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ASPERGILLUS HYPERSENSITIVITY AND ALLERGIC BRONCHOPULMONARY ASPERGILLOSIS IN BRONCHIAL ASTHMA PATIENTS

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Abstract: Introduction: Allergic bronchopulmonary aspergillosis (ABPA) is a pulmonary disease that results from hypersensitivity to aspergillus antigens mostly due to *A. fumigatus*. The majority of cases occur among people with asthma. It is the most frequently recognized manifestation of allergic aspergillosis, is an indolent disease with a protracted course, occurs worldwide, and is now seen as an important emerging disease in India. Materials and Methods: Three hundred cases in age group 15 to 65 years presenting with bronchial asthma in OPD were included in the study. Out of 300 patients included in the study Aspergillus skin test was performed on 260 patients. Patients were classified to have AH if the skin test was positive. The diagnostic criteria (Major and Minor) as given by Rosenberget *al* were followed to make the diagnosis of ABPA. Results: Out of 300 cases 154 were males and 146 were females. 31% patients were diagnosed to have A Hand 24% had ABPA Fungal growth was obtained in 23/64 (36%) sputum specimens, of which *A. flavus* was most common followed by *A. fumigatus*. Conclusion: This study demonstrated that many asthma patients were highly sensitized to Aspergillus antigen. Incidence of ABPA was 24% which was significant. Early diagnosis can prevent the complications like bronchiectasis, lung fibrosis and in a minority of patients, end-stage lung disease and respiratory failure.

Keywords: Asthma, Allergic Bronchopulmonary Aspergillosis, Skin Test, Total IgE



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INTRODUCTION

Aspergillus, a genus of spore forming fungi which is ubiquitous, occur worldwide and affects the respiratory tract in many ways. The spectrum of *Aspergillus* associated respiratory disorder comprises three well defined clinical categories: allergic manifestations, saprophytic colonization of the respiratory tract and invasive disseminated disease. ^[1] There are four distinct clinically recognizable forms of hypersensitivity induced respiratory disorders caused by *Aspergillus* i.e. allergic bronchopulmonary aspergillosis (ABPA), allergic aspergillus sinusitis, IgE-mediated asthma and hypersensitivity pneumonitis. ^[2] Amongst the allergic aspergillosis disorders, ABPA is the most recognized form.

A hypersensitivity mediated lung disease, ABPA predominately affects patients with asthma and cystic fibrosis. However, the precise incidence of ABPA in patients with asthma is not known. Approximately 2% of patients with asthma and 1 to 15% of patients with cystic fibrosis develop ABPA. ^[3] The major and minor diagnostic criteria for the diagnosis of ABPA have evolved over time. ^[4-6] However, The lack of a uniform diagnostic criterion and standardized tests has further hampered efforts on this score. ^[7]

ABPA is under diagnosed in India. As chest x-rays are not routinely done in asthma patients, there is lack of awareness of ABPA and the myco-serological tests required for diagnosing ABPA are not widely available. Therefore, the aim of the study was to determine the incidence of aspergillus hypersensitivity (AH) and occurrence of ABPA in asthma patients & also to study the clinical, mycological and serological aspects of allergic bronchopulmonary aspergillosis.

MATERIAL AND METHODS

This was a prospective study conducted in the Department of Microbiology, Dayanand Medical College and Hospital. A total of 300 consecutive cases of bronchial asthma which presented to OPDs and IPDs of DMC&H were included in the study. Pregnant women, patients with age less than 15 years & more than 65 years and already diagnosed cases of ABPA were excluded from the study. The demographic profile and clinical data were collected. Out of 300 patients included in the study *Aspergillus* skin test was performed on 260 patients. Patients with *Aspergillus* skin test positive were labeled as AH. ^[8] The diagnostic criteria (Major and Minor) as given by Rosenberget *al* ^[4] were followed to make the diagnosis of ABPA. The major criteria included: bronchial asthma, immediate skin test reactivity to *Aspergillus* antigen, elevated total serum IgE levels, pulmonary infiltrates on chest X-ray central bronchiectasis, peripheral blood eosinophilia, positive serum precipitins against *Aspergillus* antigen. The minor criteria included: expectoration of golden brownish sputum plugs, positive sputum culture for *Aspergillus* species and late skin reactivity to *Aspergillus*. ^[4,9] A minimum of four major criteria and one minor

criterion were fulfilled before making the diagnosis of ABPA. High Resolution Computerised Tomography was done (HRCT) (if required).

