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Allergic Bronchopulmonary Mycosis due to fungi other than *Aspergillus*

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Summary

*Allergic bronchopulmonary mycosis (ABPM) is a clinical syndrome associated with immune sensitivity to various fungi that colonize the airways. Early diagnosis and treatment with systemic corticosteroids is the key in preventing the progression of the disease to irreversible lung fibrosis. Although *Aspergillus* has progressively gained recognition as a causative agent in past few decades, other fungi, that have been reported to cause ABPM, are not yet widely evaluated.*

*We studied hundred and two patients with asthma for occurrence of ABPM. Patients were tested for cutaneous hypersensitivity and serum precipitin to 12 common fungal antigens. The positive cases were further evaluated for ABPM using standard criteria. Out of 102 asthma patients screened, 18 patients had either skin prick test (SPT) and/or serum precipitin positive. While 14 patients were SPT positive for one or more fungal antigen, two patients were serum precipitin positive for one or more fungi. Two patients had both serum precipitin positive as well as SPT positive. Six (5.8%) patients were diagnosed as ABPM as they fulfilled the criteria. Three of these were because of *Aspergillus* sp. Two were because of fungi other than *Aspergillus* namely *Schizophyllum* and *Curvularia*. One patient had ABPM because of both *Aspergillus* and *Curvularia*. In our study absolute eosinophil count (AEC), total IgE, serum precipitin and SPT had sensitivity of 100%, 100% 50% and 83.3% respectively for diagnosing ABPM. The specificity of these tests was 44.79%, 64.58% 98.96% and 88.54% respectively. Specific IgE was positive in 50% of patients with either serum precipitin or SPT positivity. SPT or serum precipitin followed by specific IgE had sensitivity of 100% and specificity of 96.88% for diagnosing ABPM. SPT alone followed by Specific IgE had a sensitivity of 83.33% and specificity of 96.88% for diagnosing ABPM.*

*We found that fungi other than *Aspergillus* such as *schizophyllum*, and *curvularia*, can be implicated in ABPM. Multiple fungal agents may be responsible for ABPM in an individual. There is a subset of patients of BA who have fungal sensitization but do not fulfil the criteria for ABPM. SPT was the single most sensitive and specific test, AEC >350 and total IgE more than 417IU were most sensitive tests and SPT followed by specific IgE was most effective strategy for diagnosing ABPM.*

Introduction

Allergic bronchopulmonary mycosis (ABPM) is a hypersensitivity mediated disease of the lower airways caused by environmental fungi, the most common being *Aspergillus fumigatus*. The other etiologic agents include *Candida albicans*, *Schizophyllum commune*, species of *Alternaria*, *Bipolaris*, *Clado-*

sporium, *Curvularia*, *Fusarium*, *Penicillium*, *Pseudallescheria*, *Rhizopus*, *Saccharomyces*, *Stemphylium* and *Trichosporon* (1). While allergic bronchopulmonary aspergillosis (ABPA) has been extensively studied worldwide, there is paucity of information on ABPM due to other fungi. The most commonly accepted criteria for diagnosing ABPA are those proposed by Rosenberg et al. (1977) and Patterson et al (2,3). Early recog-

nition and treatment with corticosteroids prevents progression to fibrotic lung disease (4). The term severe asthma with fungal sensitisation (SAFS) has been proposed for those patients who have persistent severe or brittle asthma (despite standard treatment) and evidence of fungal sensitisation, as defined by positive prick testing, or fungal antigen-specific blood IgE testing, and do not meet the criteria for ABPA (5).

The paucity of information on sensitization by variety of fungal allergens other than *Aspergillus* spp. in patient of asthma and their clinical profile prompted us to undertake the present study. The present study is aimed to observe the occurrence of ABPM in bronchial asthma.

