

## Original Research Article

# Diagnostic utility of skin prick test in fungal asthma

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### ABSTRACT

**Background:** Allergic bronchopulmonary mycosis (ABPM) is a clinical syndrome associated with immune sensitivity to various fungi. *Aspergillus spp.* predominates in colonizing the airways of asthmatics. Early and accurate identification of fungus in such cases can prevent worsening of asthma. Also, can help in retarding the progression of ABPM. Objectives of this study were to evaluate different fungal allergens associated with clinically diagnosed Asthma patients by Skin Prick testing (SPT), to study total IgE in asthmatic patients by serological testing and to characterize the fungal isolate associated with SPT+ cases by conventional mycological culture.

**Methods:** A prospective study of known asthma cases was done. Their sensitivity to fungal allergens was tested by SPT. The total IgE levels were measured by ELISA. Sputum collected from SPT+ cases were subjected for fungal identification.

**Results:** Out of 175 patients, 25 (14.2%) showed positive reaction against fungal antigens in which fungal growth was seen in 21 (84%) sputum specimens. *Aspergillus fumigatus* was isolated from 16 (76%) specimens followed by *Candida albicans* in 3 (14%) and *Penicillium spp* in 2 (9.5%) cases. Out of 25 SPT+ asthmatics, 21 patients with fungal growth had total IgE levels >600 IU/ml and 4 patients with negative culture had IgE levels 400-500 IU/ml.

**Conclusions:** A significant prevalence of fungal asthma is seen among asthmatics. Thus, it is essential to screen asthma patients for fungal allergy. SPT seems to be a good screening test. SPT is easy to perform, less time consuming and inexpensive however needs to be performed under pulmonologist's supervision.

**Keywords:** Allergic bronchopulmonary mycosis, *Aspergillus fumigatus*, *Candida albicans*, Enzyme linked immunosorbent assay, Skin prick test

### INTRODUCTION

Asthma is an allergic respiratory illness that is characterized by the airway's hypersensitivity to external stimuli.<sup>1</sup> Chronic airway inflammation, deteriorating airway function, and tissue remodeling are all characteristics of asthma.<sup>2</sup> Several factors have been identified as asthma risk factors.<sup>3</sup> One of the triggers is ongoing contact with allergens that are fungi.<sup>4</sup> Moulds have a considerably greater impact on patients' immune

systems than pollen or other allergy triggers.<sup>5</sup> It involves a number of airborne fungi, such as *Alternaria*, *Aspergillus*, *Cladosporium*, and *Penicillium species*, and can happen indoors, outdoors, or both.<sup>6</sup> *Aspergillus fumigatus* is the species of fungus that affects the lungs most frequently.<sup>1</sup>

The diagnosis of ABPM is a significant clinical concern because ABPM may result in lung fibrosis and ongoing decline in lung function, and early treatment is necessary to prevent long-term tissue damage.<sup>7</sup> Lack of a gold

standard for diagnosis makes it difficult to assess the sensitivity and specificity of the diagnostic criteria as a whole and its individual components.

The tests most frequently used are SPT, *in vitro* measurement of particular IgE antibodies. Other tests used are *A. fumigatus* specific IgE, total eosinophil count, chest radiographic/CT scan, high attenuation mucus present, reduced FEV1, FEV1/FVC ratio.<sup>8</sup>

## METHODS

The prospective cross-sectional study was conducted in Department of Microbiology, Grant Government Medical, Mumbai. A total of 175 asthma patients attending Pulmonary Medicine OPD were selected for the study for over a period of 18 months from February 2021 to July 2022.

### Inclusion criteria

All patients of asthma attending the outpatient Department of Pulmonary Medicine, no age specific restriction, both genders were included.

### Exclusion criteria

Patients with history of smoking, patients with previous history of tuberculosis, pregnancy, and patients currently on oral glucocorticoids or those who have been treated with the same for more than three weeks within the last six months were also excluded were excluded.

### Sample collection and storage

Skin prick testing was carried out in all (n=175) clinically diagnosed Asthma patients. Early morning sputum specimens in duplicate were collected from patients (n=25) who were found to be SPT+ in wide mouth screw capped sterile container. Blood samples from 35 known cases of asthma (25 SPT+ samples, 10 SPT negative) attending pulmonary medicine OPD were collected using aseptic technique in plain bulbs. Five healthy controls also were included in this study. Serum separated from the blood were stored at -200C at Dept of Microbiology till the serological studies were complete.

### Skin prick test

All patients underwent SPT for selected 5 fungal antigens (*Aspergillus fumigatus*, *Candida albicans*, *Alternaria* species, *Cladosporidium* spp, *Penicillium* spp.) and the positive results in the form of wheal and flare was recorded. The kits used were supplied from AllVac Pharmaceuticals Pvt. Ltd. The test was done on volar aspect of the forearm, at least 2-3 cm away from the wrist and the antecubital fossae. The wheal size is measured at its largest diameter after 15 to 20 minutes. Positive reactions are defined as wheals that are at least >3 mm in diameter than the negative control. The results of the test

were measured according to Shivpuri's criteria.<sup>9,10</sup> A drop of saline was used as negative control.

