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Association between Mite Allergen (Der p 1, Der f 1, Blo t 5) Levels and Microscopic Identification of Mites or Skin Prick Test Results in Asthmatic Subjects

Manoel Medeiros Jr.[a] Joanemile P. Figueiredo[a] Maria C. Almeida[a]
Ajax M. Atta[a] Ernesto A. Taketomi[c] Deise A.O. Silva[c] Silvia A. Terra[c]
Welma W. Amorim[b] Ricardo S. Pinho[b] Maria I. Araujo[a, b]
Edgar M. Carvalho[a, b]

[a]Serviço de Imunologia, Hospital Universitario Professor Edgar Santos, Federal University of Bahia and [b]Escola Baiana de Medicina e Saude Publica, Salvador, and [c]Division of Immunology, Federal University of Uberlândia, Brazil

Key Words
House dust mite · Mite allergens · Asthma · Skin prick test · Atopy

Abstract

Background: Mite allergens have been involved in airway sensitization and allergic diseases. Immunoassays for the identification and quantifiction of house dust mite (HDM) allergens are useful to improve the knowledge of regional mite fauna and the remediation of mite allergens in allergic diseases. The present study analyzed the association between levels of HDM allergen and results of mite identification or skin prick test (SPT) in two different areas of Bahia, Brazil. ***Methods:*** Forty-two asthmatic subjects from a rural area (group I; n = 21) and a slum (group II; n = 21) were evaluated through SPT with HDM allergens and had dust samples collected at their homes for mite identification and allergen measurements. ***Results:*** Positive SPT to *Dermatophagoides pteronyssinus, Dermatophagoides farinae* and *Blomia tropicalis* allergens were observed in 42.9, 38.0 and 42.9% subjects from group I and in 47.6, 19.0 and 33.3% subjects from group II, respectively. *D. pteronyssinus* and *B. tropicalis* were identified in approximately 76 and 50% of samples from both groups, respectively. *D. farinae* was identified in 38.0 and 9.5% of samples from groups I and II, respectively ($p < 0.005$). Der p 1, Der f 1 and Blo t 5 detection were associated with mite identification ($p < 0.05$). Association between HDM allergen levels over 2 μg/g of dust and positive SPT occurred only with *D. pteronyssinus* ($p < 0.0001$). ***Conclusions:*** *D. pteronyssinus* was the most prevalent mite species in this study followed by *B. tropicalis* and *D. farinae*. Immunoassays done to measure mite allergens were associated with mite-species identification. We conclude that these three mite species must be included on panels for the diagnosis of allergic airway diseases in subjects living in such regions.

Introduction

House dust mite (HDM) allergens are involved in sensitization and development of allergic airway diseases, particularly bronchial asthma and allergic rhinitis [1]. Considering that mite fauna could change depending on climate or housing conditions, regional studies on poten-

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Correspondence to: Dr. Manoel Medeiros Jr., Servico de Imunologia
Hospital Universitario Professor Edgar Santos, Federal University of Bahia
Rua Joao das Botas, s/n – 5 ° andar-Canela
41110.160 – Salvador-Bahia (Brazil)
Fax +55 71 245 7110, E-Mail imuno@ufba.br

tial constituents of HDM fauna could contribute to the improvement of the diagnosis and the management of allergic diseases.

Different mite species have been identified in Brazil, but there is an agreement that *Dermatophagoides pteronyssinus* and *Blomia tropicalis* are the most frequent species found in house dust [2–5]. In Salvador, Bahia, a state in northeastern Brazil, we demonstrated that 86% of individuals with bronchial asthma and/or allergic rhinitis had a positive skin prick test (SPT) to different HDM allergens, and 63% were sensitized to *D. pteronyssinus* and *B. tropicalis* [6]. In another study we demonstrated that *D. pteronyssinus* and *B. tropicalis* were found in 70 and 30%, respectively, of the house dust samples from Salvador, while *Dermatophagoides farinae* was found in only 8% of these samples [7], supporting previous reports that this species is rarely found in Brazil [8]. More recently, a high exposure index of the Der f 1 allergen was reported in Uberlandia, a central western region of Brazil [9]. These contrasting findings in Brazil have contributed to stimulating new research to determine the regional mite fauna, which could be useful to define allergen panels for the diagnosis of allergies and the remediation of mite allergens in allergic diseases.

