

Incrimination of *Blomia tropicalis* as a Potent Allergen in House Dust and Its Role in Allergic Asthma in Kolkata Metropolis, India

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Background: The increasing trend in allergic diseases has become obvious in the present day, especially in developing countries like India, because of many factors such as change in ambient air quality, increased air pollution, metamorphic change in living habits and lifestyle, and climate.¹ Mites present in house dust represent a major source of allergens, resulting in different allergic manifestations all over the world, and hypersensitivity to these dust mites may play a pivotal role in pathogenesis of several allergic complaints including bronchial asthma. The present study evaluated the sensitization toward house dust and house dust mites among patients residing in Kolkata metropolis, India, who are suffering from allergic asthma.

Methods: The skin prick test was performed on a total of 1079 patients (585 males and 494 females) between the age group 5–50 years and 50 healthy controls using a variety of 16 common aero-allergenic extracts including 4 allergens of interest, viz. *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, and *Blomia tropicalis* and total house dust allergens. Total serum IgE level was measured by using the EIA technique and specific IgE levels against aforesaid allergens were detected with the Pharmacia ImmunoCAP 100 System. The influence of age and sex, if any, on allergen sensitivity was also investigated. All statistical analyses were performed using SPSS 10.0 for Windows and Zar.²

Results: The responses among patients with asthma to house dust and house dust mite allergen tests were as follows: house dust (96.22%), *D. pteronyssinus* (75.06%), *B. tropicalis* (72%), and *D. farinae* (63.72%). The frequency of positive skin response was found to be independent of age and sex. The total serum IgE levels in patients varied between 7.3 and 4040 IU/ml (mean 369 ± 26.51 IU/ml). Specific IgE antibody test proved that 83% patients showed sensitivity toward at least 1 of the allergens tested.

Discussion: The results indicate that patients are highly sensitive to house dust and 3 other allergenic mites, namely, *D. pteronyssinus*, *D. farinae*, and *B. tropicalis*, as evidenced by the skin prick test, quantification of total serum IgE, and detection of allergen-specific IgE antibodies among patients of Kolkata. Although mites belonging

to the genus *Dermatophagoides* have already been incriminated as a major source of allergen in house dust in India, this is the first time the role of *B. tropicalis* mites causing allergic asthma has been reported from an Indian population. Thus, the importance of *B. tropicalis* mite as an aetiopathological agent in causing various allergic manifestations among the Kolkata population should not be undermined and the allergen should be included in routine allergy testing.

Key Words: allergy, *Dermatophagoides* spp., *Blomia tropicalis*, house dust, skin prick test, IgE

(WAO Journal 2010; 3:182–187)

House dust mites have been known to play an important role in the pathogenesis of bronchial asthma and other atopic diseases.^{1,3,4} Mites are a very diverse and widespread group of animals that can be found in almost all habitats; however, in different parts of the world, they differ greatly in faunal makeup.⁵ During the 1960s, it was established that house dust mites belonging to the family Pyroglyphidae are the source of allergens that play a significant role in different allergic disorders including bronchial asthma.^{5–9} The role of house dust mites belonging to the genus *Dermatophagoides* in asthmatic patients of Kolkata was already established by Saha¹⁰ and Podder et al.¹¹ Surprisingly, there is not a single published work from India incriminating the role of *Blomia tropicalis* mites in causing allergic disorders, though the sensitivity toward this mite species has been proven by several workers from different corners of the world.^{12,13} To provide patients with the best possible diagnosis and treatment, identification of offending allergen(s) is of prime importance. The present article deals with the incrimination of *B. tropicalis*, reported as important allergenic mites from elsewhere around the world, in the etiology of bronchial asthma among the Kolkata population, India.

MATERIALS AND METHODS

Selection of Patients and Control Subjects

A total of 1079 patients clinically diagnosed as suffering from bronchial asthma in the age group 5–50 years were selected from the Outpatient Department of the Allergy & Asthma Research Centre and other private clinics in the Kolkata metropolitan area for this purpose. The personal

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and/or family history of individual patients were recorded in a well-prepared questionnaire including age of onset of symptoms, frequency of attacks, months of worst suffering, type of food intake, exposure to pet animals, influence of dusts, food, and nonspecific stimuli such as cold, exercise, and other irritant factors. Both male and female patients were categorized into 3 age groups ranging as follows: <15 years (Gr. A), 16–40 years (Gr. B), and >40 years (Gr. C). Cases with other organic and systematic diseases such as hypertension, diabetes, and also pregnant and breastfeeding woman were excluded. Selection of patients was done on the basis of the criteria mentioned earlier by Saha.¹⁴ A complete record of the patients' physical and clinical condition was also maintained.

