



Clinical significance of *Aspergillus* sensitisation in bronchial asthma

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Summary

Numerous studies have suggested a link between fungal sensitisation and severity of asthma. However, few studies have specifically evaluated the relationship between *Aspergillus* sensitisation and asthma severity. This study was aimed at investigating the clinical significance of *Aspergillus* sensitisation in asthma. In this prospective cross-sectional study, patients with asthma were subjected to pulmonary function test and an intradermal *Aspergillus* skin test (AST) apart from a detailed clinical history and physical examination. Assessment of asthma severity was carried according to the Global Initiative for Asthma (GINA) recommendations, Asthma Control Test (ACT) and the mini Asthma Quality of Life Questionnaire (mini AQLQ). Based on AST, the cases were dichotomised into *Aspergillus*-sensitive and AST-negative groups. There were 417 (193 males, 224 females; mean age, 34 years) asthmatic patients of whom 219 (52.5%) showed *Aspergillus* sensitisation. The severity of disease as per the GINA criteria and the dose of ICS required for asthma control were similar in the two groups. The *Aspergillus*-sensitive group had poorer pulmonary function than the AST-negative group [AST positive vs. negative: percentage predicted mean (SD) forced expiratory volume in the first second : 73.1(23.8) vs. 77.9(22.7), $P = 0.04$; mean (SD) FEV₁/forced vital capacity (FVC) ratio: 68.2(13.3) vs. 74.3(15.7), $P = 0.0001$]. The mini AQLQ scores were similar in the two groups. In the ACT, the numbers of patients in the domain pertaining to nocturnal awakenings were significantly higher in the *Aspergillus*-sensitive group ($P = 0.014$). There was a weak association between *Aspergillus* sensitisation and severity of asthma. Whether *Aspergillus* sensitisation is causally linked to asthma severity remains to be seen.

Key words: Fungal sensitisation, asthma, SAFS, *Aspergillus*, ABPA, allergic bronchopulmonary aspergillosis.

Introduction

Asthma is an inflammatory disease of the airways characterised by airway hyperresponsiveness and air-flow limitation.¹ It is a significant public health problem with more than 300 million affected individuals.² The severity of asthma varies from patient to patient and the reasons for this are not fully understood. About two-thirds of asthmatic patients are atopic to common

allergens,^{3–5} and individuals with severe asthma have a greater degree of atopy than other asthmatic patients.⁶ Numerous environmental factors are known to worsen asthma control with a common factor being the continuing exposure to an allergen. The most frequent environmental allergens implicated in worsening asthma control are house dust mite, animal dander from pets and environmental fungi.^{1,7}

The link between fungi and asthma has been extensively investigated.⁸ However, the role of fungal allergens as the primary extrinsic factor leading to asthma severity has been incompletely explored. This is possibly because exposure to fungi is universal in contrast to other extrinsic agents such as animal dander. The role of house dust mite as the dominant exogenous agent for asthma in adults has been

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questioned by the recently published randomised cohort mite avoidance study.⁹ Hence, fungi as the dominant extrinsic trigger for asthma are now far more widely being considered.

Fungi can be linked to the severity of asthma in many ways such as inhalation of fungal spores, through fungal sensitisation (defined as presence of immediate cutaneous hyperreactivity to fungal antigen or increase in specific IgE antibodies to a particular fungus) or through causation of allergic bronchopulmonary mycosis, a severe degree of fungal sensitisation with resultant irreversible bronchopulmonary damage.¹⁰ In the recently published European Community Respiratory Health Survey (ECRHS), the frequency of sensitisation to *Alternaria* or *Cladosporium*, or both, was a powerful risk factor for severe asthma in adults.¹¹ Previous studies have shown that exposure to environmental fungi increased the risk of death from asthma¹² and also acute attacks of asthma requiring intensive care unit (ICU) admission.¹³ However, few studies have specifically evaluated the relationship between *Aspergillus* sensitisation and severity of asthma.^{14,15}

In previous studies, we had demonstrated a high prevalence of *Aspergillus* sensitisation and allergic bronchopulmonary aspergillosis (ABPA) in asthmatic patients in the North Indian population.^{16–19} The aim of this study was to ascertain the clinical significance of *Aspergillus* sensitisation in patients with asthma without ABPA.

Material and methods

This was a prospective cross-sectional study conducted between July 2008 and November 2009 in the Chest Clinic of this institute. The study was approved by the Institute Ethics Committee, and a written informed consent was obtained from all subjects prior to enrolment.