Material and Methods

This was a cross sectional observational study of 102 subjects (age >18 years) of asthma attending Dr. RML Hospital from Nov 2014 to March 2016, who were diagnosed as per GINA guidelines. Patients were subjected to Skin Prick Test (SPT) with 12 common fungal allergens (*Alternaria alternata*, *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus terreus*, *Curvularia lunata*, *Scedosporium apiospermum*, *Schizophyllum commune*, *Bipolaris hawaiiensis*, *Cladosporium*, *Rhizopus* species and *Candida*). Serum precipitin for these fungal agents was done for all patients. Those found positive on SPT or those with serum precipitin positive for a specific fungus were further investigated for specific IgE. Total IgE, Absolute eosinophil count (AEC) and X-ray Chest PA view were done for all the patients. CECT/HRCT chest was done wherever indicated.

Pregnant ladies, patients who were on systemic steroids any time during last 2 weeks, patients who were on leukotriene inhibitors or antihistaminics any time during last 6 weeks were excluded from the study. Patients with pulmonary tuberculosis were also excluded.

Approval was obtained from the ethics committee of our institute and written informed consent was taken from all patients. The skin prick test was performed in accordance with guidelines (6). A reaction was graded as a negative (equal to negative control), 1+ (<3mm), 2+(3-5mm), 3+ (5-7mm), 4+ (7-9mm). A response equal to or greater than that of positive control was considered significantly positive for that antigen.

Specific IgE in the patients serum was determined by Enzyme Linked Immunosorbant Assay (ELISA). Serum precipitins (specific IgG) were detected using 'Ouchterlony's immunodiffusion. Serum total IgE was measured by the Calbiotech IgE ELISA Kit according to manufacturer's instruction.

Spirometry was performed using SpiroPro v2.0 spirometry machine. Readings were taken before and 15 min after the inhalation of salbutamol and was interpreted in accordance with the American Thoracic Society guidelines.

Those patients who fulfilled the modified Patterson-Greenberger criteria were labelled as ABPM (4).

Results

SPT was done for 12 fungal antigen in 102 patients in which 15.7% (16 out of 102) patients showed positive type I hypersensitivity reaction to at least one fungal antigen. Serum precipitins were positive in 4 patients. Six (5.8%) patients fulfilled the modified Patterson Greenberger criteria (4) and were diagnosed ABPM. One of our patients fulfilled 7 criteria, one had 6 while the other four fulfilled 5 criteria. All patients had Bronchial Asthma and fulfilled at least 5 criteria for diagnosing ABPM as suggested in a recent review by Gupta et al (7). Details of diagnostic criteria for 18 patients with Skin prick test and/or serum precipitin positive are given in **Table I**. Clinical characteristics and asthma control is given in **Table II**.

In 16 patients, a total of 35 positive skin reactions were observed, of which 11 were positive for *A. flavus* and 6 were positive for *A. fumigatus*, 4 were positive for *A. niger* and *A. terreus* respectively and 2 were positive for *Schizophyllum commune*, *Rhizopus* spp, *Candida* spp, *Cladosporium* spp, each. Only 1 SPT was positive for *Curvularia* spp and *Bipolaris hawaiiensis*. Correlation of skin prick test positivity with specific IgE is as per **Table III**.

In 4 patients Serum precipitins were positive in seven instances. One patient had serum precipitin positive for all four aspergillus species. The same patient had three SPT positive for *Aspergillus* spp. and specific IgE was positive for *Aspergillus fumigatus* only. One patient each had serum precipitin positive for *Curvularia lunata*, *Scedosporium apiospermum* and *Schizophyllum commune*. Only 7 out of 102 patients had radiological abnormality. Out of these 7 only 3 fulfilled ABPM criteria. One patient had central bronchiectasis with mucus plug. In other 2 cases one had fibrosis in left lung and other had paraseptal emphysema and fibrosis. Total IgE was >417 in 39.2% (40 out of 102) and ≤417 in 60.8% (62 out of 102) patients. Specific IgE was done in eighteen patients who had SPT and/or Serum precipitin positive. It was positive in 9 (50%) and negative in 9 (50%). In our study AEC was most sensitive test. It had sensitivity of 100%. Serum precipitin was most specific test. It had specificity of 98.8% SPT was single most sensitive and specific (83 and 89%) test for diagnosing ABPM. SPT followed by specific IgE was most effective strategy (83% sensitivity and 97% specificity) for diagnosing ABPM (**Table IV**).

