

Association between indoor fungi in Delhi homes and sensitization in children with respiratory allergy

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Indoor fungi are potential sensitizing agents in children and their detection and quantification in indoor air are important in the diagnosis and environmental management of fungal allergies. The objective of this investigation was to assess the prevalence of fungal allergies in children in Delhi and to study the association between mold counts in the homes of children and their sensitization to respective fungal extracts. Fungal concentrations and seasonality were studied at two-week intervals for one year using Andersen Volumetric and Burkard Slide samplers. Sensitization to fungi frequently encountered in patients' homes was assessed by Skin Prick Tests (SPTs). Total fungal specific IgE was measured by ELISA in the sera of patients positive to fungal extracts. Skin Prick Tests revealed that 39.3% (33/84) of patients were markedly positive (2 + and above) to one or the other fungal allergens. Raised serum IgE to predominant indoor fungal species was observed in patients with marked SPT results. Highest marked skin reactivity (2 + and above) was obtained with *Alternaria alternata* allergens in 17.9% of the children, which was followed by the response to fungal antigens of *Aspergillus fumigatus* and *Penicillium citrinum* (15.5%). Exposure to high fungal counts of some dominant fungi (*Penicillium*, *A. nidulans* and *A. fumigatus*) was found associated with increased fungal sensitization in the patients. Total serum IgE level was revealed to be significantly linked with the intensity of skin reactions, as well as with skin index ($r^2 = 0.052$; $P < 0.05$). We concluded that children in Delhi are exposed to high concentrations of fungi in the indoor environment and that respiratory allergies were connected with higher prevalence of skin sensitization.

Keywords allergy, asthma, indoor fungi, allergens, Delhi, India, sensitization, Immunoglobulin E

Introduction

The incidence of allergic diseases has been on the rise in recent decades all over the world. Over a 20-year period, the global prevalence of allergies has grown from 10% to more than 30% due to rapid urbanization and industrialization [1,2]. Approximately 300 million people worldwide suffer from asthma with estimates suggesting that the prevalence will increase globally by 50% every decade [3]. In India, the occurrence of respiratory allergies across urban

regions has been observed to be from 1.9–15.7% of those inhabiting these areas [4,5]. The important factors that might be responsible for the increased incidence are exposure to environmental pollutants and Westernized lifestyle associated with increased urbanization [6,7].

Indoor exposure to allergens is important in the development of allergy and asthma as most people spend more than 90% of their time indoors, more than half of which is spent at home. Fungal spores are one of the most common sources of indoor allergens, in addition to house dust mite, cockroaches, and pets [8]. Allergic reactions that are linked with fungi include asthma, allergic rhinitis, hypersensitivity pneumonitis, allergic bronchopulmonary aspergillosis, and atopic eczema [9]. Most of the inhaled fungal spores ($> 10 \mu\text{m}$ in diameter) are deposited in nasopharynx, but spores of $\leq 5 \mu\text{m}$ in size penetrate the

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lower airways where allergic reactions tend to manifest as allergic asthma [10].

The prevalence of respiratory allergy to fungi is estimated to be 20–30% among atopic individuals and up to 6% in the general population [11]. Children spend most of their time indoors and those living in damp mud houses are prone to suffer more from respiratory allergy symptoms. Since fungi are one of the important components of the environment in dwellings, a study of fungal sensitization of local atopic children in Delhi was considered to be needed. A number of investigations regarding sensitization to fungi in allergic children have been reported [12,13], but investigations of the association of high concentrations of fungi in Delhi homes and sensitization of children to fungi has been lacking. Therefore, we have assessed the spectrum and concentration of airborne fungal flora inside and in the immediate vicinity of the homes of allergic children in Delhi [14]. Sensitization of children to these indoor fungi was assessed by clinico-immunologic studies using the Skin Prick Test (SPT) and the demonstration of fungal-specific IgE in patients' sera. The association of fungal concentration in the homes of children and skin sensitization to the respective fungi has been critically analyzed as potential risk factors.

Material and methods

Subjects

Children having respiratory symptoms ($n = 84$) in the age group of 3–16 years reporting at Vallabhbhai Patel Chest Institute, Delhi, were recruited for the present study. A standard questionnaire validated previously by Singh *et al.* [15] was used to record the medical histories of the patients and their housing conditions. The questionnaires were administered to those patients who had been clinically diagnosed as having bronchial asthma (BA), allergic rhinitis (AR) or BA with AR. Clinical history regarding respiratory dysfunction (breathlessness, cough, wheezing, etc.), family history, personal history, time and duration of the symptoms, length of stay in the house were all carefully recorded under the supervision of clinicians.

Characteristics of atopic children

A total of 84 children were skin tested with the panel of fungal antigens along with common inhalant pollen and house dust mite (HDM) allergens (Table 1). The average age of the patients was 11.3 years, with the patient population composed of 47/84 (55.95%) males and 37/84 (45.05%) females. The majority of patients suffered from bronchial asthma with allergic rhinitis (54/84), followed by bronchial asthma alone (22/84) and allergic rhinitis alone

Table 1 Characteristics of the study subjects.

Total number of subjects	84
Age, mean $\pm$ SD {range} yrs	11.3 $\pm$ 3.29 (3–16)
Age groups, no. (%), mean $\pm$ SD yrs	
3–7	14 (16.7%), 6.0 $\pm$ 0.78
8–12	36 (42.9%), 10.4 $\pm$ 1.42
13–17	34 (40.5%), 14.5 $\pm$ 1.33
Gender, no. (%)	
M	47 (55.95%)
F	37 (44.05%)
Diagnosis, no. (%)	
BA	22 (26.2%)
BA + AR	54 (64.3%)
AR	08 (9.5%)
Parental smoking, no. (%)	26 (32.1%)
Family history of asthma, no. (%)	41 (51.6%)
Family history of allergy, no. (%)	08 (9.9%)
Cough, no. (%)	
Seasonal (S)	43 (52.4%)
Perennial (P)	32 (39.0%)
Intermittent (I)	07 (8.5%)
Breathlessness, no. (%)	
Seasonal (S)	30 (38.9%)
Perennial (P)	30 (38.9%)
Intermittent (I)	17 (22.1%)
Rhinitis, no. (%)	
Seasonal (S)	26 (37.7%)
Perennial (P)	20 (28.9%)
Intermittent (I)	23 (33.3%)
Asthma symptoms, no. (%)	
Daytime	27 (32.1%)
Nighttime	38 (45.2%)
Daytime or nighttime	19 (22.6%)
Increased respiratory symptoms, no. (%)	
Summer	24 (30.0%)
Rain	11 (13.8%)
Autumn	51 (63.8%)
Winter	45 (56.3%)

