

Mite allergen (Der p 1) levels in houses of children with atopic dermatitis: the relationship with allergometric tests

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Summary

Several studies, in particular in adult groups, have evaluated the involvement of mites in the pathogenesis of atopic dermatitis (AD). This still remains controversial. The objective of this study was to determine the level of house dust mites (HDMs) in the beds of a group of children with AD and correlate these levels with their allergometric assessment. Forty-one children with AD underwent allergometric tests (prick test, patch test and radioallergosorbent test, RAST) and the concentration levels of HDMs in their homes were evaluated. Our data show that about half of the children (51%) with AD presented *Dermatophagoides pteronyssinus* positivity (prick test and/or RAST and/or patch test). Dust was collected in the period October–November from the children's beds, by the same two operators, using a dust-collection device. The dust mite level was tested by an enzyme-linked immunosorbent assay with antibody against Der p 1 allergen. Ten children (24%) presented a Der p 1 concentration $> 2 \mu\text{g/g}$ of dust (the value assumed to be a risk level for sensitization), 20 (49%) between 0.1 and $2 \mu\text{g/g}$ and 11 (27%) $< 0.1 \mu\text{g/g}$ of dust. In the group with the highest Der p 1 concentration ($> 2 \mu\text{g/g}$ dust) nine children (90%) presented an allergometric *D. pteronyssinus* sensitivity, the difference with the other two groups being statistically significant at $P < 0.018$. The results of the present study show that the highest HDM concentrations were observed in the group with an allergometric *D. pteronyssinus* positivity (prick test and/or RAST and/or patch test). It is hypothesized that a higher HDM concentration may elicit *D. pteronyssinus* IgE sensitization and delayed hypersensitivity in children with AD.

Key words: atopic dermatitis, *Dermatophagoides pteronyssinus*, house dust mite, patch test, prick test, radioallergosorbent test.

Atopic dermatitis (AD) is a chronic inflammatory skin disease with an early onset. It frequently represents the first clinical manifestation of atopy in infants. The role of food allergens seems to be relevant in the first years of life of many children with AD,¹ while the role of aeroallergens is less well known, even though clinical and laboratory data show their possible involvement. Mites, in particular the house dust mite (HDM) *Dermatophagoides pteronyssinus*, are the major environmental allergen and are suspected to be an important factor in the pathogenesis of AD. Current studies indicate that skin contact with environmental aeroallergens mediates an allergic specific immune response via Langerhans cells expressing the high affinity Fc-receptor for specific IgE.² Activation of the Langerhans cells results in the production of atopy-specific T-helper cells (Th2-like cells), which play a pathogenetic role by producing

and releasing cytokines responsible for the allergic inflammation.³

Furthermore, the marked clinical improvement of AD following intensive eradication of mite allergens in the home⁴ supports the hypothesis that mites are important in the pathogenesis of AD, in the same way that asthma severity appears to be related to the degree of mite allergen exposure.⁵ The aim of our study was to evaluate the HDM level in the beds of a group of children with allergic AD and correlate these levels with their allergometric assessment.

Subjects and methods

Study population

We studied 41 children aged 2–10 years (mean 3.9; median 3) affected by AD who were prick test positive

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and/or radioallergosorbent test (RAST) positive for food or inhalant allergens. All the children live in the same geographical area (Bologna and surroundings) with a continental climate, in the same houses where they were born. At the start of the study no child had any respiratory atopic symptoms. A clinical evaluation of AD was performed by the same operator using the severity scoring of AD (SCORAD) index:⁶ the mean score value observed in the children was 29 points, representing a moderate intensity of the disease.

Allergometric assessment

Skin prick tests (Bayropharm, Italy) were performed in all the children for food allergens (cow's milk, hen's egg, cereals, peas, fish), inhalant allergens (grass pollen, cat, dog, *D. pteronyssinus*, *D. farinae*) and positive (histamine) and negative (saline) controls. Allergens eliciting a mean weal diameter of 3 mm or more than the negative control were considered positive. The children had not taken antihistamines for at least 7 days before the prick tests were performed. Circulating specific IgE was determined using RAST (CAP-system, Pharmacia, Uppsala, Sweden). A child was considered sensitized if IgE antibody concentrations were higher than 0.35 kU/L (i.e. CAP class 1).