Discussion

This study was done to see the occurrence of ABPM in cases of bronchial asthma irrespective of the severity of asthma. In our study we found prevalence of ABPM in BA was 5.8%. This was

Table I - Detailed diagnostic profile of asthmatic patients with serum precipitin and/or SPT positive.

S No	Serum Precipitins	Skin Prick Test	AEC	Total IgE	Specific IgE	CT Chest	Fulfilling ABPM criteria
1.	A f, An, Afl, At	Af, Afl, At	900	6128	A f	CB	Yes (Af)
2.	Schizo	Schizo, Cand	12000	6400	Schizo	ORF	Yes (Schizo.)
3.	Scedo	-	930	359	-	-	No
4.	Curvularia	-	420	836	Curv.	-	Yes (Curv.)
5.		An, At, Cand	789	1675	-	-	No
6.		A fl	483	1258	-	-	No
7.		Af	760	1125	-	-	No
8.		Af, An, Afl	1024	1014	An	-	Yes (An)
9.		Af, An, Afl, At	828	1256	Af, An, At	ORF	Yes (Af, An, At)
10.		Rhizo	868	1631	-	-	No
11.		An, Afl, Clado	560	75	Afl	-	No
12.		Afl, Clado	1200	2480	-	-	No
13.		Af,	16	740	-	-	No
14.		Afl	800	42	Afl	-	No
15.		Afl	1716	191	-	-	No
16.		Afl, Bipolaaris, schizo	360	44	Bipolaris, Schizo	-	No
17.		Afl, At, Curvularia, Rhizo	480	864	At, Curv.	-	Yes (At, Curv.)
18.		Af, Afl	790	1272	-	-	No

Abbreviations- AEC (absolute eosinophil count), Af (aspergillus fumigates), An (aspergillus niger), Afl(aspergillus flavus), At (aspergillus terreus), Schizo(Schizophyllum commune), cand(candida), Scedo (Scedosporium apiospermum), Curv. (Curvularia lunata), Rhizo (rhizopus oryzae), Clado (cladosporium spp.), CB (central Bronchiectasis), ORF (other radiological features)

more than a study done in Saudi Arabia which showed period prevalence close to 3% for ABPM (8). In this study all patients were screened for ABPM with skin prick test (SPT) using a panel of fungal antigens. Panel included Aspergillus fumigatus, A. niger, A. versicolor, A. clavatus, A. repens, Alternaria, Cladosporium, Rhizopus, Penicillium, Mucor, Trichophyton, Candida, Herbarum, Phoma, Fusarium. A study done in Ireland over four years showed period prevalence of ABPM was a little above one percent (9). In this study patients were checked for only A fumigatus and candida species. A study similar to our study was done in a tertiary care hospital of Kolkata. Antigens for the following fungi namely Aspergillus fumigatus, Aspergillus flavus, Aspergillus niger, Aspergillus tamari, Alternaria alternata, Cladosporium herbarum, Curvularia lunata, Penicillium sp., Fusarium solari, Rhizopus nigricans, Candida albicans, Phoma tropicalis were used in this study. They found prevalence of ABPM to be 7.9 % which is similar to our study but more than the studies done outside India (10). However, they found that

in their 10 cases of ABPM, 9 cases were of ABPA and only 1 case was of ABPM because of fungi other than Aspergillus (Penicillium spp). In our study out of 6 cases 2 cases were of ABPM due to fungi other than Aspergillus and one patient fulfilled criteria both for Aspergillus and Curvularia while 3 patients had ABPA. Out of these one patient had SPT and specific IgE positive for three aspergillus species namely, A. Niger, A. terreus and A. fumigatus. Two other cases had SPT and specific IgE positive for A.fumigatus and A. niger respectively. Two cases that had ABPM because of fungi other than Aspergillus, the fungi implicated were Schizophyllum and Curvularia lunata respectively. There was a subset of cases that had low total IgE but SPT and specific IgE were positive for fungal antigens. It was either because the disease was not in active stage at the time of study or they were cases of fungal sensitization only.