(8/84). In 32.1% (26/84) of the homes parental smoking was observed. Family history of asthma/allergy was present in as high as 58.3% of the children. Most individuals were found to have seasonal symptoms of cough (52.4%) and 38.9% (30/84) of patients showed seasonal and perennial symptoms of breathlessness. Nighttime symptoms (45.2%) of breathlessness were more common as compared to daytime or daytime/nighttime. The highest pulmonary complaints were received in the autumn (63.8%), followed by winter (56.3%) seasons, i.e., from September to February.

Indoor fungal survey

The fungal surveys in homes of symptomatic/control children were carried out with the help of the Andersen Volumetric Sampler for colony counts, while the Burkard Slide Sampler was used for spore counts at two-week intervals as per details previously published [14].

Fungal extract

Dominant fungal strains encountered indoors that affected children were selected and cultured. Fungal antigens were extracted as per standard protocol in 1:20 (w/v) ammonium bicarbonate buffer (50 mM, pH 8.1) with 5 mM EDTA (ethylene diamine tetra acetate) and 1 mM PMSF (phenyl methyl sulfonyl fluoride). Besides fungi, pollen and house dust mites (*Dermatophagoides farinae*), extracts were procured from Drug Controller approved antigen manufacturing unit of Institute of Genomics Integrative Biology, Delhi, India.

Skin Prick Tests

Skin Prick Tests (SPTs) with antigenic extracts from fungal, common pollen and dust mites (*D. farinae*) allergens (Table 2) were carried out on the 84 children with respiratory symptoms. The patients were advised not to take antihistamines (for 48 h) and other sympathomimetic drugs (for 8–12 h) before the skin tests. A positive control of histamine hydrochloride (1 mg/ml; Sigma, USA) and a negative control of 50% glycerinated buffer saline were used. The wheal and flare reactions were measured 15–20 min after performing the tests and graded according to the criteria outlined by Singh *et al.* [17]. A wheal diameter ≥ 3 mm was considered 1+, while ≥ 5 mm (or a positive control equivalent) were considered strongly positive ($\geq 2+$).

Estimation of total serum IgE antibody

Total serum IgE which is considered as a mark of atopy was estimated in the sera of children by using the kit procured from Bethyl Laboratories (USA). The total IgE values were expressed in IU/ml (1 IU/ml = 2.4 ng/ml) [18].

Table 2 The list of dominant fungal, common inhalant pollen and mite allergens selected for Skin Prick Test on the patients.

S. no.	Fungal antigens	S. no.	Pollen antigens
1	<i>Aspergillus niger</i>	1	<i>Artemisia scoparia</i>
2	<i>Aspergillus flavus</i>	2	<i>Amaranthus spinosus</i>
3	<i>Aspergillus fumigatus</i>	3	<i>Cannabis sativa</i>
4	<i>Aspergillus nidulans</i>	4	<i>Chenopodium album</i>
5	<i>Alternaria alternata</i>	5	<i>Cynodon dactylon</i>
6	<i>Penicillium citrinum</i>	6	<i>Holoptelia integrifolia</i>
7	<i>Cladosporium</i> <i>cladosporoides</i>	7	<i>Imperata cylindrica</i>
8	<i>Trichoderma lignorum</i>	8	<i>Morus alba</i>
9	<i>Mucor mucedo</i>	9	<i>Prosopis juliflora</i>
		10	<i>Xanthium strumarium</i>
	Mite		
1	<i>Dermatophagoides</i> <i>farinae</i>		

Estimation of fungal-specific IgE antibodies

Specific IgE antibodies against fungal antigens were estimated in the sera of children showing SPT positive (2+ and above) reactions. In short, polystyrene, 96-well microtiter plates (Nunc AS Roskilde, Denmark) were coated with fungal extracts (20 µg/well), incubated overnight [O/N] at 4°C then blocked with 1% BSA, after which, 100 µl of sera samples (diluted 1:10) were added and the plates again incubated O/N at 4°C. After washing, secondary antibody (HRP-labeled antihuman IgE antibody diluted to 1:1000) was added and then developed with enzyme substrate *o*-phenylenediamine dihydrochloride (OPD). The reaction was terminated with 5N H₂SO₄ and optical densities (OD) measured at 490 nm with Immuno-Reader (Molecular Devices, USA). In addition to patient sera, the sera from normal healthy volunteers were also assayed for different fungal extracts. The results were expressed in percentage binding according to the criteria of Kauffmann *et al.* [19]. Intensity of reaction was classified into four different classes, based on percentage binding (Class I = 0–15%; Class II = 15–30%; Class III = 30–60%; Class IV = > 60%). The formula used to calculate percentage binding is outlined below:

$$\frac{A_0 - A_y}{A_x - A_y} \times 100$$

where A_x = Mean of maximum titre obtained with 10 highly positive sera; A_y = Titre obtained with normal sera; A_0 = Titres of individual sera.

Statistical analysis

The study subjects were classified into three categories, i.e., patients exposed to <25th percentile (low count), 25th–75th percentile (intermediate count) and >75th percentile (high count) of fungal concentrations in their homes. Percentage of children with positive (1+ to 3+, as well as 2+ and above) skin reactivity with respective fungi in these categories of patients was compared. Linear regression analysis was carried out to study the association of total serum IgE with skin index, as well as intensity of skin reactions. All the statistical analyses were performed with MS EXCEL Prism II software (Graph Pad Prism, San Diego, CA, USA). The significance level was considered to be $P < 0.05$.

Results

A total of 3,146 air samples were collected from bedrooms and outdoor air of homes of symptomatic children, whereas, 1,277 samples were collected from living/other rooms (in cases in which the occupant was living in single room, only

Table 3 Indoor and immediate outdoor annual concentration and percent contribution of dominant fungal colonies (in CFU/m³) in patients' homes.

