Original Article

CLINICO-IMMUNOLOGICAL AND MICROBIOLOGICAL STUDY OF BRONCHIAL ASTHMA WITH SPECIAL REFERENCE TO ASPERGILLUS

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ABSTRACT

Background: Allergic bronchopulmonary aspergillosis (ABPA) is a hypersensitivity reaction to *Aspergillus* mycelia that colonize in the bronchi. The susceptibility of asthmatic patients to develop ABPA is not fully understood. **Aim:** To evaluate the clinical, immunological, microbiological and radiological features of *Aspergillus* infection in patients with asthma. **Methodology:** 50 Patients with Bronchial Asthma as cases and 20 age and sex matched non asthmatic individuals as controls were selected for study from OPD of Department of TB and Respiratory Diseases, University Hospital, Institute of Medical Sciences, Banaras Hindu University, Varanasi during the period of January 2013 to July 2014. Various clinical, immunological, microbiological and radiological features of aspergillus infection in patients were recorded. The observed data was analyzed and intergroup comparisons were done. **Result:** In patients of bronchial asthma with positive aspergillus the percentage of presence of breathlessness, cough, family history, allergic rhinitis, history of ATT intake, AEC, IgE, IgG, BAL and CECT Thorax lied between 62.5% to 100% and most of these showed statistically significant difference. **Conclusion** The development of ABPA is probably the combination of many genetic susceptibility factors. Understanding of the genetic risks and immuno-pathogenesis of ABPA will lead to early diagnosis and improved treatment of ABPA.

Keywords: Aspergillus, Aspergillosis, Bronchopulmonary Aspergillosis (ABPA), Bronchial Asthma

Introduction

Aspergillus species are ubiquitous spore-forming fungi present in the environment that causes a variety of clinical syndromes, ranging from saprophytic colonization to extensive life threatening invasion with a multitude of intermediary conditions. There are about 180 species of Aspergillus. Allergic bronchopulmonary aspergillosis is the best-known allergic manifestation of Aspergillus-related hypersensitivity pulmonary disorders. ABPA is a hypersensitivity lung disease due to bronchial colonization by Aspergillus fumigatus that occurs in susceptible patients with asthma and cystic fibrosis (CF). ABPA was first reported by Hinson[1] and his colleagues and all major work on ABPA was done by Pepys et al and McCarthy.[2-3]

ABPA is a hypersensitivity reaction to Aspergillus mycelia that colonize in the bronchi. Asthma and cystic fibrosis are the common illnesses associated with ABPA.[4-5]

The prevalence of ABPA is reported to be 1-2% in asthmatics, 7-14% in steroid-dependent asthmatics, and 2-15% in cystic fibrosis (CF). Both environmental factors and a genetic predisposition may be present as far as the prevalence of the disease in asthmatics is concerned. The familial occurrence of ABPA supports the same. The HLA DR molecules, especially DR2, DR5, and possibly DR4 and DR-7, are associated with susceptibility to ABPA, while HLA-DRQ2 has shown to have resistance to ABPA. Type I Aspergillus cutaneous hypersensitivity has been reported in 16% to 50% patients of bronchial asthma by the experts from other countries. It is rather difficult to estimate the prevalence of ABPA because of lack of uniform diagnostic criteria and standardized tests.

By and large, there is a lack of awareness about ABPA among general practitioners and even medical specialists and many chest specialists. The mycoserological tests and investigations, like computed tomographic (CT) scan required for diagnosing ABPA are not widely available and are rather expensive and thus the diagnosis of ABPA is often delayed in our country and a fairly large number of APBA cases may be diagnosed in advanced stage, i.e., stages IV or V only. A high index of suspicion is required to establish the diagnosis of ABPA. Bronchial asthma patients having systemic complaints and/or a history of expectorating golden brownish plugs should also be investigated for ABPA. Till a few years ago, bronchography was the "gold standard" investigation for detecting bronchiectasis in ABPA, but in recent years, high resolution CT (HRCT) scan has emerged as the investigation of choice for this purpose. The goals of treatment of ABPA are: (a) to detect and treat exacerbations promptly so as to prevent or minimize the occurrence of bronchiectasis that may develop at the site of infiltrates; (b) to manage associated asthma or irreversible lung disease; (c) to exclude ABPA in family members, and (d) to identify a potential fungal source in the environment. Oral glucocorticoids are currently the treatment of choice, as they suppress both the immune response and inflammation. Different studies and reviews have recommended different dosages and duration of therapy. In these dose schedules, high relapse rate and steroid-dependence was observed.

