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Dermatophagoides farinae (Der f 1) and *Dermatophagoides pteronyssinus* (Der p 1) Allergen Exposure among Subjects Living in Uberlândia, Brazil

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Key Words

Allergen exposure · Asthma · House dust mite · Der f 1 · Der p 1 · *Dermatophagoides farinae* · *Dermatophagoides pteronyssinus*

Abstract

Background: The role of mite allergen exposure in sensitization and development of asthma has been widely recognized. Previous studies have shown that *Dermatophagoides pteronyssinus* and *Blomia tropicalis* were the most prevalent house dust mites in Brazil, while *D. farinae* was rarely found. The aim of this study was to measure Der f 1 and Der p 1 allergen levels in Brazilian asthmatics' and controls' homes. ***Methods:*** Sixty-four houses (32 asthmatic, 32 control) were visited for dust sampling from five sites. Der f 1 and Der p 1 levels were measured by two-site monoclonal-antibody-based ELISAs. ***Results:*** The highest levels of Der f 1 and Der p 1 allergens were found in bedding samples from both asthmatics' and controls' homes. However, the geometric mean of Der f 1 levels (15.8 μg/g of dust) was significantly higher than for Der p 1 (8.2 μg/g of dust) in these samples. In addition, allergen levels ≥10 μg/g of dust were found in 60–80% of the samples for Der f 1 and about 50% for Der p 1. ***Conclusions:*** These results indicate that high levels of Der f 1 allergen are present in both asthmatics' and controls' homes, in contrast to previously reported data. Therefore, studies on exposure to mites should be performed in different cities, seasons and times, since the mite fauna might be subject to variations. Knowledge of the mite fauna will certainly improve the means of investigating the association between allergen exposure and sensitization, allowing to establish the inclusion of new mite extracts in inhalant skin test sets, and even to detect monosensitized patients with respiratory allergy.

Introduction

Mites of the family Pyroglyphidae (*Dermatophagoides* spp.) are the major source of allergens in house dust [1]. Three species, *D. pteronyssinus*, *D. farinae* and *D. microceras*, have been recognized as the most prevalent [2], although *Blomia tropicalis*, in tropical and subtropical areas, can be important [3]. Studies carried out in São Paulo, Brazil, based on mite counts and microscopic iden-

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tification of mites, have shown that *D. pteronyssinus* and *B. tropicalis*, are the most frequent mite species in house dust [4].

There are two major groups of dust mite allergens, group I (Der p 1 and Der f 1) and group II (Der p 2 and Der f 2), which have been isolated from *D. pteronyssinus* and *D. farinae*, respectively [5, 6]. The quantification of these allergens in house dust has allowed to determine an index of allergen exposure [7].

The role of mite allergen exposure in sensitization and development of allergic diseases, particularly asthma and rhinitis, has been recognized in many parts of the world [8]. Previous studies performed in São Paulo, SP [9], Recife, PE [10], Rio de Janeiro, RJ [11], Salvador, BA [12] and Campinas, SP [13] have shown that *D. pteronyssinus* and *B. tropicalis* are important causes of sensitization among asthmatic patients in Brazil. In addition, in keeping with previous reports that *D. farinae* is rarely found in Brazil, Der f 1 was undetectable or found in very low levels (< 0.5 μg/g of dust) [9]. On the other hand, Geller et al. [14] reported in a study performed in Rio de Janeiro, Brazil, that 58% atopic patients were skin prick test positive to *D. farinae*. However, exposure levels ≥ 2 μg of Der p 1 and Der f 1/g of dust were found in only 7.14% of the house dust samples [11]. Moreover, in our experiments using reverse ELISA for measuring specific IgE to *D. farinae* (Der f 1) in asthmatic patients, a high percentage of sensitization (62.8%) to this allergen was found among the subjects [unpubl. data].