Aspergillus skin test (Intradermal test)

Out of 300 patients included in the study *Aspergillus* skin test was performed on 260 patients. Skin test was performed using *Aspergillus* antigen kit commercially available from Alcure Pharmaceuticals (India). The test was performed as per the kit manual with 5 aeroallergens including *A.flavus*, *A.fumigatus*, *A.niger*, *A.tamarii* and *A.versicolor*. Intradermal testing was performed with 0.01ml of the allergens (1:500 w/v) along with negative control (Phosphate buffer saline) and positive control (Histamine-15µg/ml). 0.01ml of test allergens, buffered saline (negative control) and histamine (positive control) solution were injected intradermally on ventral aspect of the forearm raising bleb of 2 to 3 mm. Separate syringes were used for each antigen. Minimum distance kept between two injection sites was 5 cm to avoid overlapping of reactions.

The results of the intradermal test were interpreted after 1 hour and then after 6 hrs. Test was read only when the negative control showed no wheal & erythema and the positive control was ≥ 6 mm. When test appeared similar to negative control, it was labelled as negative. Any test showing wheal more than positive control and erythema about 15 to 20 mm was taken as positive. When wheal and erythema developed within a minute and reached maximum size after 10-20 minutes and resolved within 1-2 hrs, it was considered as type I reaction. Any amount of subcutaneous oedema at the end of 6 hrs was considered as type III reaction.

Total IgE levels:

Serum sample was collected aseptically in a plain vial. The test was performed on Cobas e411 (Electrochemiluminescence- ECL) a fully automated system. IgE levels were expressed in KIU/ml and more than 50 KIU/ml was taken as significant.

Microscopic examination and culture for fungus

Sputum samples were collected in a clean, leak proof, disposable, wide-mouthed container and were examined (smear and culture) for any pathogenic fungus. All the sputum samples were examined for fungal hyphae using 10% potassium hydroxide and were cultured using Sabouraud dextrose agar (SDA) with antibiotics and with/without cycloheximide. A set of two tubes- one with antibiotics (chloramphenicol 50mg/1L and gentamicin 20mg/1L) and cycloheximide (500mg/L) and other with antibiotics but without cycloheximide, was incubated at 25°C. Similarly, other set of two tubes was incubated at 37°C. The inoculated media were kept for a minimum period of three weeks. Tubes were examined daily during 1st week and on alternate days in 2nd and 3rd week. The growth obtained was identified on the basis of colony

morphology, pigment production and microscopic examination of lactophenol cotton blue (LCB) preparation.

Chest X-ray (PA view)

Chest radiographs were reviewed for pulmonary infiltrates and bronchiectasis. Fleeting shadows were considered as suggestive of ABPA. ^[10]

Peripheral eosinophilia

Peripheral blood eosinophilia >1000 cells/ μ l and >500 cells/ μ l were taken as significant for ABPA and AH respectively. ^[11]

High Resolution Computerized Tomography (HRCT) (when required)

On CT, bronchiectasis was characterized by the signet ring and string of pearls appearances. ^[12]

RESULTS

A total of three hundred consecutive cases of bronchial asthma diagnosed on the basis of history and clinical presentation were included in the study. Table 1 depicts the demographic and clinical profile of the patient. Out of the total 300 patients enrolled for the study, 154 (51%) patients were males and 146 (49%) were females. The mean age group affected was 39 ± 15.34 years. Family history of asthma was present in 5% of the patients, 3.3% were smokers and 6.7% patients had taken anti tubercular treatment (ATT) in the past. All the patients presented with cough, however type of expectoration observed in these patients was variable. Most common type of expectoration observed in asthmatic patients was mucoid in nature (76%).