Selection of Control Subject

Fifty healthy people (25 males and 25 females) belonging to the same age group as patients with no personal and/or family history of atopy and without any history of sensitivity toward dust inhalation were selected to serve as control subjects.

Stool examination for 3 consecutive days against individual patients and individual control subjects were done by flotation technique to exclude the possible interference of parasitic infections on the IgE level of patients' sera.

Allergy Skin Test To Assess Immediate Hypersensitivity Response

All patients and control subjects were subjected to conventional skin prick tests against 16 common aeroallergens including 4 distinct allergens, namely, total house dust and 3 species of house dust mite allergens, viz. *D. pteronyssinus*, *D. farinae*, and *B. tropicalis*. The mites were selected on the basis of their predominance in house dust of Kolkata metropolis.

Allergy skin prick testing was carried out by a conventional method of Gislason and Gislason¹⁵ with the help of standard allergen extracts and working protocol provided by Credisol India Limited. The test was performed on the flexor side of the forearm of each patient following the method of Saha.¹⁴ Results were taken 20 minutes after the skin test and were interpreted following the criteria suggested by Grater et al.¹⁶ All antiallergic and steroid drugs were withdrawn from each patient 72 hours before the skin testing. Allergens giving 1⁺ to 4⁺ reactions were considered clinically significant.

Quantification of Total IgE Level

Measurement of total serum IgE was carried out by employing Enzyme Immuno Assay (EIA) using chemicals: Pathozyne Immunoglobulin (Ref: OD 417) supplied by Glaxo SmithKline Pharmaceuticals Ltd., Mumbai, India. The concentration of IgE is directly proportional to the color intensity of the test and the assay was calibrated against the World Health Organization¹⁷ standard for IgE.

Detection of Allergen-Specific IgE Antibodies

Detection of allergen-specific IgE antibodies in patients' sera against house dust (HD), *D. pteronyssinus* (DP), *D. farinae* (DF), and *B. tropicalis* (BT) was carried out by using the Pharmacia ImmunoCAP 100 System. The CAP was

performed in collaboration with Pharmacia-authorized Metropolitan Laboratory, Mumbai, India. The assay was calibrated against the World Health Organization Standard for IgE with a range of 0.35–100KU/L for specific IgE. Specific IgE was measured against the house dust and 3 house dust mites (*D. pteronyssinus*, *D. farinae*, and *B. tropicalis*).

Statistical Analysis

Paired Student *t* test with equal and unequal variances, Z-test, and contingent χ^2 test were used to compare the allergic response toward different allergens among different age groups of patients and between sexes. Statistical analysis was performed using SPSS 10.0 for Windows and Zar.²

RESULTS

Incidence of Allergic Response to House Dust and House Dust Mites (*D. pteronyssinus*, *D. farinae*, and *B. tropicalis*)

Results of skin tests against 16 aeroallergens are presented in Table 1. Because the primary objective for the present article is to establish the role of house dust and common house dust mites, results in that respect were analyzed separately and data in great detail is presented here. A total of 1079 patients comprised of 585 males and 494 females with a definite history of allergic asthma and 50 normal individuals serving as control subjects were selected on the basis of criteria mentioned earlier.

Results of skin tests against total house dust and selected house dust mites (DP, DF, and BT) on 1079 patients revealed that 1035 (96.22%) patients showed sensitivity toward house dust. Because total house dust is a complex

TABLE 1. Allergic Response to Common Inhalants (Pollens, Molds, and Others Including House Dust and Three Mites) in Bronchial Asthmatic Patients of Kolkata (n = 1079)

Allergens	No. Positive Patients	Percentage of Positive Patients
Pollens		
<i>Cocos nucifera</i>	783	72.58
<i>Brassica nigra</i>	598	55.42
<i>Delonix</i> sp.	522	48.38
<i>Azadirachta indica</i>	467	43.25
<i>Caesalpinia</i> sp.	431	40.02
Molds		
<i>Aspergillus fumigatus</i>	240	22.28
<i>Aspergillus niger</i>	198	18.32
<i>Candida albicans</i>	166	15.39
<i>Cladosporium</i> sp.	125	11.58
<i>Alternaria alternate</i>	44	4.1
Others		
Dog dander	99	9.23
Cat dander	61	5.71
House dust	1035	96.22
<i>Dermatophagoides pteronyssinus</i>	809	75.06
<i>Dermatophagoides farinae</i>	688	63.72
<i>Blomia tropicalis</i>	778	72.00