Based on studies of prevalence, allergen exposure and sensitization of children and adults with asthma, it has been proposed that exposure at levels ≥ 2 μg of Der p 1/g of dust is a risk factor for sensitization in mite-allergic subjects, while the comparable level for nonatopic children was approximately 50 μg/g [15, 16].

The aim of the present study was to measure Der f 1 and Der p 1 allergen levels in asthmatics' and controls' homes in Uberlândia, MG, Brazil. In addition, we investigated the correlation between exposure index of Der f 1 and Der p 1 as well as the prevalence of these mites, particularly *D. farinae*, in this region.

Materials and Methods

Houses and Subjects

Sixty-four houses in Uberlândia, MG, Brazil, were enrolled for a mite indoor environmental study. Thirty-two houses had at least one adult subject aged 18–60 years with mild to severe asthma based on criteria established by National Asthma Education Program Coordinating Committee [17]. In addition, the subjects also were allergic to *Dermatophagoides pteronyssinus* on skin prick testing evaluated in the Unit of Allergy and Infectious Diseases at the Immunology Division of the Federal University of Uberlândia, Brazil. As control group, 32 houses with no medical history of atopy, age- sex- and area-matched subjects were also recruited.

Dust Sampling

Sampling visits were made to 64 households (32 from asthmatic subjects and 32 from control subjects) between January and June 1998. Samples of house dust were obtained from five sites of each subject's house: sofa, TV room floor, bedding, bedroom floor and kitchen (including cabinet and floor dust), using a hand-held vacuum cleaner (Arno SA, São Paulo, Brazil) modified with a cotton filter, according to the standard procedure [18]. Fine dust was obtained by sieving through a 0.3-mm mesh screen. Samples of 100 mg were extracted overnight at 4°C in 2 ml borate-buffered saline, pH 8.0, and after centrifugation, supernatants were stored at –20°C prior to immunoassays.

ELISA for Measuring Levels of Mite-Allergen

Group 1 *Dermatophagoides* allergens (Der f 1 and Der p 1) were measured by two-site monoclonal-antibody-based ELISA as described by Luczynska et al. [19], with some modifications. Briefly, microtiter plates were coated with mouse monoclonal antibodies anti-Der f 1 (clone 6A8) and anti-Der p 1 (clone 5H8) at 1 μg/well in 0.06 *M* carbonate buffer, pH 9.6, overnight at 4°C. Plates were washed with 0.01 *M* phosphate-buffered saline (PBS), pH 7.2, containing 0.05% Tween 20 (PBS-T) and blocked with PBS-T plus 1% bovine serum albumin (PBS-T-BSA) for 1 h at room temperature (RT). Subsequent steps were carried out using PBS-T-BSA as diluent, and washings in PBS-T were done between the steps of the reaction. The plates were incubated with dust extracts (undiluted and 1:5) for 1 h at RT. Subsequently, biotinylated antibody anti-group 1 *Dermatophagoides* allergens (clone 4C1) was added (1:1,000) and incubated for 1 h at RT, and streptavidin-peroxidase conjugate (Sigma Chemical Co., St. Louis, Mo., USA) diluted at 1:1,000 was incubated for 30 min at RT. The assay was developed by adding the enzyme substrate (0.01 *M* ABTS and 0.03% H_2O_2), and the reaction was read at 405 nm in a plate reader (Titertek Multiskan, Flow Laboratories, USA). Reference standards containing known levels of each allergen were included in each plate in duplicate to obtain control curves ranging from 125 to 0.5 ng/ml. Absorbance results were expressed as μg/g of dust [20].

Cross-Reactivity Assays between Der p 1 and Der f 1

In order to verify the existence of cross-reactivity between Der p 1 and Der f 1, ELISAs were carried out as described above, using only reference standards. Briefly, microtiter plates were coated either with anti-Der f 1 (clone 6A8) or anti-Der p 1 (clone 5H8) capture monoclonal antibodies, and further incubated with both Der f 1 and Der p 1 allergens. These reference standards were added in duplicate, in serial twofold dilutions to make control curves ranging from 125 to 0.122 ng/ml. Next, biotinylated antibody to mite group 1 allergens (clone 4C1) was added for both allergens. The subsequent steps of the reaction were equally performed as described previously.