Patients

Patients were included in the study if they were aged 15 years or more and met two of the following criteria for the diagnosis of bronchial asthma: (i) history of recurrent or episodic attacks of chest tightness, breathlessness and cough (especially nocturnal); (ii) documented wheeze on auscultation of the chest and (iii) obstructive defect on spirometry with or without the presence of bronchodilator reversibility. The following were the exclusion criteria: (i) age more than 65 years; (ii) diagnosis of ABPA or chronic obstructive pulmonary disease; (iii) pregnancy; (iv) other immunosuppressive

states such as chronic liver disease, chronic renal failure, uncontrolled diabetes mellitus, chronic heart failure, immunosuppressive drugs other than glucocorticoids for controlling asthma and (v) failure to give informed consent.

Methods

A comprehensive clinical history was elicited, and all patients underwent detailed physical examination. The following details were recorded: age, gender, residence, presence of pets, tobacco smoking, symptoms (cough, expectoration, wheeze, dyspnoea, chest tightness) and family history of asthma/allergy. Assessment of severity of asthma was carried according to the 2002 Global Initiative for Asthma (GINA) recommendations, which include the effect of treatment on disease severity.²⁰ In addition, the subjects were also required to fill in two self-administered questionnaires, the Asthma Control Test (ACT) and the mini Asthma Quality of Life Questionnaire (mini AQLQ, Hindi version). The details of pulmonary function test and an intradermal test for *Aspergillus* were also recorded.

The Aspergillus skin test

The *Aspergillus* skin test (AST) was performed using an *Aspergillus* antigen that was prepared as follows: (i) the reference strain of *Aspergillus fumigatus* (ATCC 204305) obtained from stock culture was incubated at 25 °C in two Sabouraud's dextrose agar slants; (ii) after obtaining typical growth upon incubation, the organism was inoculated in the glucose asparagine broth and incubated at 30/25 °C for 3–4 weeks. The culture was then treated with merthiolate and then checked for microbial contamination; (iii) the broth culture was then filtered using Seitz filter and then sterilised using Millipore filters; (iv) the precipitate was dissolved in distilled water and dialysed against distilled water. The dialysate was then freeze dried after ensuring that it is endotoxin (limulus amoebocyte lysate assay) and bacteria (blood agar, brain heart infusion broth and thioglycollate broth) free; (v) the total protein content was assessed by the Lowry method and the final concentration of the protein was standardised to 100 PNU ml⁻¹ (1 PNU = 0.00001 mg ml⁻¹).

The intradermal skin test was performed as follows: (i) the skin test was performed by injecting 0.2 ml of the *Aspergillus* antigen (100 PNU ml⁻¹) intradermally in the forearm. For negative control, 0.2 ml of phosphate-buffered saline was injected intradermally in the other forearm. No positive control was used; (ii) the injection

site was examined every 15 min for 1 h. *Aspergillus* sensitisation was defined if a wheal and erythema developed within 1 min, reached a maximum after 10–20 min and resolved within 1 h; with the antigen arm skin reaction diameter being at least 8 mm more than that on the control arm.

Pulmonary function test

All subjects performed spirometry on a dry rolling seal spirometer (Spiro RS-232; P.K. Morgan Limited, Kent, UK). Spirometric indices such as forced expiratory volume in the first second (FEV₁), forced vital capacity (FVC) and FEV₁/FVC ratio were measured before and after administration of 400 µg of inhaled salbutamol using the American Thoracic Society guidelines.²¹ The highest measurements from among the three technically acceptable and reproducible manoeuvres were expressed at body temperature and pressure saturated with water vapour. Age, gender, height and spirometric data were recorded for all patients using software previously developed in our laboratory.²² The spirometer was frequently calibrated to ensure performance. Predicted values for FVC, FEV₁ and FEV₁/FVC ratio were generated using previously defined prediction equations.²³ Bronchodilator reversibility was considered significant if, after inhalation of 400 µg of salbutamol, the FEV₁ and/or FVC increased by ≥12% and 200 ml.²¹

The Asthma Control Test

The ACT is a validated, self-administered questionnaire with five items that has been developed as an easy method for patients and clinicians to assess symptoms (daytime and nocturnal), use of rescue medications and the effect of asthma on daily functioning. Each item includes five response options corresponding to a five-point Likert-type rating scale. In scoring the ACT, responses for each of the five items are summed to yield a score ranging from 5 (poor control of asthma) to 25 (complete control of asthma)²⁴. An ACT score of 19 or less is suggestive of difficulty in controlling asthma.²⁴

The mini AQLQ, Hindi Version

The mini AQLQ (Hindi version) is also a validated, self-administered, disease-specific quality of life (QoL) questionnaire.²⁵ The questionnaire is a shorter and simpler version of the original AQLQ and consists of 15 items divided into the following categories: symptoms, activity limitation, emotional function and environmental stimuli.²⁶ The responses to individual items are scaled from 1 to 7, with one representing the worst and 7

representing the best condition. The weighting, scoring and analysis of mini AQLQ are similar to AQLQ in all respects.