One case each of Schizophyllum and Aspergillus fumigatus fulfilled all the seven criteria of diagnosis and both AEC and total IgE were very high. Similarly other cases also fulfilled 5 criteria

Table II - Comparison of Clinical characteristics and control in ABPM and Non ABPM group.

Category	ABPM(6)	Non ABPM(96)
Day time symptoms		
None	3	56
Once a month	2	20
Once a week	0	4
Twice a week	1	7
>Twice a week	0	9
Night time awakening		
None	3	60
Once a month	1	10
Once a week	1	10
Twice a week	1	10
>Twice a week	0	6
Previous hospitalization		
None	4	91
1-2	2	3
2-5	0	1
>5	0	1
Acute episodes		
None	3	79
1-2	2	9
2-5	1	7
>5	0	1
Fev1		
>80	1	43
60-79	5	46
50-59	0	5
<50	0	2
Control		
Controlled	3	56
Partially controlled	2	23
Uncontrolled	1	17

at least 4. These results are similar to study by Ishiguro et al (11) who showed that presence of 6 or more than 6 diagnostic criteria had a sensitivity of 97.6% and specificity of 98.3%. However if only more than 6 criteria were taken as diagnostic criteria the sensitivity dropped to 57%. However Ishiguro et al included patients without asthma in their analysis as 33.3% of their patient did not have asthma. Analysing for ABPA

Table III - Correlation between skin prick test and specific IgE positivity.

Fungal antigen	Skin Prick Test positivity	Specific IgE positivity
A flavus	11	2
A fumigates	6	2
A terreus	4	2
A niger	4	2
Schizophyllum	2	2
Curvularia	1	1
Bipolaris	1	1
Rhizopus	2	0
Candida	2	0
Cladosporium	2	0

Table IV - Sensitivity and specificity of Different Criteria for diagnosing ABPM.

Criteria	Sensitivity	Specificity
Absolute eosinophil count>350	100%	45%
Total IgE >417	100%	65%
Skin Prick Test	83%	89%
Serum Preciptin	50%	99%
SPT/Serum Preciptin followed by Specific IgE	100%	97%
SPT followed by Specific IgE	83%	97%

in asthmatic patients only they found that 96.5% of patients showed positive result for specific IgE or SPT. As discussed in a review by Gupta et al, we considered patients with at least 5 criteria positive for the diagnosis of ABPM(7).

In our study we came across one patient with ABPA because of 3 Aspergillus fungal antigens, and one case of ABPM because of Aspergillus and a non-aspergillus fungi. Our study suggests that ABPM may be caused in an individual because of multiple antigens. In our literature review we came across only few cases of ABPM where more than one fungal antigen was implicated. Multiple causative fungi have been seen in a retrospective study by Ishiguro et al (11). In their study one patient had multiple Aspergillus species as cause and another patient had ABPM because of A.fumigatus and Schizophyllum commune. Whether the

identification of multiple agents in our study could have been caused by cross-reaction among antigens or other similar factors, is a subject of further investigation and needs a larger study.

Chaudhary et al (1) have done review of globally reported cases of ABPM and they found India accounted for 47% of globally reported cases which is much higher than any country in the world, although number of fungi tested in the previous studies were limited to few. In our study we have investigated for fungi like *Schizophyllum*, *Curvularia* and *Bipolaris* also and we found cases of ABPM or fungal sensitization because of these fungi. In previous studies these fungi have not been evaluated. This could account for the high prevalence of ABPM in our study compared to other studies done outside and similar to study done in India (10).