S. no.	Genera/species	Annual % contribution	Annual % contribution
		Indoor	Outdoor
1	<i>Alternaria</i> spp.	13.1	15.0
2	<i>Aspergillus flavus</i>	6.6	7.0
	<i>A. fumigatus</i>	1.1	0.9
	<i>A. nidulans</i>	1.7	1.7
	<i>A. niger</i>	12.4	13.8
	<i>A. versicolor</i>	10.3	8.8
	<i>Aspergillus</i> spp.	11.0	8.2
3	<i>Cladosporium</i> spp.	25.7	24.1
4	<i>Curvularia</i> spp.	1.9	2.1
5	<i>Drechslera</i> sp.	0.5	1.3
6	<i>Fusarium</i> spp.	1.6	2.6
7	<i>Mucor</i> sp.	0.3	0.3
8	<i>Penicillium</i> spp.	6.8	6.8
9	<i>Rhizopus</i> sp.	1.6	1.5
10	Others	5.4	5.9

one indoor sample was collected and was considered as a bedroom).

Seasonal variations in fungal concentrations in homes of atopic children

Species of *Aspergillus*, *Cladosporium*, *Alternaria* and *Penicillium* were the most commonly encountered fungi and represented, 88.6% and 86.3% of total CFUs in indoor and outdoor environments, respectively (Table 3). The highest average annual concentrations inside the homes were recorded for the genus *Aspergillus* (43.1%), followed by *Cladosporium* spp., *Alternaria* spp. and *Penicillium* spp. (Table 3). The highest concentrations were recorded in the month of January (12,484 CFU/m³), followed by

Table 4 Skin positivity pattern to fungal allergens tested on patients (*n* = 84).

S. No.	Name of antigen	Skin reactivity			
		1 + to 3 +		2 + and 3 +	
		<i>n</i>	%	<i>n</i>	%
1	<i>Aspergillus niger</i>	15	17.9	3	3.6
2	<i>A. flavus</i>	18	21.4	8	9.5
3	<i>A. fumigatus</i>	35	41.7	13	15.5
4	<i>A. nidulans</i>	23	33.3	7	10.1
5	<i>Alternaria alternata</i>	26	31.0	15	17.9
6	<i>Cladosporium cladosporioides</i>	28	33.3	11	13.1
7	<i>Penicillium citrinum</i>	40	47.6	13	15.5
8	<i>Mucor mucedo</i>	23	27.4	7	8.3
9	<i>Trichoderma viride</i>	18	21.4	7	8.3

Table 5 Average monthly concentration (CFU/m³) of various fungal allergens (tested on the children) in the homes of patients with high, intermediate and low colony count.

	High count (CFU/m ³)	Intermediate count (CFU/m ³)	Low count (CFU/m ³)	<i>P</i> value
<i>Aspergillus niger</i> (<i>n</i>)	20	38	19	
Mean ± SD	21120 ± 332	13110 ± 1811	6641 ± 2083	
95% CI	22680–19570	13710–12520	7645–5637	< 0.001
<i>A. flavus</i> (<i>n</i>)	20	38	19	
Mean ± SD	11490 ± 3174	6703 ± 1117	3714 ± 1012	
95% CI	12980–10010	7071–6336	4242–3266	< 0.001
<i>A. fumigatus</i> (<i>n</i>)	20	38	19	
Mean ± SD	3232 ± 3232	576 ± 208	162 ± 89	
95% CI	4848–1615	645–506	205–119	< 0.001
<i>A. nidulans</i> (<i>n</i>)	20	39	18	
Mean ± SD	3372 ± 855	1675 ± 401	681 ± 331	
95% CI	3772–2971	1805–1544	846–516	< 0.001
<i>Alternaria</i> (<i>n</i>)	20	38	19	
Mean ± SD	26800 ± 5350	12600 ± 3698	5044 ± 1553	
95% CI	29300–24300	13820–11380	5793–4296	< 0.001
<i>Cladosporium</i> (<i>n</i>)	20	38	19	
Mean ± SD	51170 ± 19940	25280 ± 6424	10290 ± 3540	
95% CI	60500–41840	27400–23170	12000–8586	< 0.001
<i>Penicillium</i> (<i>n</i>)	20	38	19	
Mean ± SD	13890 ± 3526	6428 ± 1476	2969 ± 737	
95% CI	15540–12240	6913–5942	3324–2614	< 0.001
<i>Mucor</i> (<i>n</i>)	20	38	19	
Mean ± SD	11440 ± 2739	2770 ± 1731	204 ± 83	
95% CI	12730–10060	3339–2201	182–0	< 0.001

High count = Fungal concentration above 75th percentile; Intermediate count = Fungal concentration between 25th and 75th percentile; Low count = Fungal concentration below 25th percentile; *n* = number of patients.

April (12,323 CFU/m³) and February (12,070 CFU/m³), while the lowest concentrations were noted in the month of June (4,262 CFU/m³) when temperatures in Delhi are high.

Almost 58% of the cases had severe asthmatic complaints in winter months (December to January) when high mold concentrations were observed. The trend of worsening respiratory symptoms was observed to be in parallel with the seasonal distribution of total fungi.

Skin Prick Test

Of the 84 patients tested, 39.3% (33/84) showed marked positive (2+ and above) reactions to one or the other

fungal allergen tested, but 75% (63/84) had mild to strong positive skin reactions. *Penicillium citrinum* elicited 1 + to 3 + skin test responses in 47.6% of the patients, followed by *Aspergillus fumigatus*, *A. nidulans* and *Cladosporium cladosporioides*. However, marked skin reactivity (2 + and above) was obtained in 17.9% of the children with *Alternaria alternata*, followed by fungal antigens of *A. fumigatus* and *P. citrinum* (15.5%). The least marked skin reactivity was recorded with *A. niger* extracts and was found in only 3.6% of the children (Table 4).

Among pollen allergens selected for skin testing (including two grasses, five weeds and three trees predominant in Delhi region), the highest marked skin positivity (2 + and above) was observed with the grass pollen, *Cynodon dactylon* in 33.3% patients, followed by *Holoptelia integrifolia*

(28.6%), *Chenopodium album* (23.8%), *Imperata cylindrica* (20.2%), *Amaranthus spinosus* (17.9%), *Prosopis juliflora* (16.7%), *Artemisia scoparia* (15.5%), *Cannabis sativa* (10.7%) and *Morus alba* (10.7%). Markedly positive skin responses to *D. farinae* (a common HDM in Indian homes) was recorded in 17.9% of the patients.