TABLE 2. Intensity of Skin Reaction Toward House Dust and Mites Among Asthmatic Patients of Kolkata

Allergen Used	No. (%) Patients With Different Degrees of Skin Test Intensity			
	1+	2+	3+	4+
HD	356 (34.39%)	315 (30.43%)	251 (24.25%)	113 (10.91%)
DP	240 (29.70%)	265 (32.71%)	197 (24.32%)	107 (13.01%)
DF	177 (25.76%)	223 (32.45%)	175 (25.47%)	113 (16.56%)
BT	179 (23.03%)	226 (29.08%)	229 (29.16%)	144 (18.53%)

TABLE 3. Total Serum IgE Level in Patients and Control Subjects Residing in Kolkata

Study Group	Range of IgE IU/ml	Mean IgE IU/ml	Student <i>t</i> Test	Remark
Patients (n = 337)	7.3–4040	369 ± 26.51	1.499	<i>P</i> < 0.05
Control (n = 50)	15–120	56 ± 34		

heterogeneous mixture of substances of both plant and animal origin, a high degree of sensitivity toward house dust is not uncommon.¹⁴ In the case of pyroglyphid mites the sensitivity was highest toward *D. pteronyssinus* (75.06%) followed by *D. farinae* (63.72%). Interestingly, the other mite *B. tropicalis* is responsible for causing allergies in 72% of the Kolkata population (Table 1).

The intensity of skin reaction against 4 allergens (house dust and 3 species of mites) tested varied between 1⁺ and 4⁺, the details of which are depicted in Table 2.

Total Serum IgE Levels Among Patients and Control Subjects

For this purpose, of 1079 primary selected patients, 337 patients were selected finally on the basis of high positive reaction to skin prick test against 4 allergens, namely, house dust, *D. pteronyssinus*, *D. farinae*, and *B. tropicalis* mite species. The total serum IgE levels in 337 asthmatic patients and 50 control subjects of this study group varied from 7.3 to 4040 IU/ml (mean 368.67 ± 26.51) and 15–120 IU/ml (mean 56 ± 34), respectively (Table 3). Paired sample Student *t* test was done to investigate the differences, if any, in mean IgE value between the patients' sera and control sera and the result showed that the difference between the 2 mean values was statistically significant (*P* < 0.05). Only 6.5% of the patients in our study group had serum IgE levels within the normal limits, whereas the remaining 93.5% patients showed elevated serum IgE levels (more than 300 IU/ml).

Comparison of Total Serum IgE Levels Among Different Age Groups

For this study the patients and control subjects were categorized into 3 age groups as mentioned earlier. The results revealed that the lower and upper limits of total serum IgE levels in 55 (16.32%) patients <15 years were 7.3 and 1799 IU/ml, respectively, and the mean value was 415.47 ± 64.39 IU/ml. In the next age group, that is, 16–40 years (n =

TABLE 4. Total Serum IgE Levels in Different Age Groups of Patients

Age of Patients	No. Patients	Range of IgE (IU/ml)	Mean IgE (IU/ml)
<15 years	55 (16.32%)	7.3–1799	415.47 ± 64.39
16–40 years	92 (27.29%)	36–4040	424.47 ± 65.88
>40 years	190 (56.37%)	26–2538	315.64 ± 35.44

TABLE 5. Statistical Analysis on Total Serum IgE Levels in Different Age Groups of Patients

Age Group	Student <i>t</i> test	df	Remark
Gr. A vs. Gr. B	0.278	138	<i>P</i> > 0.05
Gr. A vs. Gr. C	1.0087	78	<i>P</i> > 0.05
Gr. B vs. Gr. C	1.334	127	<i>P</i> > 0.05

df, degree of freedom.

92, 27.20%), the total serum IgE levels varied between 36 and 4040 IU/ml (mean 424.47 ± 65.88 IU/ml). Serum IgE levels of 190 (56.37%) patients belonging to the >40 years age group ranged between 26 and 2538 IU/ml (mean 315.64 ± 35.44 IU/ml) as shown in Table 4. Statistical analysis (Student *t* test with unequal variance) revealed that the mean IgE values of patients in the different age groups were not statistically significant (Table 5), although the mean IgE value in the middle age group, that is, Gr. B, was apparently higher in comparison to the other two groups of patients.

Comparison of Total Serum IgE Levels Among Two Sexes

Appropriate data in this respect were analyzed and the results revealed that the total serum IgE levels in 175 (51.92%) male patients ranged from 7.3 to 4040 IU/ml (mean 383.74 ± 38.15 IU/ml). In the case of female patients, the corresponding value was 23–2953 IU/ml (mean 356 ± 36.73 IU/ml). Statistical analysis (Student *t* test with unequal variance) was performed to find out if the variation exists, if any, in mean total serum IgE levels between two sexes, which revealed that the difference was not statistically significant (*P* > 0.05), as depicted in Table 6.