Patch tests were performed in 33 children using purified extracts of *D. pteronyssinus* and grass pollen kindly supplied by Bayropharm. The extracts had a starting concentration of 2608 units RAST activity (RUA)/mg corresponding to 9298 protein nitrogen units (PNU)/mg for *D. pteronyssinus* and 5983 RUA/mg corresponding to 11,143 PNU/mg for grass pollen. In each child, three different concentrations of each allergen were used in a 20- μ L dose: *D. pteronyssinus* at 1600, 800 and 400 RUA/dose of lyophilized extracts dissolved in glycerosaline solution and grass pollen at 1600, 800 and 400 RUA/dose, again in glycerosaline solution. The concentrations used were chosen in accordance with previous studies.⁷⁻⁹ In addition, a control test with glycerosaline solution alone was performed. The extracts were applied to blotting paper and put in a Finn Chamber. Epicutaneous patch tests were applied on clinically normal skin on the back which had not been treated with disinfectants. The chambers were removed after 48 h. The test reactions were read at 48 and 72 h and also, when the reaction was doubtful, at 96 h. Patch test reactions showing erythema with induration or papules, or both, or spreading beyond the test area, or (less frequently) a vesicular reaction,

were recorded as positive. Vehicle controls were used to exclude false positive reactions in the same subjects.

Collection and handling of dust

The dust was collected in the period October–November from the children's beds, by the same two operators, with a dust-collection device (ALK, Denmark) as described by Dreborg *et al.*¹⁰ The collecting device was a suction mouthpiece which is constructed for mounting on common European vacuum cleaners. The air was sucked through a cellulose filter (ALK), which retains about 75% of particles 0.3–0.5 μ m in size, 80% of particles 0.5–1 μ m in size, 95% of particles 1–10 μ m in size, and 100% of larger particles.¹⁰

The mattress of each child's bed was vacuumed at 2 min/m². The samples collected were weighed on an electric balance and stored at –18 °C until extraction. The dust was extracted together with the filter paper by agitation in dust extraction buffer (0.125 mol/L ammonium hydrogen carbonate buffer + 0.1% sodium azide), in a 1:10 dust/buffer (w/v) ratio for 2 h. The suspension was centrifuged for 15 min. Extracts were stored at –18 °C until determination assay.

Assay procedure

The HDM level was tested by an enzyme-linked immunosorbent assay (ELISA) test (ALK). Ready-to-use microwell plates were precoated with antibodies against Der p 1 allergen. The extracted dust samples and the standards were incubated for 2 h with the immobilized antibodies. After unbound material was washed away, a horseradish peroxidase conjugate antibody was added to each well. The resultant sandwich was washed and a chromogenic substrate for the horseradish peroxidase was added to each well. The colour reaction was stopped by adding a stop buffer. The microwell plate was assessed on an automated photometer at 490 nm. A standard curve was plotted and the sample concentrations of major allergen were calculated. The allergen levels were expressed as μ g/g of dust. The detection limit of the ELISA was 0.1 μ g/g of dust. The dust allergen levels were then stratified into three classes: <0.1 μ g/g (not detectable), 0.1–2 μ g/g and >2 μ g/g of dust.

Statistics and ethical consideration

Statistical analysis used the Statistical Package for Social Science. χ^2 analysis and Fisher's exact test were used for comparison between groups; correlation

between variables was assessed using Spearman's rank correlation. Informed consent was obtained from the parents before the children were included in the study.

Results

The results of allergometric tests are reported in Table 1. Skin prick tests for *D. pteronyssinus* were positive in 11 children (27%); RAST was also positive in 11 children (27%). Patch tests, performed only in 33 children, were positive in 17 (52%); of these 17, six (35%) presented only *D. pteronyssinus* patch test positivity, while the others also showed prick test or RAST positivity. A positivity in at least one of the tests (prick test, patch test, RAST) was present in 21 children (51%). No difference was found in the clinical evaluation between the 20 subjects without *D. pteronyssinus* sensitivity (median SCORAD index 33) and the 21 subjects with *D. pteronyssinus* sensitivity alone or combined with other allergens (median SCORAD index 28).

