

Comparative detection of mite allergens in house dust of homes in Moscow by enzyme-linked immunosorbent assay and acarologic analysis

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The present study revealed that 73% of surveyed apartments in Moscow whose residents included children with the atopic form of bronchial asthma and sensitization to *Dermatophagoides pteronyssinus* allergens were infested with the pyroglyphid mites *D. pteronyssinus* and *D. farinae*. The number of mites in the surveyed apartments varied between 0 and 154 mites/g of dust for *D. pteronyssinus* and between 0 and 162 mites/g of dust for *D. farinae*. The levels of mite allergens in these apartments ranged from 0.5 to 165.8 µg/g for *Der p* I and from 0.3 to 91.3 µg/g of dust for *Der f* I. The *Der p* I allergen was found to predominate, and its concentration in one-third of the apartments was more than 10-fold greater than that of *Der f* I. Correlation between the number of pyroglyphid mites and the concentration of group I allergens was established for both *D. pteronyssinus* ($r = 0.4932$; $P < 0.01$) and *D. farinae* ($r = 0.6748$; $P < 0.01$). In most of the apartments, high and moderate levels of *Der* I allergens were detected.

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Mites of the family Pyroglyphidae are well known as the source of highly potent house-dust-mite allergens. Two widespread species of this family, *Dermatophagoides pteronyssinus* (Trouessart, 1897) and *D. farinae* Hughes, 1961, endemic all over the world, are of particular importance. So far, three main clinically significant groups of allergens have been isolated from each of the two main species of pyroglyphid mites: *Der p* I, *Der p* II, and *Der p* III from *D. pteronyssinus*, and *Der f* I, *Der f* II, and *Der f* III from *D. farinae* (8). Of patients sensitized to pyroglyphid mites, 40–75% react to group I allergens. *Der p* I and *Der f* I occur mainly in the fecal pellets of mites (at an average concentration of 0.1–0.2 ng per particle of 1 µm in diameter), constantly accumulating in house dust even if the number of mites is low (8, 10, 13).

Exposure to mites is estimated by the number of mites and the concentration of mite allergens in the

patient's environment. However, the acarologic method for the detection of mites, one of the most reliable methods used in medical practice, could give an underestimate, because the total number of pyroglyphid mites is not constant, being subject to seasonal changes (20).

The presence of mite allergens in house dust can be determined by various immunochemical methods (3–6, 10–14, 18–20). The immunoassays which use monoclonal antibodies (mAb) against *Der p* I and *Der f* I are one of the most specific and sensitive methods (1, 8, 9).

The aim of the present investigation was to compare the detection of mites and the mite allergens *Der p* I and *Der f* I in house dust of homes of bronchial asthma patients, sensitized to pyroglyphid mites, by two methods, acarologic analysis and enzyme-linked immunosorbent assay (ELISA).

Material and methods

Acarologic analysis and preparation of house-dust extracts

Dust samples were collected from January to December 1991 in 15 apartments in Moscow. The residents of these apartments included children with the atopic form of bronchial asthma and pronounced sensitization to pyroglyphid mites. Skin prick tests for pyroglyphid mites had yielded positive results in all children. Altogether, 33 dust samples were collected with a household vacuum-cleaner from the bedclothes and beds of children and their parents.

Mites were removed by flotation on saturated NaCl from the collected dust samples and counted. The preparations were prepared in Hoyer-Berlese fluid.