Statistical Analysis

As the allergen concentration results followed a non-Gaussian distribution, calculations were performed on log-transformed data and allergen levels expressed as geometric mean (GM). Statistical

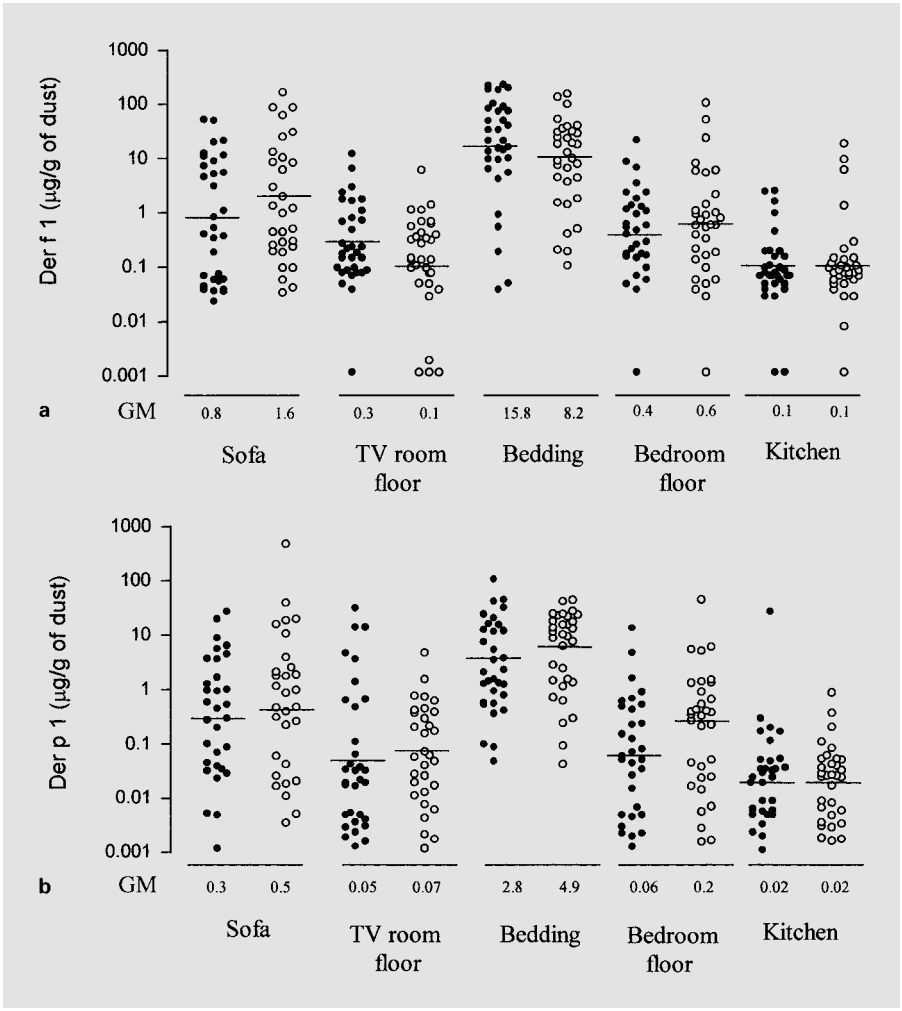

Fig. 1. Levels of *D. farinae*, Der f 1, (**a**) and *D. pteronyssinus*, Der p 1, (**b**) expressed in µg/g of dust, in dust samples collected from five sites: sofa, TV room floor, bedding, bedroom floor, and kitchen from 32 asthmatics' (●) and 32 controls' (○) homes. The horizontal bar shows the GM.

analysis consisted of determinations of geometric means with 95% confidence intervals and the differences between the means were analyzed by the unpaired Student t test. The group comparisons were performed using the differences between two proportions by Z statistic. Data sets were analyzed by Pearson's correlation test. Values of $p < 0.05$ were regarded as statistically significant.