The geometric mean dust load in mattresses was 288 mg/m² (range 50–1900) in the total group; that in the mattresses of children with dust-positive allergometric tests was 273 mg/m² (range 50–1900), which was not significantly different from that in mattresses of children with negative tests at 308 mg/m² (range 70–1310). The geometric mean Der p 1 concentration, expressed as amount of dust/m², was 199 ng/m² [95% confidence interval (CI) 126–316] in the total group; in the children with dust-positive allergometric tests it was 245 ng/m² (95% CI 123–489), while in the children with dust-negative allergometric tests it was

128 ng/m² (95% CI 81–204). This difference is not statistically significant ($P = 0.16$).

The Der p 1 concentration in the children's beds, expressed as µg allergen/g dust, was distributed as follows: 10 children (24%) presented a Der p 1 concentration > 2 µg/g of dust (the value assumed to be a risk level for sensitization), 20 (49%) between 0.1 and 2 µg/g and 11 (27%) < 0.1 µg/g of dust.

The relationship between HDM concentration and *D. pteronyssinus* tests is reported in Table 2. RAST positivity shows a significant ($P < 0.002$) difference between the groups stratified on the basis of mite concentrations. Moreover, the number of positive responses to *D. pteronyssinus* in one or more of the allergometric tests differed between the three dust groups considered: in the group with the lowest Der p 1 concentration (< 0.1 µg/g dust) four children (36%) were positive, in the intermediate group (0.1–2 µg/g dust) eight children (40%) were positive and in the group with the highest Der p 1 concentration (> 2 µg/g dust) nine children (90%) were positive; the difference between these groups is statistically significant ($P < 0.018$).

We observed an isolated contact sensitivity to *D. pteronyssinus* in only six subjects: five of these were included in the group with the highest Der p 1 concentration (> 2 µg/g dust) and the other was included in the intermediate concentration group (0.1–2 µg/g dust). However, the number of subjects is too small to obtain statistical significance. Spearman's rank correlation showed no significant correlation ($P = 0.6$) between specific IgE antibodies (kU/L) to *D. pteronyssinus* and the concentration of Der p 1 (µg/g) in the bed. We did not find any significant relationship between the

Table 1. Allergometric assessment in 41 children with atopic dermatitis. A radioallergosorbent test (RAST) greater than 1 was considered to be positive. A patch test showing erythema with induration or papules, or greater change, was defined as being positive. A prick test giving a weal diameter of 3 mm or more than the physiological saline control was defined as being positive

	Allergen	No. of positives (total $n = 41$)	(%)
Prick test	Food ^a	15	37
	Inhalants ^b	18	44
	<i>D. pteronyssinus</i>	11	27
RAST	Food ^a	23	56
	Inhalants ^b	23	56
	<i>D. pteronyssinus</i>	11	27
Patch test ($n = 33$)	Inhalants ^c	22	67
	<i>D. pteronyssinus</i>	17	52
Combined			
Prick test and/or RAST and/or patch test	<i>D. pteronyssinus</i>	21	51

D. pteronyssinus, *Dermatophagoides pteronyssinus*. ^aFood allergens tested: cow's milk, hen's egg, cereals, peas, fish. ^bInhalant allergens tested: grass pollen, cat, dog, *D. pteronyssinus*, *D. farinae*. ^cInhalant allergens tested: grass pollen, *D. pteronyssinus*.