Table 2 shows the results of lab investigations done on these patients. Chest X-ray showed pulmonary infiltrates in 41 % of cases. Aspergillus (Intradermal) skin test was performed on 260 patients. Rest of the cases were either lost on follow up or the test was not performed on them due to non-compliance and invasiveness of the procedure. Out of the total cases on which skin test was done, 143 patients (55%) were positive for both type I and type III hypersensitivity reaction (biphasic reaction). The most common reaction was to *A.flavus* antigen (39.8%) followed by *A.fumigatus* (28.6%) then *A.tamarii* (11.8%), *A.niger* (10.4%) and *A.versicolor* (9%).

Total IgE level was significantly higher in 87% of cases i.e. >50 KIU/ml. (Table-2) AEC was significantly higher in 92% cases, of which 52% patients had levels >1000 cells/ μ l and 40% had levels between 500 to 1000 cells/ μ l.

In direct microscopic examination of the sputum samples using KOH mount, hyaline septate hyphae were found in 33% and budding yeast like cells with pseudohyphae in 28% of the samples. However, 39% of the samples tested did not reveal any fungal element.

Out of 64 sputum samples subjected to fungus culture, 42 (66%) showed fungal growth (*Aspergillus* species 23 and *Candida* species 19). Out of 23 *Aspergillus* species *A. flavus* was the most common followed by *A. fumigatus*.

Allergic bronchopulmonary aspergillosis (ABPA) & Aspergillus hypersensitivity (AH)

Out of the total cases on which skin test was done, 143 patients (55%) were positive for both type I and type III hypersensitivity reaction (biphasic reaction). Eighty of these patients were diagnosed to have AH whereas 63 had ABPA. However, of the total 300 patients with asthma, 64 cases were labeled as having ABPA as per Rosenberg criteria. In 22 cases, five major and 2 minor (skin test type III and culture) were positive. In 41 cases five major and one minor (skin test type III) criteria were positive. One case had four major and one minor criteria positive (culture). (Table -3)

DISCUSSION

Despite the description of ABPA in India as early as 1971,^[12] this disease entity is still under recognized,^[9,13] although there are reports of ABPA complicating the course of severe asthma^[14] and presentation of ABPA as acute asthma.^[15] The development of ABPA in bronchial asthma is known to worsen asthma control^[16] and can cause repeated exacerbations, and was the prime reason for the conduct of this study. Despite several published series from various parts of the country, this disease is misdiagnosed as pulmonary tuberculosis^[9,17, 18] and has serious clinical implications as patients with ABPA often receive antitubercular therapy for a long time while lung damage continues to progress relentlessly.

In the present study, mean age of patients was 39+15.34 years and was slightly more common in males. Similar observation was reported by Chakrabarti *et al.*^[9] In contrast, a study by Aggarwal *et al* observed that females were more commonly affected.^[19] Asthma is well known to run in families but familial occurrence of ABPA remains a rarity.^[20,21] In our study family history was seen in 5% of the total patients. In a study conducted at a tertiary referral center, on 164 patients over a period of 22 years, familial occurrence was detected only in 4 (4.9%) pairs of first degree relatives. The prevalence of ABPA was evaluated in smokers and non smokers. Only 10/300 patients recruited in the study were smokers and 8 (5.6%) out of 143 skin test positive patients were smokers. In a similar study out of 176 ABPA patients 10 (8.1%) were smokers.^[17] In the present study cough was present in all patients with 76% of the patients having mucoid expectoration while 95% of the patients presented with wheeze and dyspnea/breathlessness

was seen in 86.7% patients. Mucopurulent expectoration has also been described by Chakrabartiet *al* and Prasad *et al* in 68.5% and 57.1% of the patients respectively and dyspnea was seen in all patients. ^[9,18]

Constitutional symptoms like fever and chest pain was not a significant finding in the present study in asthmatic patients as seen in 14.7% and 6% patients respectively. In AH patients pleuritic chest pain was found in 13.6% patients and fever in 25.9% patients whereas in ABPA patients pleuritic chest pain and fever was seen in 4.8% and 15.8% patients respectively. These symptoms were more commonly seen in AH patients as compared to ABPA patients.