Fine-dust samples obtained from the bedclothes and beds of children and their parents were extracted in ammonium bicarbonate buffer solution (w/v 1:40; pH, 8.2) for 24 h, and were then centrifuged at 4000 rpm for 40 min. The supernatant thus obtained was filtered and stored at -20°C .

mAb assays

Der p I and *Der f I* were determined in ELISA (5, 8). Polystyrene strips (Nunc F-8 module, A/S Nunc, Denmark) were coated with $1\text{ }\mu\text{g/well}$ of either mAb 5H8 against *Der p I* or mAb 6A8 against *Der f I* in 50 mM carbonate-bicarbonate buffer, pH 9.6, overnight at 4°C . The strips were washed and incubated for 1 h with 0.1 ml 1% BSA. After addition of 0.1 ml extracts of dust and incubation for 1 h, the wells were washed with PBS-T. The wells were filled with 0.1 ml of mAb against *Der f I* (4C1), conjugated with biotin diluted 1:1000, peroxidase-labeled ovidin (Sigma Chemical Company, USA) diluted 1:2000, and substrate (1 mM ABTS, Sigma 1888) diluted in 70 mM citrate-phosphate buffer solution (pH 4.2). The optical density of the solution thus obtained was determined at wavelength 405 nm.

Results

Fifteen Moscow apartments whose residents included children with the atopic form of bronchial asthma and pronounced sensitization to *D. pteronyssinus* allergens were studied by acarologic analysis. The study revealed that 73% of these apartments were infested with pyroglyphid mites – *D. pteronyssinus* and *D. farinae*. The former species was detected most frequently, occurring in 67% of apartments, while the latter species was detected in only 40% of apartments. In 27% of the apartments, mixed populations of *D. pteronyssinus* and *D. farinae* were registered (Table 1). The number of mites in the

Table 1. Numbers of *D. pteronyssinus* and *D. farinae* and concentrations of mite allergens *Der p I* and *Der f I* in house dust of Moscow homes

Apt nos.	Biotores	Mite species				
		<i>D. pteronyssinus</i>		<i>D. farinae</i>		<i>Der p I</i> : <i>Der f I</i> [#]
		Mite count (mites/g)	<i>Der p I</i> [@]	Mite count (mites/g)	<i>Der f I</i> [@]	
1	1*	0	2.2	0	0.5	4.4
	2	0	1.3	0	0.6	2.2
2	1	13	29.1	0	1.5	19.4
	2	81	34.3	0	2.5	13.7
3	1	0	–	0	1.5	
	2	0	3.5	0	0.7	5.0
4	1	0	11.8	0	2.7	4.4
	2	126	5.2	0	1.1	4.7
5	1	0	12.4	0	0.8	15.5
	2	0	–	0	0.8	
6	1	0	2.3	0	0.7	3.3
	2	0	4.3	0	0.7	6.1
7	1*	56	2.0	0	7.3	0.3
	2	120	4.6	0	4.1	1.1
8	1	0	32.1	11	27.1	1.2
	2	0	15.2	4	6.6	2.3
9	1	3	10.1	0	91.3	0.1
	2	0	10.2	0	0.9	11.3
10	1	0	2.2	162	–	
	2	0	2.3	65	–	
11	1	0	18.4	48	2.0	9.2
	2	0	1.2	21	1.6	0.8
12	1	0	7.9	0	1.9	4.2
	2	0	28.2	5	43.5	0.6
13	1	0	11.4	0	0.3	38.0
	2	154	9.2	77	36.3	0.3
	1	31	5.1	0	3.9	1.3
	1	0	6.7	0	5.6	1.2
14	1	0	2.5	80	54.0	0.05
	1	0	4.7	32	76.1	0.06
	2	0	0.5	0	1.9	0.3
15	1	99	136.1	0	1.3	104.7
	2	126	164.8	0	1.4	117.7

* 1) Child's bed; 2) parents' bed.

[#] Index of distribution of two mite species in dust sample.