### Fungal identification

Microscopic techniques like KOH and gram staining were used to look for fungal elements. Also, these were cultured on Sabouraud's dextrose agar (plain and chloramphenicol-cycloheximide containing) and incubated at two different temperatures (30°C and 37 °C). Culture readings for yeasts were taken at 24, 48, 72 hours, 5<sup>th</sup> day and 7<sup>th</sup> day and till one month for filamentous fungi. Genus and species level of identification of the yeast colonies obtained was done by germ tube test and slide cultures was the main stay for filamentous fungi.<sup>11,12</sup>

### Total serum IgE levels

The blood samples collected were subjected to Total IgE testing by ELISA [enzyme linked immunosorbent assay]. Kits provided by AlkorBioAllergo EIA-Total IgEkit. As per kit literature the samples had to be processed in duplicate. As a result, 40 samples were processed in 96-well plate as the kit consisted of 1 control and six calibrators. Out of 40 samples processed, 25 were SPT+ samples, 10 were SPT negative and 5 were healthy controls. The samples and reagents were brought to room temperature before processing. Mean OD of calibrators are plotted verses their respective IgE concentrations using standard curve.

## RESULTS

Out of 175 asthma cases, 96 (54.8%) belonged to age group of 25-50 years, 54 (30.8%) in the age group of >50 years and 25 (14.2%) in the age group of 0-25 years. Thus, it was observed that maximum number of asthma patients were observed in age group of 25-50 years (Table 1). Also, it was seen that out of 175 asthma cases, 104 (59.4%) were males and 71 (40.5%) were females (Figure 1).

**Table 1: Age-wise distribution of cases.**

Age in years	Total cases	Percentage (%)
0-25	25	14.3
25-50	96	54.9
>50	54	30.8
<b>Total</b>	<b>175</b>	<b>100</b>

### Skin prick test

All the 175 asthma patients were subjected to SPT. The fungal antigens tested were for *Aspergillus fumigatus*, *Candida albicans*, *Penicillium* species, *Alternaria* species, *Cladosporium* species. Out of 175 patients, 25 (14.2%) showed positive reaction against fungal antigens. Results interpretation as shown in Table 2. Rest all 150

(85%) showed no reaction which were termed as SPT negative.

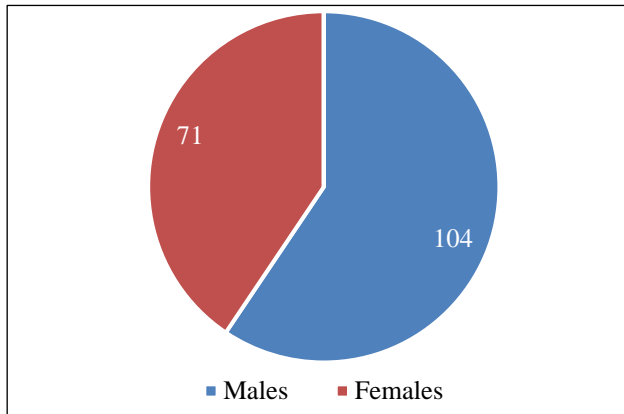


Figure 1: Asthma cases.

Table 2: Skin prick test results as per Shivpuri’s criteria.

Grading	Interpretation [wheal size in comparison with negative control]	Total numbers	Percentage (%)
4+	>8mm	11	44
3+	>6mm	7	28
2+	>4mm	4	16
1+	>2mm	2	8
Doubtful	<1+	1	4

**Conventional mycological identification**

Sputum specimens were collected from 25 SPT+ cases in duplicate, out of 25 SPT+ cases, 11 (44%) were KOH positive (fungal elements seen) and 14 (56%) were

negative. While fungal growth was seen in 21 (84%) out of 25 cases and 4 (16%) cases showed no growth. Out of the 21 fungi grown, predominant fungus was *Aspergillus fumigatus* (76%) followed by *Candida albicans* (14%) and *Penicillium* species (9.5%) (Figure 2).

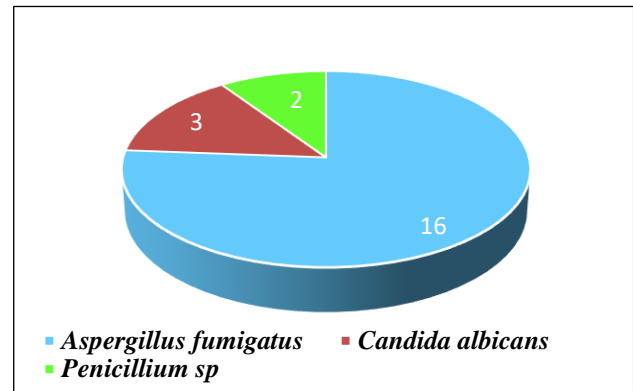


Figure 2: Fungus grown.