Detection of Allergen-Specific IgE Antibodies

For detection of specific IgE antibodies against 4 allergens, namely, house dust, *D. pteronyssinus*, *D. farinae*, and

TABLE 6. Variation in Total Serum IgE Levels in Relation to Sex of Patients

Sex	Range of IgE (IU/ml)	Mean IgE (IU/ml)	Student <i>t</i> test	Remarks
Male n = 175 (51.92%)	7.3–4040	383.74 ± 38.15	0.3854 (df = 335)	<i>P</i> > 0.05
Female n = 162 (48.07%)	23–2953	356.07 ± 36.73		

TABLE 7. Allergen-Specific IgE Reactivity Against HD, DP, DF, and BT Allergens by ImmunoCAP 100 System

Allergens	No. and Percentage Sensitivity
HD	71 (79.77%)
DP	64 (71.91%)
DF	79 (88.76%)
BT	94 (90%)

TABLE 8. Frequency of Positive Pharmacia ImmunoCAP Results Against HD, DP, DF, and BT in Patients of Different Age Groups

Age	No. Patients	Total Tests	No. Positive Tests	χ^2	Remark
Gr. A	16	64	36 (56.25%)	3.7967 (df = 2)	$P > 0.05$
Gr. B	66	264	187 (70.71%)		
Gr. C	23	92	64 (69.60%)		

B. tropicalis mites, 105 patients were selected finally, who showed higher total IgE values and maximum reactivity towards the allergens of interest by a skin prick test. Analysis of Pharmacia ImmunoCAP results against the 4 allergens of interest revealed that of 105 serum samples tested, 89 (84.76%) showed the presence of allergen-specific IgE antibodies against at least 1 of the 4 allergens tested, whereas 16 (15.23%) samples did not show any positive response against any of the 4 selected allergens. Among the positive samples, 71 (79.77%) had specific IgE antibodies against HD, 64 (71.91%) against DP, 79 (88.76%) against DF, and 94 (90%) against BT allergen (Table 7).

Pharmacia ImmunoCAP Sensitivity in Relation to Patients' Age

Analysis of ImmunoCAP results against HD, DP, DF, and BT allergens in relation to age of patients revealed that of 64 tests performed on 16 patients in the age group <15 years, 36 (56.25%) showed positive ImmunoCAP response and of the 264 tests performed on 66 patients in the age group 16–40 years, 187 (70.71%) were ImmunoCAP positive, whereas in the remaining 23 patients in the age group >40 years, 92 tests were done, of which 64 (69.56%) showed positive response (Table 8).

Statistical analysis (2×2 contingent χ^2 test) indicated that the frequency of positive ImmunoCAP response to different allergens tested were not age dependent ($P > 0.05$), though the frequency of ImmunoCAP sensitivity in subjects aged 21 years and older was apparently higher.

ImmunoCAP Response in Relation to Sex of Patients

Analysis of ImmunoCAP 100 results in relation to 2 sexes (Z test) revealed that the frequency of total positive responses to at least 1 of the 4 allergens tested and against each of the individual allergens did not differ significantly between the two sexes (Table 9).

TABLE 9. Prevalence of ImmunoCAP Sensitivity Against HD, DP, DF, and BT Allergens in Two Sexes

Allergens	No. and Percentage of Positive Patients			Z	Remarks
	Male	Female			
HD	47 (81.03%)	23 (74.19%)	1.285	$P > 0.05$	
DP	41 (70.68%)	24 (77.41%)	0.0602	$P > 0.05$	
DF	53 (91.37%)	28 (90.32%)	1.003	$P > 0.05$	
BT	59 (89.39%)	35 (89.74%)	1.007	$P > 0.05$	
At least 1	58 (87.87%)	31 (79.48%)	1.156	$P > 0.05$	