Association between indoor fungal count allergic sensitization to fungi

As discussed in the Methods, patients were categorized according to high, intermediate or low fungal count in their homes (Table 5). The differences in the mean fungal counts of all three groups were statistically significant ($P < 0.001$). Homes with high colony counts of *Aspergillus flavus*, *A. nidulans*, *Alternaria* spp. and *Penicillium* spp. had a higher

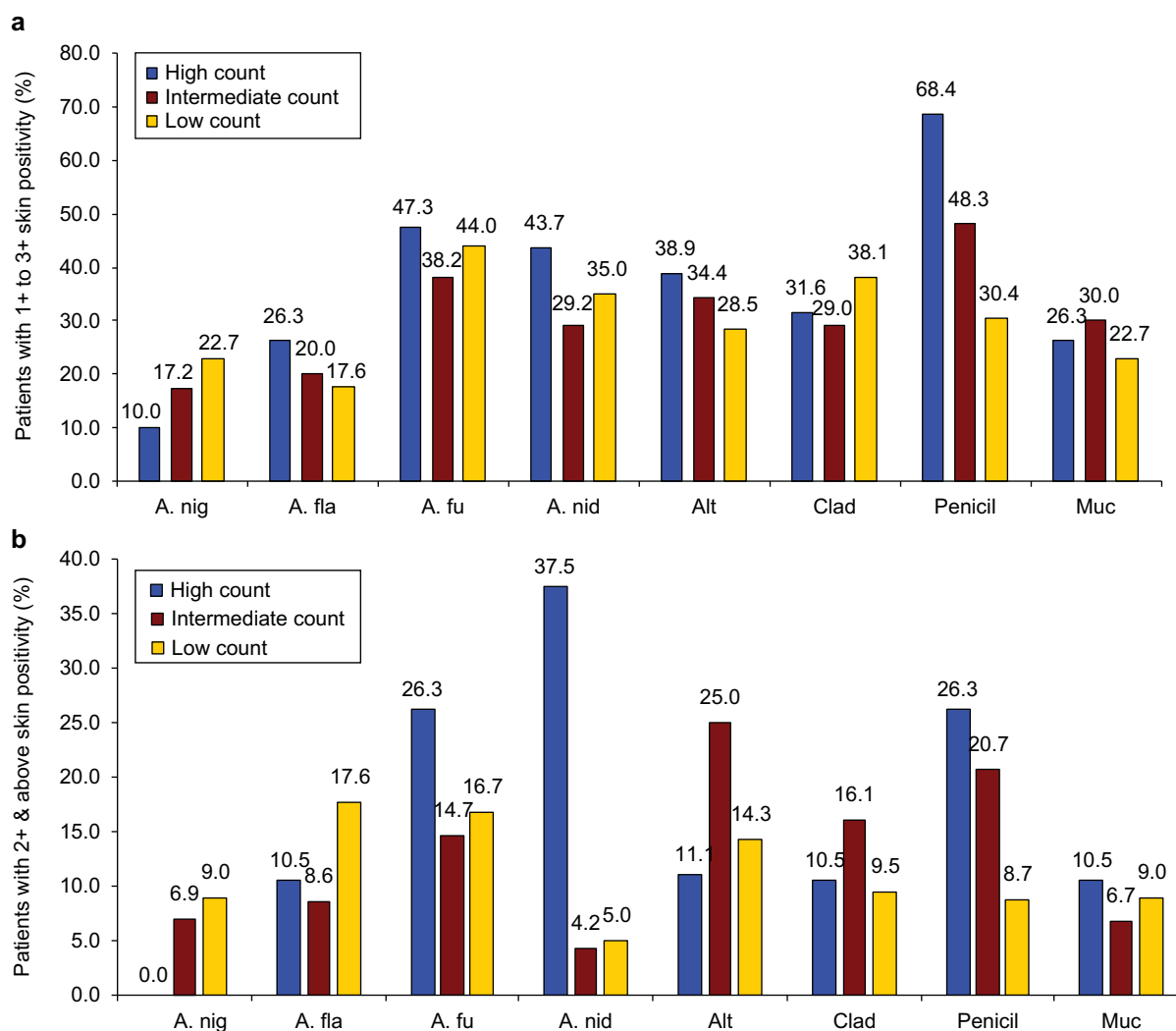


Fig. 1 Seasonal variation in total fungal concentrations (CFU/m³) in indoor and outdoor environments of patients homes (based on two sampling in a month) and comparison with the percentage of patients reported with increased respiratory complaints in each month.

percentage of children with positive skin test (1 + to 3 +) as compared to children in homes with intermediate and low colony counts (Fig. 1a). However, the homes with a high colony count of *A. fumigatus*, *A. nidulans*, *Penicillium* spp. and *Mucor* spp. and intermediate fungal concentrations of *Alternaria* spp. and *Cladosporium* spp. had higher percentages of children with marked (2 + and above) skin positive reactions (Fig. 1b). High concentrations of *Aspergillus niger* and *A. flavus* in homes did not seem to be associated with the skin reactivity of children in these dwellings. On the other hand, *Penicillium* spp. were observed to have the best trend of positive associations between indoor concentrations and skin positive reactions. However, these associations were not statistically significant, but a tendency towards positive skin tests was noted in patients' homes with high mold counts.

Total serum IgE

Total serum IgE in the sera of patients ($n = 81$) ranged from 30 IU/ml to as high as 2,083 IU/ml, with mean serum IgE level of 638 IU/ml. Males ($n = 43$) had higher mean IgE levels (686.4 IU/ml) as compared to females ($n = 38$; 583.9 IU/ml). However, this difference was not found to be statistically significant ($P > 0.05$). Mean IgE level (638.3 IU/ml) in patients is significantly higher ($P < 0.001$) than that in control subjects (158.8 IU/ml; Fig. 2).