Out of the 260 Aspergillus intradermal skin tests performed 143 (55%) came out to be positive for type I and type III hypersensitivity reaction and all had biphasic response. However in a previous study 97.6% patients were positive for type I hypersensitivity reaction and 69.1% patients were positive for type III reaction. ^[22] Further studies conducted showed that 56% of the cases had Type I skin reaction and 17% had biphasic response. ^[9] Long bottom and Pepys in 1964 demonstrated that 38% of their 238 asthmatic subjects had a positive skin test result for Aspergillus antigens. ^[23] Henderson *et al* found that 24% of their 39 asthmatic patients had skin test results that were positive for Aspergillus antigens. ^[24] Overall, Aspergillus skin test reactivity has been recorded in 16 to 38% of asthmatic patients from different parts of the world. ^[20-25]

Out of 143 skin test positive in the present study, 57 (39.8%) patients were sensitive to *A.flavus*, 41 (28.6%) were sensitive to *A.fumigatus*, 17 (11.8%) to *A.tamaritii*, 15 (10.4%) to *A.niger* and 13 (9%) to *A.versicolor*. Another study from United Kingdom reported that 16% of their 656 patients had a skin test that was positive for *A.fumigatus*. ^[25] In another study it was seen that 28.5% patients showed immediate hypersensitivity reaction to one or more Aspergillus antigens. Of these 97% patients were sensitive to *A.fumigatus*, 70% patients were sensitive to *A.flavus* and *A.tamaritii*, and 64% patients were sensitive to *A.niger*. Late cutaneous reactions were seen in 64% patients. ^[2] In the present study no subject was sensitive to more than one Aspergillus antigen.

Total IgE level is one of the major criteria given by Rosenberg *et al* to diagnose AH and ABPA. According to ISHAM working group the cut off level of total IgE is >1000 IU/ml for diagnosing ABPA and >500 IU/ml for diagnosis of AH. ^[5] In our study total IgE levels were performed in 229 patients, among them 84 (37%) patients had total IgE levels >500 IU/ml and 114 (50%) patients had levels >1000 IU/ml. The data regarding the correlation between IgE levels and AH/ABPA is limited, however in a previous study, comprising of 16 patients it has been reported that all the asthmatics had IgE>1000IU/ml. ^[18]

Absolute eosinophil count (>1000 cells/ μ L), one of the major diagnostic criteria, is often the initial diagnostic indicator in a patient with asthma. ^[1] In present study AEC levels were

between 500-1000 cells/ μ l in 40.3% patients and 52.3% patients had value >1000 cells/ μ l on the basis of ISHAM working group. Whereas in two other studies conducted in north India, the peripheral blood absolute eosinophil count was >1000 cells/ μ l in all patients and in 88% of patients respectively.^[14,18]

In the present study 64 sputum samples were screened for fungus culture in which 33% patients came out to be positive for *Aspergillus* with *A.flavus* as the commonest species. In a study conducted in a tertiary care centre sputum culture was positive in 63% and 69% patients respectively and *A.flavus* was the most common. Although *A. fumigatus* is the organism implicated in the causation of the disease in its original descriptions, *A.flavus* was more commonly isolated in these series.^[18,26]