DISCUSSION

A gradual increase in the incidence of bronchial asthma among the population of Kolkata during the last few years tempted us to identify the etiologic agents responsible for allergic complaints. The pilot screening test against 16 allergens of interest clearly showed that positive response to house dust and house dust mites were higher than the rest of the allergens tested. The skin prick test results against 4 selected aeroallergens of interest account for about 76% of allergic asthma among the Kolkata population. Skin test results on 1079 patients against HD, DP, DF, and BT showed that 96.22% patients were sensitive to HD, 75.06% to DP, 63.72% to DF, and 72% to BT allergens. These results confirm the earlier observation of Saha¹⁴ who also reported the high degree of reactivity toward house dust and *Dermatophagoides* mites among patients with asthma from Kolkata. The present study also agrees quite well with other investigators¹⁸ who also established the correlation between allergy and positive skin reaction. It seems that the frequency of allergic responses to different inhalants is the same in different age groups and for both sexes. However, these findings disagreed with Hannaway et al¹⁹ who came to the conclusion that the rate of skin reactions was highly age dependent and peak reactivity occurred during adulthood with a sharp decline in the older age group. Murray et al²⁰ interpreted it as the natural decline in reactivity that occurs in older patients that may be attributed to either an actual decrease in immunologic reactivity or a decrease in the capacity of the skin to respond to immunologic challenge.

IgE was considered as the prime carrier of reaginic hypersensitivity in humans.²¹ According to Johansson²² and Gleich et al,²³ the total serum IgE level is high in bronchial asthmatic patients. A wide range of serum IgE levels (7.3–4040 IU/ml) had been observed in this study group and difference of mean serum IgE levels between patients and control subjects was observed to be statistically significant in the present study. According to Ganju et al,²⁴ the basis of variations in IgE levels was polygenic with a possible effect of environmental and socioeconomic factors.

In the present study group it is to be noted that patients between the age of 16 and 40 years showed higher mean IgE levels in comparison to the other two groups, though the difference was not statistically significant. These findings are in conformity with the findings of Johansson²² and Zetter-

strom and Johansson,²⁵ who also reported that the serum IgE level did not depend on age. However, Khatua et al²⁶ suggested that the age of patients has a significant effect on the increase of total serum IgE level. However, the difference in mean serum IgE levels in male and female patients is not significant. This finding agrees with Zetterstrom and Johansson²⁵ and Batabyal et al.²⁷ However, the present findings disagree with those of Wood and Oliver,²⁸ Sharma et al,²⁹ and Barbee et al³⁰ who had the opinion that total serum IgE levels were influenced by sex and age. In conclusion, it can be mentioned that the increase of total serum IgE levels is neither age nor sex dependent.

Although skin testing is an excellent screening test to evaluate immediate hypersensitivity reaction, allergen-specific IgE antibodies test is definite proof of IgE-mediated diseases.²⁵ Hence, the determination of specific IgE provides important information regarding the therapy to be adopted.

Analysis of allergen-specific IgE antibodies against house dust, *D. pteronyssinus*, *D. farinae*, and *B. tropicalis* results in relation to patients' age and sex revealed that the frequency of sensitivity toward 4 selected allergens is neither age nor sex dependent ($P > 0.05$). This finding correlated well with the observation made by Chew et al,¹³ Kerkhof et al,³¹ and Simpson et al.¹⁸ The present findings revealed that about 76% of the patients in Kolkata are sensitive to house dust and 3 mite allergens tested. Among these positive patients, 93.5% have elevated serum IgE levels. This proves the atopic status of those patients. In the present study allergen-specific IgE antibodies were detected in the patients as follows: 79.77% to HD, 71.91% to DP, 88.76% to DF, and 90% to BT allergens. In the case of skin response 96.22% of the patients were sensitive to HD, 75.06% to DP, 63.72% to DF, and 72% to BT allergens. This difference in sensitivity against 4 allergens of interest by 2 diagnostic methods may be due to the appearance of false-positive or false-negative results by skin prick test whereas demonstration of specific IgE antibodies to a defined allergen is a definite proof of IgE-mediated disease.³¹

Thus, the present study established the role of 3 allergenic mites, namely, DP, DF, and BT, in house dust allergy as evidenced by the skin prick test, total serum IgE, and detection of allergen-specific IgE antibodies among patients of Kolkata. This is the first time the role of *B. tropicalis* mite causing various allergic diseases including bronchial asthma has been reported from the Indian population. The study suggests that, in areas where *B. tropicalis* forms part of acarofauna, sensitization to this mite is an independent risk factor for asthma and as a matter of fact the role of *B. tropicalis* in causing bronchial disorders cannot be ruled out or underestimated. In conclusion, it can be said that sensitization to pyroglyphid and nonpyroglyphid mites is evident in the Kolkata population. These findings indicated that in vivo and/or in vitro testing for sensitization to these 3 species of mites should be considered in routine allergological diagnosis procedures.

ACKNOWLEDGEMENTS

The authors are grateful to the University Grants Commission, New Delhi, India, for providing financial assistance (sanction no. F3-132/2003/SR). Thanks are also due to H. Biswas and I. Roy for their technical assistance.

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