Total serum IgE skin reactivity

Patients with 2 + and above skin reactivity had higher mean IgE (727.7 IU/ml) levels as compared to the other two groups. While patients with strong skin reactions (2 + and above) to fungi had higher level of total serum IgE (761.9 IU/ml), this association was not statistically significant ($P > 0.05$; Fig. 3). Similar results were obtained

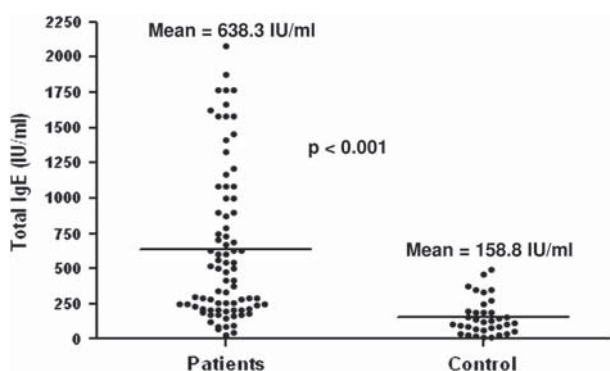


Fig. 2 Total serum IgE levels (IU/ml) in asthmatic/allergic children displaying negative positive skin reactivity against fungal allergens ($n = 74$) compared to healthy controls (HV).

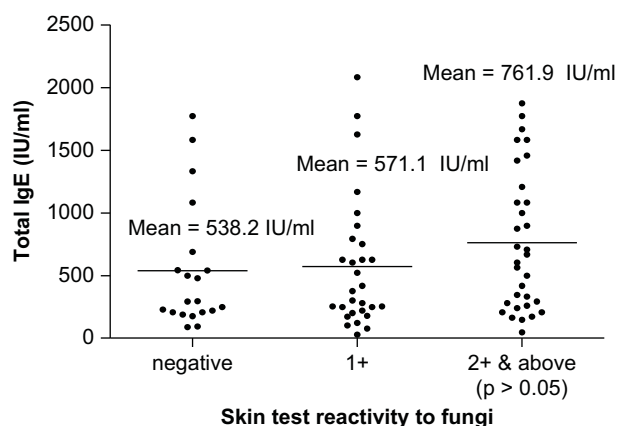


Fig. 3 Correlation between total serum IgE and intensity of skin reactivity (1 + to 3 +) to fungal antigens in patients.

with the mean IgE levels in pollen sensitive cases. Interestingly, significantly high mean IgE levels were observed in the patients with 2 + and above skin positivity (965.9 IU/ml) to mites as compared to those with negative skin reactivity (522.9 IU/ml; $P < 0.05$).

Total serum IgE skin index (number of allergens to which patients have positive responses)

Linear regression showed significantly positive association between total serum IgE skin index to fungi ($r^2 = 0.052$; $P < 0.05$) (Fig. 4a). The association was more intense with 1 + to 3 + skin test responses against fungal allergens ($r^2 = 0.143$) with P value less than 0.001 (Fig. 4b).

Fungal-specific IgE in the sera of skin test positive patients

The highest number of children with raised (greater than 30% binding of sera) specific IgE were to *Aspergillus nidulans* (66.7%), followed by *A. fumigatus* (55.0%), *Alternaria alternata* (44.0%), *C. cladosporioides* (20.8%) and *P. citrinum* (18.5%). One hundred percent of children with 2 + and above positive reactions to *Trichoderma viride* and *Mucor mucedo* were found to have elevated (greater than 15% binding only) specific IgE against these two molds. Significantly high mean OD values were obtained in 2 + and reactions against all the fungal allergens ($P < 0.001$), as compared to healthy volunteers. Linear regression analyses carried out between the OD values obtained in the sera of patients with intense skin reactions showed a statistically significant increase in specific IgE OD values against the above fungal antigens with the increase in the intensity of skin reactions (Table 6). Skin reactivity against *P. citrinum* and *C. cladosporioides* did not correlate with OD values for specific IgE against these fungi.

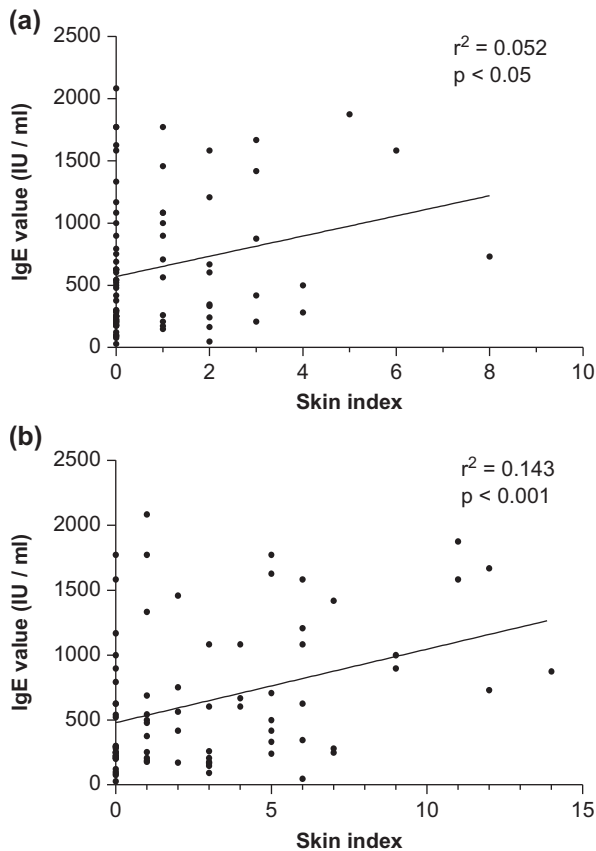


Fig. 4 Comparison of mild to marked skin test positivity (1+ to 3+) to various fungi in the patients (represented as %) with the respective concentrations of fungi in their homes (which are categorized into high, intermediate or low colony counts).

Is asthma associated with fungal allergy?

Patients suffering from BA displayed high skin positivity (50%) to fungi as compared to with BA + AR (37.8%) and AR (28.6%) and a completely reverse pattern was obtained with negative skin reactivity to fungi. Thus, it was speculated

as to whether asthma is associated with fungal sensitization (Fig. 5).