Results

Mite allergens were measured in 315 dust samples from the 64 houses visited (five sofa dust samples were not available). The highest levels of Der f 1 allergens were detected in bedding samples from both asthmatics' (GM: 15.8 µg/g of dust; 95% CI: 6.9–36.1 µg/g) and controls' homes (GM: 8.2 µg/g of dust; 95% CI: 4.1–16.4 µg/g), with slightly higher levels in asthmatics' homes, but without significant differences ($p > 0.05$). GM <2 µg of Der f 1/g of dust were found in the other analyzed sites, in both the asthmatics' and controls' homes (fig. 1a). Similarly, the highest levels of Der p 1 allergens were also found in bedding samples from asthmatics' (GM: 2.8 µg/g of dust; 95% CI: 1.4–5.7 µg/g) and controls' homes (GM: 4.9 µg/g of dust; 95% CI: 2.5–9.6), with no statistically significant difference between them ($p > 0.05$) (fig. 1b). The Der f 1 levels in these bedding samples were significantly higher than those observed for Der p 1 ($p < 0.05$) only for the asthmatics' homes. There were no statistically significant differences between the levels of the Der f 1 and Der p 1 allergens found in the different sites of both asthmatics' and controls' homes.

When analyzing the results, the highest level for each allergen (from the five sites) in a house was taken as an index of exposure to that allergen. Thus, a correlation between the levels of Der f 1 and Der p 1 in dust samples

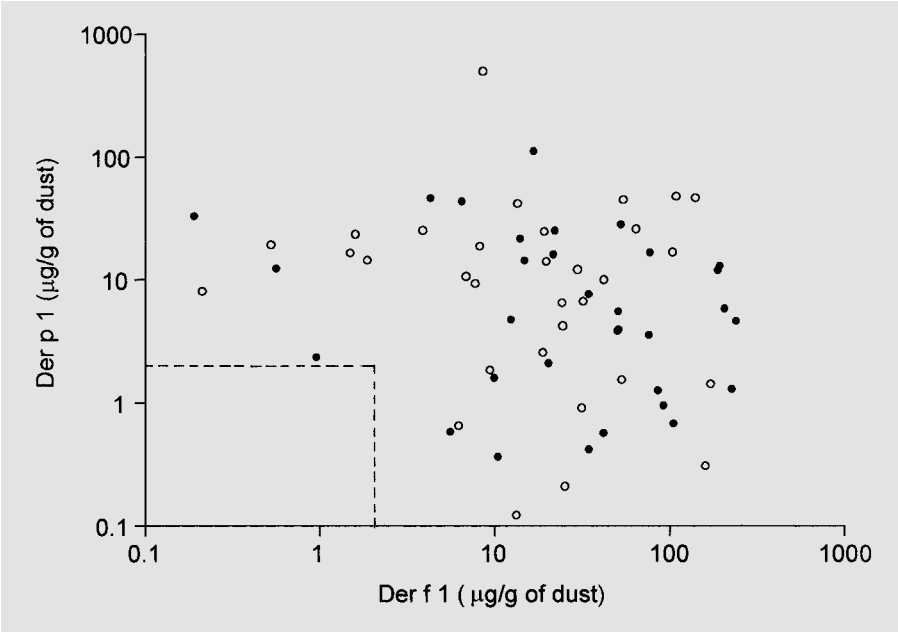

Fig. 2. Correlation between levels of *D. farinae* (Der f 1) and *D. pteronyssinus* (Der p 1) expressed in μg/g of dust, in dust samples from 32 asthmatics' (●) and 32 controls' (○) homes. Extracts of house dust were obtained from five sites. The highest value in the house was taken as exposure index. The dashed line represents the level of 2 μg/g of dust, which has been proposed as risk factor for sensitization in mite-allergic subjects.