The aim of this study was to evaluate the association between mite allergen (Der p 1, Der f 1 and Blo t 5) levels and the identification of mite species or the SPT results with HDM, analyzing asthmatic subjects and their house dust samples in the state of Bahia, Brazil.

Subjects, Material and Methods

Forty-two subjects with a history of bronchial asthma in the preceding 12 months were selected from two villages with the same low socioeconomic status and asthma prevalence as previously reported [10]. Twenty-one individuals, 10 males and 11 females, aged between 6 and 35 years, were selected from a rural area in the inner state (group I) and another 21 subjects, age- and sex-matched with group I subjects, were selected from a slum close to the sea in Salvador, the capital of the state of Bahia (group II). None of the individuals were taking antiallergic medicine that might interfere with the SPT; they were able to cooperate and had not been given immunotherapy. The ethical committee of the Hospital Universitario Professor Edgar Santos approved the protocol, and all participants or their guardians gave written informed consent.

Skin Prick Test

SPT were performed on the right forearms of all individuals using *D. pteronyssinus, D. farinae* and *B. tropicalis* glycerinated allergen extracts (IPI-ASAC, Spain). Histamine (1/1,000) and saline (glycerinated solutions) were used as positive and negative controls, respectively. A positive skin reaction was defined as a wheal with mean diameter greater than 3 mm with or without a surrounding flare. The SPT results were read 20 min after application. An SPT reaction was considered positive if at least one of the three tested allergens induced a positive reaction.

HDM Analyses: Mite Identification and Mite Allergen Levels

House dust samples from all subjects' homes were collected during 1 week in April 2000, since meteorological conditions were very similar in the two areas. According to official data, the mean annual temperature was 25.5 ± 1.2 and 26.0 ± 1.3°C in the costal area and in the inner state, respectively ($p > 0.05$). The mean annual relative humidity was also similar, ranging from 71.3 to 81.4% throughout the year ($p > 0.05$). The samples were collected from bedding (mattresses and pillows) of the individuals enrolled in the study, using a 1,200 W vacuum cleaner as previously described [11]. Briefly, 1 m^2 of the top of the mattress and the surface of the pillow were vacuumed for 1 min. The bags were sealed and identified, and the samples stored at 4°C until assayed for the microscopic identification of the mites and the mite allergen level measurement. Mites were microscopically identified with a dust aliquot obtained by sieving through a 1-mm mesh screen in accordance with morphological descriptive guidelines [12]. Fine dust was obtained by sieving through a 0.3-mm mesh screen for the determination of the allergen level. Samples of 100 mg from this fine dust were extracted overnight at 4°C in 2 ml borate-buffered saline, pH 8.0, centrifuged and the supernatants were stored at –20°C prior to performing the immunoassay.

Group 1 *Dermatophagoides* (Der f 1 and Der p 1) and *B. tropicalis* (Blo t 5) allergens were measured by a two-site monoclonal antibody-based ELISA as described by Luczynska et al. [13] with some modifications. Briefly, microtiter plates were separately coated with mouse monoclonal antibodies anti-Der f 1 (clone 6A8), anti-Der p 1 (clone 5H8) and anti-Blo t 5 (clone 4G9) 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 a 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. Plates were washed again, and the correspondent antibodies were added as biotinylated mouse monoclonal anti-group 1 *Dermatophagoides* allergens (clone 4C1) and anti-Blo t 5 (clone 4D9) diluted at 1:1,000. After incubation for 1 h at RT, streptavidin-peroxidase conjugate (Sigma Chemical Co.) diluted at 1:1,000 was added and incubated for 30 min at RT. Enzyme substrate (0.01 *M* ABTS plus 0.03% H_2O_2) was added for assay development, 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 make reference curves in two-fold serial dilutions range from 0.5 to 125 ng/ml for Der f 1, Der p 1, and Blo t 5. Allergen concentrations were expressed as micrograms per gram of dust. The 2 μg of allergen/g of dust for group I allergen was considered as the sensitizing level as previously established [14, 15]. Regarding Blo t 5, there is no consensus about the threshold for sensitization, and the same concentration was used.