Discussion

The development of allergic sensitization and atopic diseases in children/adults is a function of the extent of the patients' exposure to allergens, as well as their genetic predisposition to react to antigens. Allergens in both the indoor and outdoor environments are responsible for this exposure. Studies in different countries indicate that indoor dampness and mold growth are associated with respiratory health issues [20]. In atopic patients, a relationship has been shown to exist between severity of asthma and/or rhinitis and the extent of exposure to fungi. Such contact can occur as much outdoors as inside the homes or places of work of the patients [21,22]. The contribution of allergens to respiratory diseases in children and adults varies in different parts of the world because patients with allergy asthma tend to become sensitized to local allergens prominent in their living environments.

When the relative prevalence of the fungi recovered from indoor sources in the present study is considered, it seems comparable to that reported by other investigators [23,24]. Prevalence of the dominant fungi varied between 6.8% for *Penicillium* spp. to 43.1% for *Aspergillus* spp. in the indoor environments of Delhi. Khan and co-workers reported a wide variety of fungi as the main constituents in the air of Kuwait, including *Cladosporium* spp. (22.8%), *Aspergillus* spp. (20.9%), *Penicillium* spp. (14.6%) and *Bipolaris* (10.6%) [25]. Thus, the prevalence rate of fungal aerospora differs in various countries depending on indoor environmental conditions, as well as the application of different sampling strategies. In India, *Cladosporium*, *Ustilago* (smuts), *Aspergillus flavus*, *Alternaria*, *Aspergillus niger* have been found to be the more prevalent fungi in Delhi's outdoor atmosphere [26]. Thus, the presence of different species of *Aspergillus*, *Penicillium*, *Alternaria* and *Cladosporium* as the major

Table 6 Association between skin test positivity in the patients and their respective specific IgE OD values.

S. no.	Name of antigen	Mean specific IgE OD value in the sera of 2+ & above cases	Mean specific IgE OD value in the sera of 1+ cases	Mean specific IgE OD value in the sera of healthy volunteers (HV) (n)	r^2 value and P value
1	<i>Aspergillus fumigatus</i>	0.376	0.204	0.145 (10)	0.2388; $P < 0.001$
2	<i>A. nidulans</i>	0.279	0.286	0.084 (10)	0.2362; $P < 0.001$
3	<i>A. flavus</i>	0.418	0.295	0.145 (11)	0.2090; $P < 0.05$
4	<i>Alternaria alternata</i>	0.395	0.131	0.076 (14)	0.3433; $P < 0.001$
5	<i>Cladosporium cladosporioides</i>	0.268	0.206	0.095 (14)	0.0004; ns
6	<i>Penicillium citrinum</i>	0.109	0.102	0.072 (11)	0.0288; ns

ns = not significant; $P < 0.05$ = significant; $P < 0.01$ = highly significant; $P < 0.001$ = very highly significant.

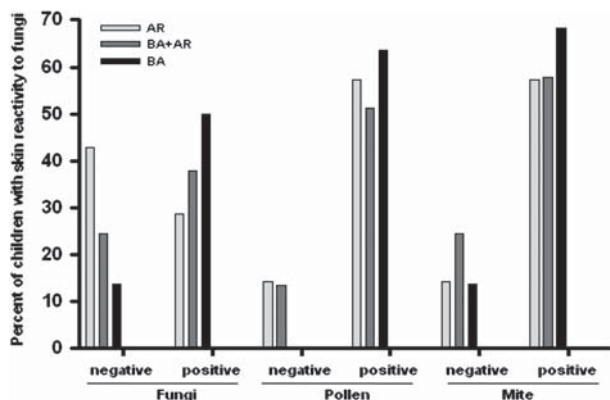


Fig. 5 Trend of positive (2+ and above) negative skin reactivity to fungi, pollen mite (represented as %) in the children suffering from BA, BA + AR and AR.

component of the indoor fungal aerospora worldwide is consistent with the results of a previous study [14].

There is an increase in respiratory symptoms of patients in winter and summer which coincides with the peak levels of fungi. Bacterial infections are not ruled out, but the role of fungi in the development or aggravation of allergy/asthma should not be underestimated. Various other workers have also analyzed the levels of indoor fungi-associated respiratory symptoms and found that high levels of *Penicillium* [27], *Cladosporium* and *Aspergillus* [20,22] were suspected sources of atopic symptoms, as well as asthma.

Skin testing is an important tool for the clinical diagnosis of allergy and the immediate wheal and flare reaction to an allergen tested is a measure of the preponderance of mast cell/basophil fixed IgE antibodies [28]. We recorded 39.3% of the patients tested had marked positive skin reactions (2+ and above) to one or more of the fungal allergens found indoors. Sensitization to molds is a risk factor for allergic diseases as demonstrated by Shivpuri and his colleagues in studies of skin test responses to various fungal antigens in Delhi, finding 2–34% fungal sensitivity in the Indian population [29]. *Alternaria*, *Aspergillus*, *Cladosporium* and *Fusarium solani* have been reported as important fungal allergens in India [30–32]. Black *et al.* [33] have observed that asthmatic patients admitted to the ICU were more likely to have positive skin tests for fungal allergens (54%), as compared to those in the community and hospitals (30%). In an investigation conducted in the UK it was found that 76% of patients with multiple admissions for severe respiratory symptoms had positive skin test results to at least one mold [34]. In contrast, in Kuwait, overall positivity to at least one mold was observed to be 20.9% in children [35]. Sensitivity to multiple fungi is more common in children as compared to mono sensitization to fungi, in that it was observed in 30% of the fungal sensitized children. This might be the consequence of cross-reactive

protein(s) which have been observed among different fungi [9]. Variations in sensitization to fungal allergens could possibly be due to differences in environmental conditions, the number of allergens tested, the age of patients, batch-to-batch variations in the properties of allergen extracts employed, e.g., low protein and high carbohydrate contents and the presence of potent proteolytic enzymes.

Beside sensitization to fungi, Delhi children also showed positivity against dust mites (*D. farinae*), which is another important indoor allergen. However, we have found that the sensitivity to mites in patients (17.9%) was lower than to one or the other fungal allergen (39.3%). Various independent workers have reported high sensitivity (52–80%) against mites (*D. farinae* and *D. pteronyssinus*) in the patients suffering from respiratory allergies [36–38]. This suggests that the homes in Delhi contain lower concentrations of HDM and thus low sensitization is observed to these allergens as compared to the results reported in other studies. This could be due to high temperatures in Delhi in the summer and autumn seasons, and low humidity in winters.