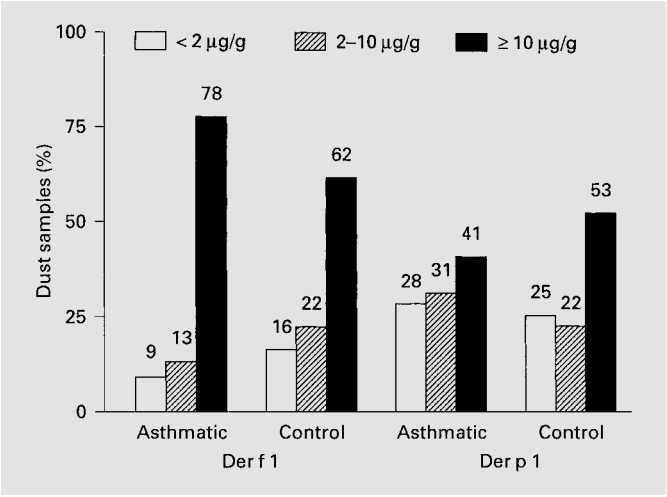

Fig. 3. Percentage of dust samples, analyzed as exposure index, containing Der f 1 and Der p 1 allergens at different ranges (<2 μg/g; 2–10 μg/g; ≥10 μg/g of dust) in 32 asthmatics' and 32 controls' homes.

from asthmatics' and controls' homes was done (fig. 2). There was no significant correlation between these allergens in both asthmatics' and controls' homes; in contrast, there was a tendency to a negative correlation ($r = -0.23$; $p = 0.2032$ for asthmatics' and $r = -0.16$; $p = 0.3887$ for controls' homes). In addition, the great majority of the dust samples from asthmatics' homes had levels of Der f 1 and Der p 1 ≥2 μg/g of dust (29/32 and 23/32, respectively), which have been proposed as risk factor for sensitization in mite-allergic subjects. However, Der f 1 was found in high concentrations (≥10 μg/g) in the majority of the analyzed samples (25/32) as compared with Der p 1 (13/32) ($p = 0.0037$). This fact reflects a higher exposure index for Der f 1 in the studied asthmatics' homes. Similar results were obtained with dust samples from controls' homes at exposure levels ≥2 μg/g of dust.

Concerning the exposure index for Der f 1 and Der p 1 allergens found in each asthmatics' and controls' home, the percentages of dust samples obtained were analyzed according to the different allergen levels and are demonstrated in figure 3. Thus, 78% of the asthmatics' and 62% of the controls' homes had levels of Der f 1 ≥10 μg/g of dust, as compared with those obtained for Der p 1 (41% and 53% of the respective homes), values significantly higher only for Der f 1 in the asthmatics' homes ($p = 0.0037$). On the other hand, lower percentages (9%) were observed for levels of Der f 1 < 2 μg/g of dust in the asthmatics' homes as compared with Der p 1 (28%), although with no statistically significant difference ($p = 0.0548$).

As demonstrated in figure 4, when comparing the standard curves of ELISA for the determination of cross-reactivity between Der f 1 and Der p 1, no cross-reactivity was detected by using anti-Der f 1 (clone 6A8) as capture monoclonal antibody for measuring the Der p 1 reference

allergens (fig. 4a). In parallel, the sensitivity of the assay for measuring Der f 1 was 2.0 ng/ml with an interassay coefficient of variation of 9.9%. Similarly, by using anti-Der p 1 (clone 5H8) as capture monoclonal antibody for measuring the Der f 1 standard allergens, no cross-reactivity was observed (fig. 4b). The sensitivity of the assay for the measurement of Der p 1, in parallel, was 3.9 ng/ml, and the interassay coefficient of variation was 7.1%.

Discussion

Dust mites are doubtless the most common sensitizing among the indoor allergens. Mite growth and proliferation are dependent on several conditions, particularly indoor temperature and humidity, which seem to be decisive and limiting factors for their development [21]. Thus, regional variation in house dust mite allergen levels depends largely on climate differences. Since mites rely on high humidity for their survival, dry or very cold climates are generally low in allergen, while areas with high humidity and mild winters have the highest allergen levels [22].