For the purpose of the study, the cases were dichotomised based on the AST into *Aspergillus*-sensitive and AST-negative groups.

Statistical analysis

Data are presented in a descriptive fashion as mean (SD), median (IQR) or number (percentage). The normalcy of distribution was evaluated using Kolmogorov–Smirnov test. The differences between continuous variables were analysed using Mann–Whitney *U*-test (for median values) if not normally distributed and using Student's *t*-test (for means) if normally distributed. The differences between categorical variables were analysed using chi-square test. *P* values <0.05 were considered statistically significant.

Results

During the study period 417 (193 males, 224 females) patients with bronchial asthma with a mean (SD) age of 34 (12.2) years were included. Of these, 219 (52.5%) patients demonstrated immediate cutaneous hyperreactivity to AST. Three hundred and seventy-four (89.7%) patients resided in the urban area and 398 (95.4%) patients used liquefied petroleum gas as the main source of fuel. Five (1.2%) patients kept pets at home and only one patient smoked tobacco. The baseline characteristic of the study population is shown in Table 1. Shortness of breath was the most common complaint, followed by wheeze, cough, chest tightness and sputum production. Frequent daytime and night-time symptoms were seen, respectively, in 21.6% and 18.7% of the patients. Sputum production and nocturnal symptoms were significantly higher in the *Aspergillus*-sensitive group, whereas rural residence was higher in the AST-negative group.

Assessment of severity between the two groups

The severity of the disease in the two groups is shown in Table 2. The severity of disease assessed by the GINA criteria was similar in the two groups. The dose of ICS required for asthma control was also similar between the two groups. The pulmonary function was worse in the AST-positive group, and this group had significantly lower percentage predicted mean FEV₁ and FEV₁/FVC ratio (Table 2) when compared with the AST-negative group.

Table 1 Demographic details of the study groups.

	<i>Aspergillus</i> sensitive (n = 219)	AST negative (n = 198)	P-value
Age in years, mean (SD)	34.7 (12.6)	33.5 (11.8)	0.34
Female gender	120 (53.6)	104 (46.4)	0.64
Body mass index, kg m ⁻² , mean (SD)	1.6 (0.18)	1.59 (0.18)	0.73
Rural residence	11 (5)	32 (16.2)	0.0001
Pets	3 (1.4)	2 (1)	0.74
Tobacco smoking	0	1 (0.5)	0.29
Duration of asthma in years, median (IQR)	5 (2–10)	5 (2–10)	0.89
Cough	177 (80.8)	166 (83.8)	0.42
Wheeze	180 (82.2)	170 (85.9)	0.31
Dyspnoea	216 (98.6)	191 (96.5)	0.15
Tightness	152 (69.4)	151 (76.3)	0.12
Sputum	100 (45.7)	67 (33.8)	0.014
Daytime symptoms			0.39
≤2 week ⁻¹	91 (41.6)	87 (43.9)	
>2 week ⁻¹ , <1 day ⁻¹	75 (34.2)	74 (37.4)	
Daily	53 (24.2)	37 (18.7)	
Night-time symptoms			0.014
≤2 month ⁻¹	89 (40.6)	108 (54.5)	
>2 month ⁻¹	40 (18.3)	30 (15.2)	
>1 week ⁻¹	48 (21.9)	24 (12.1)	
Frequent	42 (19.2)	36 (18.2)	
Family history of asthma	5 (2.3)	2 (1)	0.31
Family history of allergy	9 (4.2)	5 (2.5)	0.81
Wheeze on auscultation	184 (84)	165 (83.3)	0.85

All values are expressed as number (percentage) unless otherwise stated. The bold values are significant P-values.

The mini AQLQ scores were similar in the two groups (Table 2). However, in the ACT, the numbers of patients in the domain pertaining to nocturnal awakenings due to asthma symptoms were significantly higher in the *Aspergillus*-sensitive group (Table 2).

Discussion

The result of this study suggests a weak association between *Aspergillus* sensitisation and severity of asthma. *Aspergillus*-sensitive patients had more frequent nocturnal symptoms and poorer lung function compared with the AST-negative group. However, the QoL was similar in the two groups.