The objective of the present study was (i) to study the relationship between *Aspergillus* and bronchial asthma, (ii) to evaluate the clinical, immunological, microbiological and radiological features of Aspergillus infection in patients with asthma.

Methodology

The present study was conducted in the Department of TB and Respiratory Diseases, Institute of Medical Sciences, Banaras Hindu University, Varanasi in collaboration with Department of Pathology and Microbiology during the period of January 2013 to July 2014. The hospital serves as a tertiary care center for the patients coming from eastern part of Uttar Pradesh, adjoining area of Bihar, Jharkhand, Madhya Pradesh and Chhattisgarh. Patients selected from those admitted in the Department of Tuberculosis and Respiratory Diseases.

Inclusion criteria:

(1) Any gender,

(2) Patients willing to provide signed informed consent,

(3) Clinical history suggestive of bronchial asthma,

(4) PFT criteria of airflow limitation and reversibility,

(5) Age group between 15 years and above.

Exclusion criteria:

(1) HIV,

(2) COPD,

(3) Bronchiectasis,

(4) ILD,

(5) Pulmonary koch's,

(6) Post TB sequelae,

(7) Patients on Chemotherapy/ immunotherapy,

(8) Chronic kidney diseases/chronic liver diseases,

(9) Diabetes mellitus

Specific tests for Aspergillus:

(1) Total serum IgE,

(2) IgG specific for Aspergillus,

(3) Sputum/BAL for Aspergillus

Those with post bronchodilator reversibility of >200 ml and 12% improvement in FEV1 were taken as cases for the study and others for controls. Other investigations like HIV and blood sugars were done to rule out immuno-compromised states. A chest X-Ray to rule out other conditions mimicking asthma, sputum and BAL samples were collected from the cases and controls and subjected for microscopic examination for Aspergillus fungal hyphae. Routine laboratory investigations were done for all the subjects.

There were a total of 50 patients recruited who received the diagnosis of Bronchial Asthma as cases and 20 controls.

All data were analyzed by using a statistical software. Results were expressed as Mean ± SD. Differences with p value less than 0.05 were considered as statistically significant.

Results:

Fifty patients with Bronchial Asthma (confirmed with post bronchodilator reversibility of >200 ml and 12% improvement in FEV1 on pulmonary function tests) and 20 age and sex matched non asthmatic individuals as controls were selected for study.

In this study among the 50 patients, 7 were below or equal to the Age of 20 yrs and 7 were above 50 yrs of age. Most of the patients were of middle age. The youngest patient was 17 years old and the oldest patient was 75 years old. The percentages of males and females in cases and controls were 66, 34 and 70, 30 respectively.

Mean and standard deviation of age, height, weight, BMI, blood pressure, pulse, respiration rate, TLC, DLC, haemoglobin, urea and creatinine were determined for both the groups and intergroup comparison of means using independent sample t-test resulted not statistically significant difference for all these variables except respiration rate (RR) and haemoglobin **(Table-1).**

All patients reported episodic breathlessness whereas, cough, expecto-ration and chest pain were reported by 66%, 34%, 18% cases respectively. The intergroup comparisons for breathlessness, cough & expectoration showed statistically significant difference. The history of hemoptysis was observed in 10% patients of bronchial asthama. This shows the hemoptysis is less commonly associated with asthma. If there is hemoptysis in asthmatics other causes should be excluded like LRTI, TB, Post TB sequele and of course Aspergillus infection. 34 out of 50 patients (68%) were having the family history of bronchial asthma.