Statistical Analysis

Statistical analyses were performed using the software SPSS for Windows and EPI Info. The frequencies of positive SPT results and mite species identified by microscopic examination were expressed

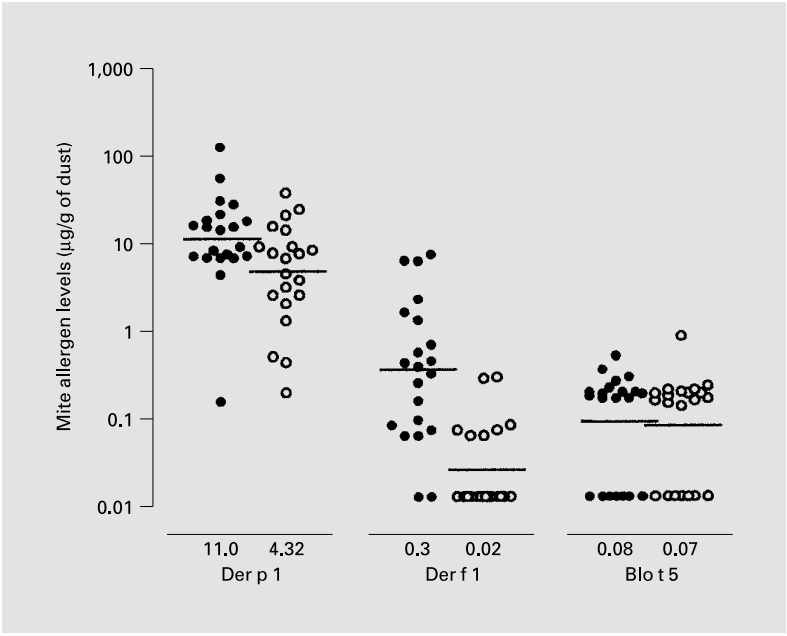

Fig. 1. Levels of mite (Der p 1, Der f 1, Blo t 5) allergens measured in dust samples collected from 21 houses of subjects living in the rural area – group I (●) and 21 houses of subjects living in the favela – group II (○). The figures given above the allergens refer to the geometric mean indicated in the figure with horizontal bars.

as percentages. Mann-Whitney and t tests were used to analyze the differences in the mean levels of HDM between the groups as well the associations between the different variables. Statistical significance was established at the 95% confidence interval.

Results

Table 1 shows the demographic data, the frequencies of positive SPT and the mite species identified through microscopic analysis. Positive SPT were observed in 14 (66.7%) and 12 (51.1%) subjects from groups I and II, respectively ($p > 0.05$). Asthmatic subjects from the rural area did not show statistical differences between the SPT responses to the different HDM tested. However, in subjects from the slum area, there was a lower frequency of *D. farinae* SPT (19.0%) compared to *D. pteronyssinus* (47.6%) and *B. tropicalis* (33.3%; $p < 0.05$). *D. pteronyssinus* was the predominant mite species identified in 76.2% of dust samples from both groups. *Cheyletus* sp. and *B. tropicalis* were identified in approximately 50% of dust samples from groups I and II, while *D. farinae* was identified in 38.0 and 9.5% of the samples of groups I and II, respectively ($p < 0.005$).