The likelihood of developing sensitivity to aeroallergens depends on the degree of atopic susceptibility, the concentration and potency of allergens to which one is exposed, and adjuvant factors [39]. In the present study, exposures to high concentrations of fungi such as *Penicillium*, *Aspergillus nidulans*, *A. fumigatus*, *Alternaria* and *Cladosporium* might act as potential sensitizers in children and adults. The most common species *Penicillium* (*P. citrinum*), *Cladosporium* (*C. cladosporioides*) *Alternaria* (*A. alternata*) in indoor environments of homes were used in skin tests of patients, who showed significant sensitization to these allergens. Other workers [40] have also reported significant associations of allergic sensitization or atopy with high counts of mold spores such as those of *Cladosporium* and *Aspergillus*. In the present study, such associations were more consistently observed for species of *Penicillium* and *A. nidulans*, where exposure above 75th percentile (13,890 CFU/m³, 3,372 CFU/m³, respectively) increased the allergic sensitization to approximately 2- to 4-fold. In contrast, high levels of *A. fumigatus* elevated the allergic sensitization only slightly (approximately 1.6-fold). However, although intermediate counts (between 25th and 75th percentile) of *Alternaria* spp and *Cladosporium* spp. were observed with higher allergic sensitizations in susceptible individuals, the lower allergenic potential of *A. niger* and *A. flavus* was indicated even at high concentrations (> 75th percentile). This is probably due to the absence of allergenic epitopes in the extracts of these fungi and is in agreement with the study of Katz and co-workers [41]. In a similar study conducted by Osborne *et al.* [42] positive associations were obtained between concentrations of basidiospores and rhinitis ($P < 0.01$) and *Penicillium/Aspergillus* SPT(+), as well as *Alternaria* SPT(+) to any allergen ($P < 0.01$).

Total serum IgE is observed to be significantly high in the age group of 8–12 years. Reports have shown that serum IgE levels are age related, with peak levels occurring during childhood, usually between the ages of 8 and 12 years, and start decreasing thereafter [18,43]. It is evident from various studies that levels of total serum IgE are associated with allergic sensitization and are found to increase several fold in atopic individuals, as compared to healthy controls [44]. A significantly positive correlation between skin index (number of allergens to which patients show skin positivity) and total serum IgE levels was observed in the present study. Patients having skin positivity to a number of fungal allergens also showed increased IgE in their sera. Similar observations have also been reported by other workers [18,44] and thus it seems a good marker for the diagnosis of allergies.

A correlation between skin positivity to fungal extracts and specific IgE to the respective fungi in the sera of patients ranged from 33–100%. We also observed a significant correlation between specific IgE OD values and intensity of skin reactions against most of the fungal allergens tested. Although elevated specific IgE was observed to *C. cladosporioides* and *P. citrinum*, the correlation was comparatively weak as compared to other extracts. Presence of elevated levels of specific IgE confirms the presence of reaginic antibodies in the sera of allergic patients to respective fungal antigens. A good correlation between the Skin Prick Test and specific IgEs suggests a reliable clinical tool that provides immunologic evidence for allergens as a causative factor in respiratory allergic disorders.

In conclusion, the study has provided important information on the association between the level of indoor fungi in Delhi homes and sensitization in allergic children. Enhanced total fungal specific IgE antibody titres were also observed to have significant correlation with intensity of the skin positivity skin index.

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References

- 1 Aubier M, Neukirch F, Annesi-Maesano I. Epidemiology of asthma and allergies. The prevalence of allergies increases and worldwide asthma has reached its highest-ever prevalence in Europe: why? *Bull Acad Natl Med* 2005; **189**: 1419–1434.

- 2 Pawankar R, Baena-Cagnani CE, Bousquet J, *et al.* State of World Allergy Report 2008: Allergy Chronic Respiratory Diseases. *World Allergy Org J* 2008; **1**: S4–17.
- 3 Masoli M, Fabian D, Holt S, Beasley R, Global Initiative for Asthma (GINA) Program. The global burden of asthma: executive summary of the GINA Dissemination Committee report. *Allergy* 2004; **59**: 469–478.
- 4 Gupta D, Aggarwal AN, Kumar R, Jindal SK. Prevalence of bronchial asthma and association with environmental tobacco smoke exposure in adolescent school children in Chandigarh. *North India J Asthma* 2001; **38**: 501–507.
- 5 Paramesh H. Epidemiology of asthma in India. *Ind J Pediatr* 2002; **69**: 309–312.
- 6 D'Amato G, Liccardi G, D'Amato M, Holgate S. Environmental risk factors and allergic bronchial asthma. *Clin Exp Allergy* 2005; **35**: 1113–1124.
- 7 Bartra J, Mullol J, del Cuvillo A, *et al.* Air pollution and allergens. *J Investig Allergol Clin Immunol* 2007; **17**: 3–8.
- 8 Erwin EA, Custis N, Ronmark E, *et al.* Asthma and indoor air: contrasts in the dose response to cat and dust-mite. *Indoor Air* 2005; **15**: 33–39.
- 9 Cramer R, Weichel M, Fluckiger S, Glaser AG, Rhyner C. Fungal allergies: a yet unsolved problem. *Chem Immunol Allergy* 2006; **91**: 121–133.
- 10 Horner WE, Helbling A, Salvaggio JE, Lehrer SB. Fungal allergens. *Clin Microbiol Rev* 1995; **8**: 161–179.
- 11 Kurup VP, Shen HD, Banerjee B. Respiratory fungal allergy. *Microbes Infect* 2000; **2**: 1101–1110.
- 12 Zureik M, Neukirch C, Leynaert B, *et al.* European Community Respiratory Health Survey. Sensitization to airborne moulds and severity of asthma: cross sectional study from European Community respiratory health survey. *BMJ* 2002; **325**: 411–414.
- 13 Mari A, Schneider P, Wally V, Breitenbach M, Simon-Nobbe B. Sensitization to fungi: epidemiology, comparative skin tests, and IgE reactivity of fungal extracts. *Clin Exp Allergy* 2003; **33**: 1429–1438.
- 14 Sharma R, Deval R, Priyadarshi V, *et al.* Indoor survey of fungi in the homes of allergic/asthmatic children in Delhi, India. *Allergy Rhinology* 2011; **2**: 21–32.
- 15 Singh AB, Singh A, Pandit T. Respiratory disorders among occupational workers in India: a cross sectional epidemiological study. *Ann of Agri Environ Med* 1999; **6**: 1–11.
- 16 Singh A, Prakash D, Singh AB. Sensitization to different species of *Aspergillus* in bakery workers and general population. *Asia Pac J Allergy Immunol* 1998; **16**: 5–15.
- 17 Singh AB, Malik P, Prakash D, Gupta CK. Biological standardization of pollen allergens from India. *Asia Pac J Allergy Immunol* 1992; **10**: 103–109.
- 18 Sharma S, Kathuria PC, Gupta CK *et al.* Total serum immunoglobulin E levels in a case-control study in asthmatic/allergic patients, their family members, and healthy subjects from India. *Clin Exp Allergy* 2006; **36**: 1019–1027.
- 19 Kauffman HF, Beaumont F, Meurs H, van der Heide S, de Vries K. Comparison of antibody measurements against *Aspergillus fumigatus* by means of double-diffusion and enzyme-linked immunosorbent assay (ELISA). *J Allergy Clin Immunol* 1983; **72**: 255–261.
- 20 Jacob B, Ritz B, Gehring U, *et al.* Indoor exposure to molds and allergic sensitization. *Environ Health Perspect* 2002; **110**: 647–653.
- 21 Andersson M, Downs S, Mitakakis T, Leuppi J, Marks G. Natural exposure to *Alternaria* spores induces allergic rhinitis symptoms in sensitized children. *Pediatr Allergy Immunol* 2003; **14**: 100–105.
- 22 Matheson MC, Abramson MJ, Dharmage SC, *et al.* Changes in indoor allergen fungal levels predict changes in asthma activity among young adults. *Clin Exp Allergy* 2005; **35**: 907–913.