**Total IgE levels**

Further this study also analyzed allergy cases serologically. The serum samples of 40 persons (25 SPT+, 10SPT negative and 5 Healthy controls) were processed by ELISA to determine total IgE levels of these patients. Out of 40 samples, 25 were SPT+ samples in which 21 showed fungal growth (as shown in Table 3), the total IgE level of these sample was >600 IU/ml and 4 samples in which no fungal growth was detected had IgE levels in the range of 400-500 IU/ml. 10 samples from SPT negative patients were taken having total IgE levels in the range of 200-500 IU/ml. Also 5 samples from healthy controls were processed for comparison which had IgE levels in between 0-60 IU/ml. Thus, a significant increase in IgE levels was detected in case of fungal asthma patients. The co-relation between SPT, total IgE levels and fungal culture shown in Table 3.

Table 3: Correlation of tests (SPT, fungal culture and Total IgE).

SPT	Fungal culture	Total no	Total IgE	Case details	
POS (21)	<i>A. fumigatus</i>	16	>600 IU/ml	Asthma patients	
	<i>Penicillium</i> species	2			
	<i>C.albicans</i>	3			
POS	No fungus grown	3	400-450 IU/ml		Asthma patients
POS	No fungus grown	1	500-600 IU/ml		
NEG	No fungus grown	1	150-550 IU/ml		
NEG	No fungus grown	2	250-300 IU/ml		
NEG	No fungus grown	2	300-350 IU/ml		
NEG	No fungus grown	2	300-400 IU/ml		
NEG	No fungus grown	1	400-450 IU/ml		
NEG	No fungus grown	1	400-500 IU/ml		
NEG	No fungus grown	1	500-550 IU/ml		
NEG	No fungus grown	5	0-60 IU/ml		

## DISCUSSION

The present study also showed that maximum number of asthma patients with fungal allergy were from age group of 25-50 (54.9%) followed by those in age group of >50 years (30.8) and 0-25 years (14.3) (Table 1). Asano et al conducted a study suggesting that ABPA typically manifests in individuals between the ages of 30 and 40, which is consistent with cases in India.<sup>13</sup> Also, in a study conducted by Prasad et al, 29 patients (69%) of the 42 total patients were between the year range of 20-40.<sup>14</sup> In the present study, out of 175 patients, 104 (59.5%) were males and 71 (40.5%) were females concluding that asthma cases were more in males compared to females. Chowdhury et al, in their study concluded that women have asthma more frequently and severely as adults.<sup>15</sup> The sex differences in asthma incidence, prevalence, and severity have been linked to a variety of critical elements, as previously mentioned, social and environmental factors, and reactions to asthma treatments. Honkamaki et al, also reported in their study that out of 63.7% of subjects, 58.4% of men and 67.8% of women concluding that prevalence of asthma was more in women than in males (Figure 1).<sup>16</sup>

### *SPT in asthma and ABPA*

In this study, out of 175 asthma patients 25 showed a positive reaction to skin prick test for fungal allergen, which is 14.2%. Out of 25 SPT positives, 16 were due to *A. fumigatus* (Table 2) concluding higher prevalence of ABPA. Maurya et al, in their study reported *Aspergillus* SPT positivity in 30 out of 105 patients (28.5%).<sup>17</sup> In study by Sarkar et al, about 25% of asthmatics with SPT positive results had an ABPM prevalence.<sup>18</sup> The study also demonstrates that 90% of ABPM cases were caused by *A. fumigatus*, supporting the fact that *Aspergillus* is the most common pathogen associated with ABPM.

### *IgE levels*

In the present study the levels for serum IgE in all 25 SPT positive cases was >600 IU/ml (since the highest range of calibrator given in kit was 600 IU/ml all the values above that range were concluded to be >600 IU/ml). Other asthmatics with negative SPT had IgE levels within range of 150-550 IU/ml and healthy controls were found to have IgE <60 IU/ml (Table 3). Sandeep et al, in their study observed that serum IgE levels in mild to moderate asthmatics was in the range of 400-700 IU/ml while in severe asthma patients it was  $\geq$ 1000 IU/ml. In healthy controls it was <150 IU/ml.<sup>19</sup> Also, they observed that in ABPA patients it was >1000 IU/ml. Ritesh et al studied the IgE levels of asthmatic patients.<sup>20</sup> They found that for diagnosing ABPA, total serum IgE levels should be >500IU/ml.

## CONCLUSION

It was concluded that Fungi are also found to be one of the important allergens causing asthma. Among them most common allergen was found to be *Aspergillus fumigatus* resulting in ABPA. Skin prick test is a good screening test to identify fungal asthma patients among asthmatics as it is easy to perform, inexpensive and is less time consuming. One of the precautions required for this testing is that this test needs to be carried out under supervision of Pulmonologist. Conventional fungal culture also seems to be promising though it needs mycology laboratory infrastructure and time taken for fungal growth is certainly more than SPT. Another interesting finding from this study was Total IgE levels measurements by ELISA. The Total IgE level was significantly high in Asthmatics with SPT+ for fungal antigen. Total IgE level testing by ELISA needs a good infrastructure of laboratory and cost of the kit is another limitation. Thus, we can conclude that prompt diagnosis for fungi as one of the causes of asthma should be done and appropriate anti-fungal treatment should be considered. Role of clinical microbiologist is equally important in management of Asthma cases.

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