In our study, only 2 out of 102 patients had FEV1 less than 50%. Eighteen patients had uncontrolled asthma, 1 in ABPM group and 17 in non-ABPM group. The relation between ABPM and severity or control of asthma would require a larger number of subjects with ABPM.

The diagnostic criteria for allergic fungal diseases are evolving (12). However, since the focus of our study was ABPM because of fungi other than *Aspergillus*, we used the standard criteria established for ABPA.

Conclusion

Fungi other than *Aspergillus* such as *Schizophyllum*, *Bipolaris* and *Curvularia*, can be implicated in ABPM. Multiple fungal agents may be responsible for ABPM in an individual. There is a subset of patients of BA who have fungal sensitization but do not fulfil the criteria for ABPM. SPT was the single most sensitive and specific test, AEC >350 and total IgE more than 417 IU were most sensitive tests and SPT followed by specific IgE was most effective strategy for diagnosing ABPM.

Conflict of Interests

The authors declare that they have no conflict of interest.

References

1. Chowdhary A, Agarwal K, Kathuria S, Gaur SN, Randhawa HS, Meis JF. Allergic bronchopulmonary mycosis due to fungi other than *Aspergillus*: a global overview, *Critical Reviews in Microbiology*. 2013;40:30-48, DOI: 10.3109/1040841X.2012.754401
2. Rosenberg M, Patterson R, Mintzer R et al. Clinical and immunological criteria for the diagnosis of Allergic Bronchopulmonary Aspergillosis. *Ann Intern Med* 1977;86:405-14.
3. Patterson R, Greenberger PA, Halwig JM et al. Allergic bronchopulmonary aspergillosis: natural history and classification of early disease by serologic and roentgenographic studies. *Arch Intern Med* 1986;146:916-8.
4. Greenberger PA. Allergic Bronchopulmonary Aspergillosis. *J Allergy Clin Immunol* 2002;110:685-92.
5. Agarwal R. Severe asthma with fungal sensitization. *Curr Asthma Allergy Rep*. 2011;11:403-13.
6. Agarwal MK. Skin testing and respiratory allergic disorders. *Indian J Chest Dis Allied Sci*. 1986;28:179-82.
7. Gupta RK, Chandra A, Gautam PB. Allergic Bronchopulmonary Aspergillosis – A Clinical Review. *J Assoc Physicians India*. 2012 Apr;60:46-51.
8. Al-mobeireek af, gad. El-rab mo, al-hedaithy ssa, alasali k, al-majed s, joharjy i. Allergic bronchopulmonary mycosis in patients with asthma: Period prevalence at a university hospital in Saudi Arabia. *Respir Med*. 2001;95:341-7.
9. Donnelly SC, McLaughlin H, Bredin CP. (1991). Period prevalence of allergic bronchopulmonary mycosis in a regional hospital outpatient population in Ireland 1985–88. *Ir J Med Sci* 160:288–90.
10. Sarkar A, Mukherjee A, Ghoshal AG, Kundu S, Mitra S. Occurrence of allergic bronchopulmonary mycosis in patients with asthma: an eastern India experience. *Lung India* 2010;27:212-6.
11. Ishiguro T, Noboru T, Ryuji U, Yuri B, Eriko K, Yoich K, et al. Diagnostic criteria that can most accurately differentiate allergic bronchopulmonary mycosis from other eosinophilic lung diseases: A retrospective, single-centre study. <http://dx.doi.org/10.1016/j.resinv.2016.01.004>
12. R. Agarwal, A. Chakrabarti, A. Shah, D. Gupta, J. F. Meis, R. Guleria, R. Moss, D. W. Denning For the ABPA complicating asthma ISHAM working group. *Clinical & Experimental Allergy*, 2013;43:850–873.