[@] Micrograms per gram dust.

surveyed apartments varied between 0 and 154 mites/g of dust for *D. pteronyssinus*, and between 0 and 162 mites/g of dust for *D. farinae*. At the same time, in only 4/15 apartments (27%) was the density of the mite population in the beds of children higher than in those of adults, while in 6/15 apartments the reverse situation was noted. In two cases (apartment nos. 9 and 14), mites were detected in only the children's beds, while in the parents' beds no mites were detected. In two apartments (nos. 4 and 12), no mites were detected in the children's beds, but they

were detected in the beds of their parents (Table 1). Thus, the mite-allergen exposure in the apartments of allergic children was caused by the presence of mites in the beds of both children and adults. This suggests that sanitary and hygienic measures (regular vacuum-cleaning, change of bed linen, etc.) should be taken not only in the child's bedroom, but also in the whole apartment.

The dust collected in practically all surveyed apartments was found to contain both *Der p* I and *Der f* I. The concentration of group I mite allergens in different apartments varied within the range 0.5–164.8 µg/g of dust and 0.3–91.3 µg/g of dust for *Der p* I and *Der f* I, respectively.

The quantitative ratio of *Der p* I to *Der f* I in individual house-dust samples is clearly shown by the index of distribution of *Der p* I:*Der f* I (1, 19), which varied greatly both in different apartments and between parents' and children's beds (Table 1). In 53% of apartments, *Der p* I levels were considerably higher; in 30% of the apartments, the concentration of *Der p* I in the dust was 10 times greater than that of *Der f* I, at least in one of the surveyed biotopes. In apartment no. 13, great variations in the distribution index were observed: 0.05–38.0.

The presence of direct correlation between the number of pyroglyphid mites and the concentration of group I allergens was established for both *D. pteronyssinus* ($r = 0.4932$; $P < 0.01$) and *D. farinae* ($r = 0.6748$; $P < 0.01$).

Discussion

According to our data, ELISA was 1.7-fold more sensitive than acarologic analysis (Table 1). This should be borne in mind in practical determinations of the presence of mite allergens in house dust. Evidently, if the mite population is depressed at a given moment and mites cannot be detected by microscopic examination, group I allergens, contained mainly in fecal pellets of mites, can still be detected by ELISA. Nevertheless, it should be pointed out that in cases of mixed *D. pteronyssinus* and *D. farinae* populations, ELISA may yield excessively high results, because the molecules of *Der p* I and *Der f* I have a common epitope (2, 7, 8).

Our data on the concentration of mite allergens of group I, obtained with ELISA, are comparable with the data obtained by a similar method in other laboratories (Table 2).

At present, an exposure to *Der p* I exceeding 10000 ng/g of dust is considered to be high, that exceeding 2000 ng/g is considered to be elevated, that less than 2000 ng/g is considered to be low, and that less than 400 ng/g is considered to be very low (8, 10, 15). In addition, levels of mite exposure of 2–10 µg of group I allergen per gram (equivalent to

Table 2. Comparison of results obtained in detection of group I mite allergens in various countries

Group I allergens (µg/g of dust)		Country	References
<i>Der p</i> I	<i>Der f</i> I		
<0.03–200	<0.01–60	Denmark	(15)
0–45	0–19	Denmark	(6)
<0.02–100	<0.02–50	Austria	(15)
<0.02–8	<0.01–0.5	Belgium	(15)
0.5–164.8	0.3–91.3	Russia	Our data
0.07–11	0.05–15	USA	(4)
<0.06–10	<0.045–15	USA	(15)
0.98–155.2	–	USA	(16)
0.02–33.0	0.1–147.1	USA	(17)
2.15	1.05	Japan	(19)
0.415–88.1	0.393–18.60	Japan	(14)

about 100–500 mites/g) of dust are implicated in asthma (13). In all apartments which were surveyed in Moscow, the exposure to *Der p* I was either high (60.0% of the apartments) or elevated (40.0% of the apartments). Simultaneously, the exposure to *Der f* I was high in 37.0%, elevated in 28.6%, and low in 35.7% of apartments. In none of the surveyed apartments was a very low level of mite allergens found.

The data obtained in this study indicate that exposure to mite allergens was present in all surveyed apartments, an exposure which may cause the development of respiratory allergy and constantly exacerbate the disease.

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