Figure 1 shows the distribution of mite allergen (Der p 1, Der f 1 and Blo t 5) levels in house dust samples from subjects living in the rural area (group I) and the slum area (group II). Der p 1 allergen was identified in all dust samples. Der f 1 allergen was detected in 90 and 38% of the

Table 1. Demographic data of asthmatic subjects from two low socioeconomic status groups and comparative data from SPT results with indoor allergens, microscopic identification of mite species and measurements of allergen levels in house dust samples, in Bahia state, Brazil

	Group I (rural area) (n = 21)	Group II (slum) (n = 21)
Age, years		
Median	14	14
Range	7–35	6–25
Sex (M:F)	9:12	11:10
Positive SPT		
Total positive results	14 (66.7)	12 (57.1)
D. pteronyssinus	9 (42.9)	10 (47.6)
*D. farinae**	8 (38.0)	4 (19.0)
B. tropicalis	9 (42.9)	7 (33.3)
Mite species identification		
D. pteronyssinus	16 (76.2)	16 (76.2)
*D. farinae***	8 (38.0)	2 (9.5)
B. tropicalis	11 (52.4)	10 (47.6)
Cheyletus sp.	11 (52.4)	11 (52.4)

Figures in parentheses represent percentage. * $p < 0.05$; ** $p < 0.005$.

dust samples from groups I and II, respectively ($p < 0.05$), and Blo t 5 allergen was detected in 67% of dust samples from both groups.

There was a statistically significant association between the detection of the levels of Der p 1, Der f 1 and Blo t 5 and the respective identification of the mite species in all dust samples studied. There was also a significant association between Der p 1 levels (≥ 2 μg/g) and positive SPT results in subjects from groups I ($p < 0.005$) and II ($p < 0.05$). No associations could be found between Der f 1 or Blo t 5 allergen levels (≥ 2 μg/g) and SPT results.

Discussion

The state of Bahia has subtropical climate conditions and official data from the Brazilian Meteorological Division show that there are no statistically significant differences in the temperature and relative humidity in the two villages studied during the year 2000. 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 [16]. The climate conditions in the areas studied were favorable for mite development. Although mite and allergen levels may vary between sites within a home [16], mattresses and pillows were chosen to collect the dust because the subjects were from a low socioeconomic background with most homes having only two rooms and no carpet. In addition some of them had no tiled floors and very few had a sofa.

In the present investigation, we confirm our preliminary results that *D. pteronyssinus* is the most prevalent mite species in Salvador [7] and now conclude that these results could be extended to the state of Bahia, Brazil. *D. pteronyssinus* is a cosmopolitan mite, identified in dust samples around the world, and related to allergic sensitivity [6–8, 16]. In this study the findings are supported by the documentation of high levels of Der p 1 and positive SPT reactions to *D. pteronyssinus*.

B. tropicalis is another important mite found in Salvador [7] and in this study it is the second most prevalent mite species in the state of Bahia. However, despite the frequent finding of *B. tropicalis* in dust samples and the documentation of positive SPT to this mite allergen in the two studied groups, low levels of Blo t 5 allergen were detected in the dust samples studied. It is possible that Blo t 5 mAb detects the most relevant epitope of *B. tropicalis,* and consequently the sensitizing threshold for Blo t 5 could be lower than for the group 1 mite allergens. Alternatively, the Blo t 5 allergen could be denaturated during the processing of dust samples or antigenic variations in this allergen could be occurring in the *B. tropicalis* species from Brazil.

The frequency of *D. farinae* was quite different in the two studied areas. Considering that the climate conditions are similar in the two places, differences in temperature and humidity do not explain the higher occurrence of *D. farinae* in the inner state. It is important to note that there was a greater prevalence of positive SPT to *D. farinae* allergen in subjects living in the inner state relative to the subjects from the costal area despite a lack of an association between the mite occurrence and the SPT results, probably due to the small sample size.

Although the predator mite *Cheyletus* sp. had been found in a large number of houses, the role of this mite species as a risk factor for the development of respiratory allergy has not been documented. In this study *D. pteronyssinus* was the most important HDM followed by *B. tropicalis* and *D. farinae*. Therefore, subjects living in the state of Bahia who are examined for allergic airway diseases must be skin prick-tested with these three mite antigens. Additionally, our results confirm the importance of assays for the measurements of HDM allergen levels, which were associated with the microscopic identification of these mite species.

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