Table 2. Allergometric *Dermatophagoides pteronyssinus* assessment related to Der p 1 allergen concentration

	Der p 1 concentration <0.1 µg/g dust (11 children) Positive/total (%)	Der p 1 concentration 0.1–2 µg/g dust (20 children) Positive/total (%)	Der p 1 concentration > 2 µg/g dust (10 children) Positive/total (%)
Prick test	2/11 (18)	4/20 (20)	5/10 (50)
RAST	1/11* (9)	3/20* (15)	7/10* (70)
Patch test (<i>n</i> = 33)	4/9 (44)	6/15 (40)	7/9 (78)
One or more of the above tests combined	4/11** (36)	8/20** (40)	9/10** (90)

RAST, radioallergosorbent test; **P* < 0.002; ***P* < 0.018.

children's age and the frequency of positive results or the Der p 1 concentration, probably because most children in the group were aged about 4–5 years.

Discussion

The relationship between AD and aeroallergens, in particular HDMs, has been suspected by many authors, even though it is difficult to quantify. We selected children with allergic AD, i.e. with positive prick test or RAST, and moderate symptoms (mean SCORAD index 29) but without allergic respiratory diseases. Our data show that about half of our children with AD presented *D. pteronyssinus* positivity (prick test and/or RAST and/or patch test). *D. pteronyssinus* patch testing showed the highest sensitivity (17 of 33 children, 52%). Moreover, more than a third of these 17 children did not show any *D. pteronyssinus* IgE-mediated response.

Some previous studies have demonstrated a positive patch test reaction for aeroallergens only in AD patients with positive prick test reactions for the same allergens and elevated IgE levels;^{11–13} in contrast, recent studies show that up to 30–40% of patients affected by AD and having positive patch test reactions do not have positive prick tests to the same aeroallergens.^{7,8,14} Our data agree with these last studies. The aim of the present study was to demonstrate a relationship in children with AD, between HDM concentrations and allergometric *D. pteronyssinus* positivity. Studies to determine risk HDM allergen levels have previously been performed in the homes of asthmatic patients.^{7,15}

Although differing collection methods have been used, sometimes with a lack of consensus as to the best way to quantify Der p 1 allergen (total allergen content or concentration per unit weight or per area sampled),¹⁶ Doull *et al.*¹⁷ recently concluded that the expression of Der p 1 levels per weight of collected dust appears to be the best method; this was the method we adopted. An association of mite sensitization with food

or other inhalant allergens was present in nearly all our subjects (88%); this could be a factor favouring sensitization even when HDM concentration is < 2 µg/g, as proposed by Kuehr *et al.*¹⁸ This tendency was also observed in our study: all the subjects with isolated *D. pteronyssinus* sensitivity were included in the group with higher Der p 1 concentrations. The minimum level of exposure to settled allergen necessary for sensitization and provocation of symptoms is still under discussion, although 2 µg/g of dust is considered by many authors as the threshold.^{5,18,19}

In previous clinical trials, a correlation between IgE-mediated responses and the levels of Der p 1 allergen in asthmatics' homes has been demonstrated.⁵ In our study we found a good correlation also between *D. pteronyssinus* sensitization and HDM levels in beds of children with AD: 90% of our subjects presenting values above such a threshold were sensitized to *D. pteronyssinus* (Table 2) and RAST positivity showed a significant (*P* < 0.002) difference between the groups stratified on the basis of mite concentrations. In contrast, no correlation was found between the level of specific IgE antibodies (kU/L) to *D. pteronyssinus* and the Der p 1 concentration. Previous studies have shown that a precocious positive allergometric reaction (skin prick test or RAST) for inhalant allergens, in particular for *D. pteronyssinus*, in patients with AD, is predictive of the appearance of allergic respiratory symptoms.^{20,21} Furthermore, some authors have reported that clinical symptoms in subjects with AD are related to HDM exposure and that HDM avoidance improves clinical skin symptoms.⁴

In conclusion, our study underlines the role of HDM levels in the induction of *D. pteronyssinus* IgE sensitization and delayed hypersensitivity in children with AD. Delayed hypersensitivity tests (patch test) showed the highest percentage of positivity in the group of children with the highest Der p 1 concentration in HDM (Table 2), although this value was not statistically

significant. However, in children with AD the role of *D. pteronyssinus* delayed hypersensitivity in triggering the clinical symptoms and/or the appearance of an allergic respiratory disease is still under discussion.

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