Mite group 1 allergen levels ≥2 μg/g and ≥10 μg/g of dust have been suggested as risk factors for sensitization and acute attacks of asthma, respectively, at the First International Workshop on dust mite allergens and asthma [20]. However, the threshold for acute attacks of asthma (10 μg/g) has never been confirmed in the epidemiological studies and has been rejected subsequently [16]. In contrast, the threshold for sensitization (2 μg/g) has been confirmed in several studies [16, 23].

In the present study, the highest levels of Der f 1 and Der p 1 allergens were found in bedding samples from both asthmatics' and controls' homes. These results are in keeping with previous reports, in that the highest level of mite allergen was found in the bedroom dust samples, such as pillows, mattresses, beds and bedroom floor [24]. Consequently, bedding is the main source of exposure to mite allergens, due to close contact for longer periods.

By analyzing GM levels, both Der f 1 and Der p 1 were found at sensitization levels (≥2 μg/g), but only Der f 1 was detected at high levels (≥10 μg/g of dust). Such occurrence should be considered in analyzing the sensitization of asthmatic patients, due to the fact that IgE antibody to mite allergens of either *Dermatophagoides* species are highly cross-reactive.

Studies carried out in the United States found that *D. farinae* and *D. pteronyssinus* were the predominant mite species with variation in their relative prevalence.

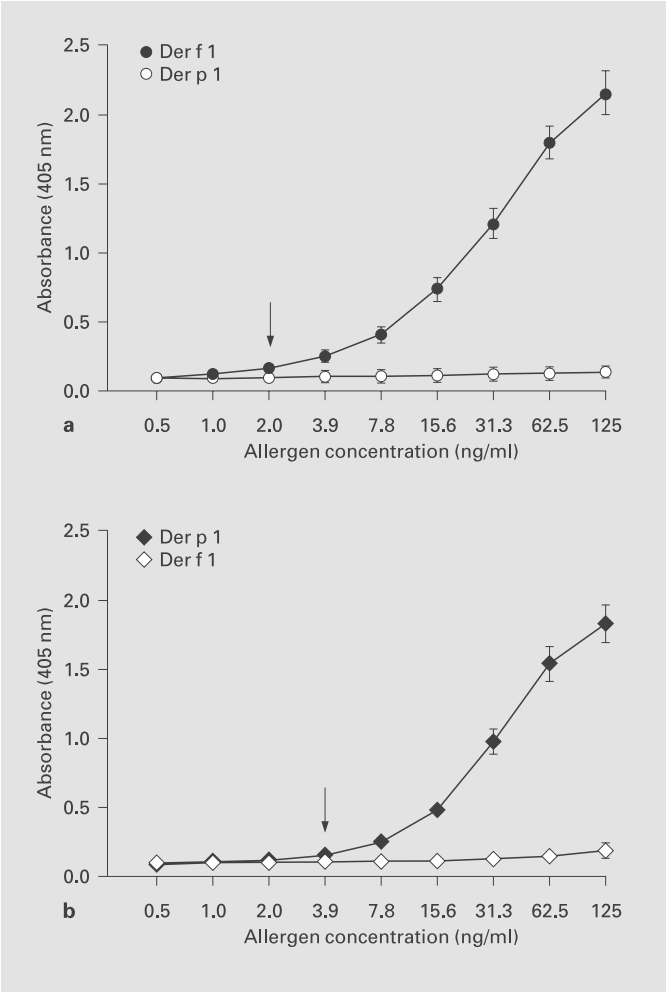

Fig. 4. Standard curves of ELISAs for the determination of cross-reactivity between Der f 1 and Der p 1. (**a**) ELISA for measuring Der f 1 and Der p 1 allergens, by using anti-Der f 1 (clone 6A8) as capture monoclonal antibody, and anti-group 1 *Dermatophagoides* allergens (clone 4C1) as biotinylated secondary antibody. (**b**) ELISA for measuring Der p 1 and Der f 1 allergens, by using anti-Der p 1 (clone 5H8) as capture monoclonal antibody, and anti-group 1 *Dermatophagoides* allergens (clone 4C1) as biotinylated secondary antibody. The arrows indicate the sensitivity of each assay. Data are representative of three separate experiments.