Based upon various laboratory findings, in the present study aspergillus hypersensitivity (AH) was found in 80 patients (55%) and allergic bronchopulmonary aspergillosis (ABPA) was found in 64 (21%) patients, out of 260 patients on which skin test was performed. A study given by Maurya *et al*, out of 30 patients with skin reactivity to *Aspergillus* antigens, 8 (26.6%) fulfilled all of the major criteria for the diagnosis of ABPA.^[2] Henderson *et al* reported that, of their 46 asthmatic patients, 11% had definite allergic aspergillosis and 22% had probable allergic aspergillosis.^[24] Greenberger and Patterson evaluated 531 patients with asthma and observed ABPA in 32 patients (6%).^[27] Schwartz and Greenberger determined that 28% of *Aspergillus* skin test-positive asthmatic subjects had ABPA;^[28] Eaton *et al* demonstrated the presence of ABPA in 25% of *Aspergillus* skin test-positive patients and supported the use of skin testing as a screening tool for ABPA in asthmatic subjects.^[29] In a study by R. Aggarwal *et al* 29 of 57 (50.9%) patients were diagnosed to have AH as evidenced by a positive *aspergillus* skin test whereas 22 out of the 57 patients (38.6%) were diagnosed to have ABPA.^[8] In a meta-analysis (20 studies, 5092 asthmatics) given, they demonstrated the prevalence of AH and ABPA in outpatients with bronchial asthma, was 28% and 17.9% respectively.^[30] This study further revealed even a higher prevalence of ABPA in patients with acute severe asthma. In a study from PGI Chandigarh there was a high prevalence rate of ABPA (27.2%). Chakrabarti *et al*, observed eighty- nine cases of ABPA over 8 years (1991-98), in comparison with the 35 cases reported in the previous 5 years (1986-90).

Early diagnosis and therapy are important for ABPA cases to prevent the progression of the disease to fibrosis of the lung.^[9] The under diagnosis of ABPA can be ascribed, in part, to a lack of routine skin testing in most asthma clinics.^[31] Another important reason is the lack of uniform diagnostic criteria, and thus the variations in diagnostic criteria between centers may be an equally important source of diagnostic uncertainty and more patients with serological ABPA were prevalent in the acute severe asthma group (45.4%) vs. the outpatient bronchial asthma group (23.9%). This is probably because patients with serological ABPA will be missed

on the chest radiograph and this condition cannot be diagnosed unless active serological screening is performed. ^[21] Thus, based on our study results; asthma clinics should adopt a policy for screening all patients with asthma for ABPA with an *Aspergillus* skin test (and probably IgE levels). Further work-up for ABPA is warranted only if screening tests are positive.

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Table 1—Demographic and clinical profile of the patients suspected of ABPA

Age (yr)	Mean(SD) - 39 (15.34)
Sex	
Male	154(51%)
Female	146(49%)
Family history of Asthma	15(5%)
Clinical features	
Wheeze	287(95.7%)
Cough	300(100%)
Dyspnea	260(86.7%)
Fever	61(14.7%)
Pleuritic Chest Pain	18(6%)
Type of Expectorations	
Mucoid	228(76%)
Mucopurulent	65(22%)
Purulent	7(2%)
History of ATT	20(6.7%)
Cigarette smoking	10(3.3%)
Occupation	
House wives	112(37.3%)
Students	62(21%)
Farmers	61(20%)

Office workers	28(9.3%)
Buissnessman	17(5.7%)
Labourers	8(2.7)
Other	12(4%)

Table 2- Diagnostic investigations in suspected cases of ABPA

Total IgE levels - KIU/mL(n=229)	
<50	31 (13%)
50-100	84(37%)
>100	114(50%)
Absolute eosinophil count, cells/μL (n=216)	
<500	16(8%)
500-1000	87(40%)
>1000	113(52%)
Aspergillus skin test(n=260)	143 (55%) – Both type I & type III
A.flavus	57(39.8%)
A.fumigatus	41(28.6%)
A.tamari	17(11.8%)
A.niger	15(10.4%)
A.versicolor	13(9%)
Chest X ray (n=300)	123(41%)
Culture Positive for Aspergillus (n=64)	23(36%)

Table 3: Distribution of various findings (clinical and laboratory) in patients labeled with ABPA (n=64), on the basis of Rosenberg criteria

Major criteria	Number of cases
Bronchial Asthma	64
Aspergillus intradermal skin test type I	63
AEC (>1000 cells/ μ L	64
Chest x ray (pulmonary infiltrates)	64
Total IgE>100 KIU/mL	64
Minor criteria	Number of cases
Aspergillus intradermal skin test type III	63
Sputum sample for fungus culture	22