Table -1: Some clinical parameters of subjects along with, age, height, weight, and BMI

Parameters	Mean ± SD		Intergroup Comparison t-test	p-value
	Case (n=50)	Control (n=20)		
Age	36.80±15.20	39.45±13.62	t = 0.678	0.500
Height	165.34±6.54	162.15±5.94	t = 1.889	0.063
Weight	55.98±8.09	55.20±7.64	t = 0.370	0.713
BMI	20.45±2.53	20.95±2.32	t = 0.768	0.445
SBP	128.76±13.61	126.60±16.35	t = 0.566	0.574
DBP	81.28±7.64	79.70±7.90	t = 0.774	0.442
Pulse	81.32±10.48	77.60±10.16	t = 1.352	0.181
RR	18.80±2.68	14.70±1.49	t = 6.436	0.000
Hb	12.14±2.45	10.83±1.66	t = 2.188	0.032
TLC	9566.0±382.45	8505.0±298.60	t = 1.112	0.270
Neutrophil	58.32±10.38	55.60±10.23	t = 0.994	0.324
Lymphocyte	32.10±10.71	37.00±11.51	t = -1.692	0.095
Eosinophil	7.40±9.91	4.75±1.83	t = 1.182	0.241
Monocyte	2.18±1.59	1.55±.99	t = 1.634	0.107
Urea	29.58±12.67	30.25±9.87	t = 0.212	0.833
Creatinine	0.96±.30	0.96±.20	t = 0.054	0.957
OT	41.46±26.77	39.00±20.43	t = 0.369	0.713
PT	37.60±20.87	42.95±21.93	t = 0.955	0.343

BMI= Body Mass Index; SBP= Systolic Blood Pressure; DBP- Diastolic Blood Pressure; RR = Respiratory rate; Hb= Haemobglobin; TLC = Total Lymphocyte Count

Table-2 : Presence of various sign and symptoms

Type of Sign and Symptoms of bronchial asthma	Sign and Symptoms Present/Positive (in percent)		
	Case (n=50) %	Control (n=20) %	Inter group comparison
Episodic Breathlessness	100	0	P<0.001 *
Cough	66	15	P<0.001 ^
Expectoration	34	5	P=0.012 ^
Hemoptysis	10	0	P=0.312 *
Chest Pain	18	0	P= 0.052 *
Family History	68	5	P<0.001 ^
Allergic Rhinitis	50	5	P<0.001 ^
Other atopic illnesses	22	0	P=0.027 *
Historty of ATT intake	32	5	P=0.028 *
X-ray chest PA view Fleeting Opacities	10	0	P=0.312 *
CECT Thorax Central Bronchiectasis	10	0	P=0.312 *
Sputum for Aspergillus	16	0	P=0.095 *
BAL Fluid for Aspergillus	10	0	P=0.312 *
Absolute Eosinophils Count >1000/mm3	18	0	P=0.042 ^
Total serum IgE >100 IU/ml	58	15	P=0.013 ^
IgG Specific for Aspergillus >8 U/ml	56	30	P=0.280 ^

*Fisher's Exact test, ^ Chi Square test

This shows the genetic association of the disease. 50% had history of allergic rhinitis which proved "one airway one disease" hypothesis. Whereas, 22% had history of other atopic illnesses like atopic dermatitis, allergic sinusitis etc. this showed the environmental association of the disease. The history of ATT intake in the past was in 32% cases and it was observed that patients with radiological shadows were also prescribed anti tubercular drugs, sometimes in the course of their illness. The intergroup comparison for family history of bronchial asthma, history of allergic rhinitis, other atopic illnesses and ATT intake showed statistically significant difference. In most of the patients chest X-ray is normal or prominent broncho-vascular markings and in few patients there is fixed opacities. In 10% patients serial chest x rays PA view shows fleeting opacities predominantly in upper zone. CECT Thorax of 10% cases showed central bronchiectasis. Out of 50 asthmatic patients 8 (16%) were found to be Aspergillus positive in sputum. This is confirmatory but sometimes due to sample contamination it may be less reliable. Further, 10% were found to Aspergillus positive in Broncho Alveolar Lavage. Absolute Eosinophils Count >1000/mm3 was observed in 18% cases and total serum IgE >100 IU/ml was found in 58% cases of bronchial asthma and 15% subjects of control group. IgG specific for Aspergillus >8 U/ml was observed in 56% cases and 30% control subjects **(Table-2).**