Thus, *D. pteronyssinus* was recovered mainly in southern states that are continuously warm and humid, and *D. farinae* recovered in central and northern states [24]. In contrast, our results demonstrated a higher prevalence of *D. farinae* than *D. pteronyssinus* in our region, which presents tropical climate, characterized by dry and mild winters, a relative humidity of 31–95% and a mean annual temperature of 18–30°C.

On the other hand, studies performed in Southeast Brazil by Arruda et al. [9] have reported that *D. farinae* was not found or detected in very low levels (<5 μg/g of dust). In addition, only 7.1% of the dust samples analyzed in a study carried out in Rio de Janeiro by Geller [11], displayed levels of Der f 1 and Der p 1 ≥2 μg/g of dust. In a recent investigation, in order to identify the mite fauna in Northeast Brazil (Salvador, BA), Serravalle and Medeiros [12] found that *D. pteronyssinus* is the most prevalent mite, being detected in 70% of the dust samples, while *D. farinae* was detected in 8% only.

Some of the variability in these results is probably caused by differences in climate between studies [22]. It is widely recognized that seasonal fluctuations in climate account for associated fluctuations in allergen levels, and the stability of reservoir mite-allergen concentrations [25]. In addition, there is also great variation in allergen levels between houses within the same climate region, probably due to housing characteristics which affect indoor humidity and, consequently, mite growth [22].

As expected, the mite allergen levels were not statistically significant different between asthmatics' and controls' homes, indicating that both groups were exposed to high levels of allergens. These results are in agreement with previous reports [15, 22, 26] emphasizing the importance of allergen exposure for sensitization, although it is not necessarily a causal factor.

No positive correlation was observed between Der f 1 and Der p 1 allergens in both asthmatics' and controls' homes; in contrast, a tendency for a negative correlation was found. This fact suggests a distinct pattern of exposure index in that there is a higher prevalence of Der f 1 allergens in the homes analyzed.

It is worth noting that Der p 1 and Der f 1 show an amino acid sequence homology of 80%. The divergence is predominantly in the N-terminal residues 1–20 (45%), the C-terminal residues 210–222 (31%) and a central region 91–130 (30%) [27]. These regions containing divergent sequences corresponded to regions exhibiting antigenic differences [28]. Extensive cross-reactivity in binding human IgE and IgG antibodies has been demonstrated between Der p 1 and Der f 1 [29]. However, surprisingly, most monoclonal antibodies produced against the group I allergens are species specific, as are the monoclonal antibody immunoassays that have been developed to measure these allergens in dust samples and extracts [30, 31].

By using sensitive two-site monoclonal-antibodies-based immunoassays for Der f 1 and Der p 1, our results reinforce the specificity of this assay, with the use of highly specific monoclonal antibodies: anti-Der f 1 (clone 6A8) and anti-Der p 1 (clone 5H8) as demonstrated in the standard curves of the ELISAs, where no cross-reactivity was observed.

Taken together, it can be concluded that studies on exposure to mites should be performed in different Brazilian cities as well as in distinct seasons and times, since the mite fauna might be subject to variations. Although the existence of extensive cross-reactivity between Der f 1 and Der p 1-specific IgE antibodies is well known, knowledge of the mite fauna will certainly improve the means of investigating the association between allergen exposure and sensitization. This will establish the necessity of including new mite extracts in inhalant skin test sets used for evaluating patients with suspicious respiratory allergy [32], allowing even the detection of monosensitized patients.

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