- 23 Shelton BG, Kirk KH, Flers WD, Morris GK. Profiles of airborne fungi in buildings and outdoor environments in the United States. *Appl Environ Microbiol* 2002; **68**: 1743–1753.
- 24 Unlu M, Ergin C, Cirit M, Sahin U, Akkaya A. Molds in the homes of asthmatic patients in Isparta, Turkey. *Asian Pac J Allergy Immunol* 2003; **21**: 21–24.
- 25 Khan ZU, Khan MA, Cy R, Sharma PN. *Aspergillus* and other moulds in the air of Kuwait. *Mycopathologia* 1999; **146**: 25–32.
- 26 Gupta SK, Pareira BMJ, Singh AB. Survey of airborne culturable and non culturable fungi of different sites in Delhi Metropolis. *Asian Pacific J Allergy Immunol* 1993; **11**: 19–28.
- 27 Gent JF, Ren P, Belanger K, et al. Levels of household mold associated with respiratory symptoms in the first year of life in a cohort at risk for asthma. *Environ Health Perspect* 2002; **110**: A781–786.
- 28 Yunginger JW. Standardization of allergenic extracts. *Ann Allergy* 1991; **66**: 107–111.
- 29 Agarwal MK, Shivpuri DN. Fungal spores – their role in respiratory allergy. *Adv Pollen-Spore Res* 1974; **1**: 78–128.
- 30 Prasad R, Verma SK, Dua R, et al. A study of skin sensitivity to various allergens by skin prick test in patients of nasobronchial allergy. *Lung India* 2009; **26**: 70–73.
- 31 Agashe SN. Public awareness of allergens. *Indian J Allergy Asthma Immunol* 2003; **17**: 33.
- 32 Singh AB, Kumar P. Common environmental allergens causing respiratory allergy in India. *Indian J Paediatr* 2002; **69**: 245–250.
- 33 Black PN, Udy AA, Brodie SM. Sensitivity to fungal allergens is a risk factor for life-threatening asthma. *Allergy* 2000; **55**: 501–504.
- 34 O'Driscoll BR, Hopkinson LC, Denning DW. Mold sensitization is common amongst patients with severe asthma requiring multiple hospital admissions. *BMC Pul Med* 2005; **5**: 4.
- 35 Ezeamuzie CI, Al-Ali S, Khan M, et al. IgE mediated sensitization to mould allergens among patients with allergic respiratory diseases in a desert environment. *Int Arch Allergy Immunol* 2000; **121**: 300–307.
- 36 Tang RB, Tsai LC, Hwang HM, et al. The prevalence of allergic disease and IgE antibodies to house dust mite in school children in Taiwan. *Clin Exp Allergy* 1990; **20**: 33–38.
- 37 Chew FT, Lim SH, Goh DY, Lee BW. Sensitization to local dust-mite fauna in Singapore. *Allergy* 1999; **54**: 1150–1159.
- 38 Su HJ, Wu PC, Lei HY, Wang JY. Domestic exposure to fungi and total serum IgE levels in asthmatic children. *Mediators Inflamm* 2005; **3**: 167–170.
- 39 Warner JA, Warner JO. Allergen avoidance in childhood asthma. *Respir Med* 1991; **85**: 101–105.
- 40 Garret MH, Rayment PR, Hooper MA, Abramson MJ, Hooper BM. Indoor airborne fungal spores, house dampness and associations with environmental factors and respiratory health in children. *Clin Exp Allergy* 1998; **28**: 459–467.
- 41 Katz Y, Verlerger H, Barr J, et al. Indoor survey of moulds and prevalence of mould atopy in Israel. *Clin Exp Allergy* 1999; **29**: 186–192.
- 42 Osborne M, Reponen T, Adhikari A, et al. Specific fungal exposures, allergic sensitization, and rhinitis in infants. *Pediatr Allergy Immunol* 2006; **17**: 450–457.
- 43 Barbee RA, Halonen M, Lebowitz M, Burrows B. Distribution of IgE in a community population sample: correlations with age, sex, and allergen skin test reactivity. *J Allergy Clin Immunol* 1981; **68**: 106–111.
- 44 Backer V, Ulrik CS, Wendelboe D. Distribution of serum IgE in children and adolescents aged 7 to 16 years in Copenhagen, in relation to factors of importance. *Allergy* 1992; **47**: 484–489.

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