Table- 3: Various characteristics of bronchial asthma cases with positive *Aspergillus*

Characteristics	**Presence in percent (among positive Aspergillus cases n=8)**
Episodic breathlessness	100%
Cough	62.5%
Expectoration	50%
Hemoptysis	50%
Chest pain	50%
Family history	75%
Allergic rhinitis	62.5%
Other atopic diseases	37.5%
History of ATT intake	87.5%
Absolute eosinophis count >1000 cells/mm^3	100%
Total serum IgE >100 IU/ml	100%
IgG specific for *Aspergillus* >8 U/ml	100%
Sputum positive for *Aspergillus*	100%
BAL positive for *Aspergillus*	62.5%
Fleeting opacities on chest X-ray PA view	62.5%
Central bronchiectasis on CECT Thorax	62.5%

Further, the presence of various above characteristics among those bronchial asthma cases who had positive Aspergillus was recorded and shown in **Table-3.**

Discussion:

ABPA is the best recognized manifestation of Aspergillus associated hypersensitivity to Aspergillus antigens in patients with long standing atopic asthma. The true prevalence of ABPA in patients of bronchial asthma is still not known. This may be due to the lack of a uniform diagnostic criterion and standardized tests. Earlier, the disease was thought to be a rarity but it has now become evident that ABPA is an important cause of significant lung damage.[6] Diagnosis of Aspergillus in bronchial asthma or ABPA was made on major and minor criteria laid down by Rosenberg .[7] Based on this, 8 patients (16%) of bronchial asthma had ABPA which included 6 males and 2 females. Their mean age was 49.5 years (20 to 75 years) and mean duration of illness was 11.3 years (7 years to 18 years) which is in correlation with previous studies.[8-9] Clinical profile of these patients showed that episodic breathlessness was predominant symptom presented in all 50 patients. In stable asthmatics there is above normal respiratory rate. As severity increases respiratory rate and pulse rate also increases. The prevalence of Aspergillus in asthmatic patients, in present study was 16% which was near to the estimate reported in other studies.[10-12] Elevated total serum IgE or IgG specific for Aspergillus are strong indicator of ABPA, but in our study, out of 29 patients with positive specific IgE to Aspergillus fumigatus, and 28 patients with positive specific IgG to A. fumigatus, only 8 (27.5% and 28.5% respectively) were found to have ABPA, which again highlights the fact that, like chest radiograph and CECT Thorax, these serologic tests also lack reliability for diagnosis of ABPA if performed alone. Efforts should be made to improve the awareness level about this disease, among general physicians who frequently confuse it with pulmonary tuberculosis, for timely diagnosis and institution of appropriate treatment so as to avoid misuse of anti-tubercular drugs and prevention of end stage irreversible lung damage.

Conclusion:

Bronchial asthma is a disease of airflow obstruction, most common in middle age groups. Episodic breathlessness is predominant symptom followed by cough, expectoration and chest tightness. In few cases hemoptysis and chest pain are also present. Environmental and genetic factors both play a role in pathogenesis. ABPA is a hypersensitivity reaction to Aspergillus mycelia that colonize in the bronchi. ABPA is associated with increased absolute eosinophils count, increased total serum IgE and increased IgG specific for Aspergillus. The susceptibility of asthmatic patients to develop ABPA is not fully understood. Serologic tests are less reliable for diagnosis of ABPA if performed alone. The true prevalence of ABPA in patients of bronchial asthma is still not known due to the lack of a uniform diagnostic criteria and standardized tests. A high index of suspicion is to be required to diagnose ABPA as early as possible, so that aggressive treatment to be given. This will prevent further lung damage and end-stage fibrosis. Oral glucocorticoids are currently the treatment of choice for ABPA. Further trials are needed to optimize the dose and duration of steroid therapy. Role of antifungal agents is being explored.

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