive results of the APT, SPT and sIgE tests in different age group. The association analysis of positive APT results with the age subgroup in our study population showed a significantly higher frequency of positive APT reactions to mite allergens in adult and adolescent patients compared with those in children. The positivity of SPT or sIgE did not show a significant association with age. The other studies also found that the dust mites is the most common and clinically relevant allergen in adult AD patients, especially in those with head-and-neck involvement.^{6,35,36}

In summary, this study was the first to investigate the APT to mite allergens in Chinese AD patients. We observed house dust mite induced cutaneous hypersensitivity with the APT in AD patients, especially in adult and adolescent patients. Both intrinsic and extrinsic AD patients showed positive APT reactions to house dust mite. The APT results were not associated with disease severity, but it might help to identify the mite allergens-elicited eczematous type-IV sensitization via the skin in AD patients.

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