The link between non-*Aspergillus* mould-related fungal sensitisation and asthma was described more than a decade ago when *Alternaria* sensitivity was associated with an increased risk of respiratory arrest in asthmatic patients.²⁷ Subsequently, studies have suggested a link between *Alternaria* sensitivity and bronchial hyper-reactivity,^{28,29} and severe asthma.^{11,30} Other fungi like *Cladosporium*, *Epicoecum* and *Helminthosporium* and

others have also been implicated with severe asthma,¹¹ recurrent hospital admissions³¹ and ICU admissions for acute asthma.¹³ In a recently published study involving 181 asthmatic patients, sensitivity to at least one fungus (*Aspergillus*, *Alternaria*, *Cladosporium*, *Penicillium*, *Candida*) was seen in a higher proportion of asthmatic patients requiring multiple hospitalisations vs. those not requiring admissions.³² On the other hand, studies have demonstrated that *Aureobasidium*, *Helminthosporium* or *Trichophyton* sensitivity but not sensitivity to other fungi (including *Aspergillus*, *Alternaria* and *Cladosporium*) was associated with asthma severity and asthma hospitalisations.^{33,34}

Although some differences between studies can be partly attributed to difficulties in the standardisation of mould allergen extracts or skin testing techniques,³⁵ the fact remains that most studies have not investigated for *Aspergillus* sensitivity despite the concept that the best model for mould sensitisation is *Aspergillus*-related allergic phenomenon. Only with *Aspergillus* species does one encounter two extreme forms of immunological phenomenon, the *Aspergillus*-sensitive asthma and ABPA.^{16,17,19} Although other fungi can occasionally cause ABPA-like syndrome, the frequency is far less when compared with ABPA and is generally documented as isolated case reports.³⁶

The prevalence of *Aspergillus* sensitisation in asthma is variably quoted in different studies.¹⁰ In a systematic review, we demonstrated a pooled prevalence of *aspergillus* sensitivity in bronchial asthma of 28% [95% confidence intervals (CI), 24–34].¹⁸ However, few studies have clarified the clinical significance of *Aspergillus* sensitisation in asthma. Schwartz *et al.* [14] suggested that *Aspergillus* sensitisation was related to the severity of airway obstruction in 200 asthmatic patients, similar to our study. Another study involving 105 asthmatic patients found that *Aspergillus* sensitivity was associated with increased steroid usage, higher eosinophil counts and IgE levels but the lung function was not different from controls.¹⁵ However, this study had divided the patients into five groups, and thus was probably underpowered to draw any relevant conclusions because of small numbers in each group. In a study published from our centre, the prevalence of *Aspergillus* sensitivity was approximately 51% in patients with severe acute asthma admitted to an ICU vs. 39% in the outpatient bronchial asthma group.³⁷

We used intradermal skin testing with an indigenous antigen for the diagnosis of *Aspergillus* sensitisation. One can question the validity and standardisation of this antigen. However, the fact that the skin test was negative in all the 100 healthy controls in a previous

Table 2 Severity of the disease in the two groups.

	<i>Aspergillus</i> sensitive (n = 219)	AST negative (n = 198)	P-value
GINA severity (with treatment), no. (%)			
Intermittent	22 (10)	19 (9.6)	0.72
Mild persistent	19 (8.7)	24 (12.1)	
Moderate persistent	46 (21)	40 (20.2)	
Severe persistent	132 (60.3)	115 (58.1)	
Lung function on spirometry, mean (SD)			
FEV ₁ (% predicted)	73.1 (23.8)	77.9 (22.7)	0.04
FVC (% predicted)	84.9 (19.4)	84.10 (17.9)	0.66
FEV ₁ /FVC ratio	68.2 (13.3)	74.3 (15.7)	0.0001
ICS doses, median (IQR)	160 (0–1000)	400 (0–1000)	0.64
Mini AQLQ			
AQLQ symptoms	4.69 (1.26)	4.89 (1.13)	0.20
AQLQ activity limitation	5.64 (1.17)	5.77 (1.09)	0.32
AQLQ emotional function	4.35 (1.61)	4.47 (1.44)	0.55
AQLQ environmental stimuli	4.17 (1.32)	4.27 (1.22)	0.46
AQLQ total	4.71 (1.24)	4.85 (1.1)	0.41
Asthma control test			
ACT 1 – In the past 4 weeks, how much of the time did your asthma keep you from getting as much done at work, school or at home			
All of the time (1)	5 (2.3)	2 (1.0)	0.26
Most of the time (2)	16 (7.3)	8 (4.0)	
Some of the time (3)	25 (11.4)	30 (15.2)	
A little of the time (4)	52 (23.7)	39 (19.7)	
None of the time (5)	121 (55.3)	119 (60.1)	
ACT 2 – During the past 4 weeks, how often have you had shortness of breath			
More than once a day (1)	32 (14.6)	19 (9.6)	0.65
Once a day (2)	19 (8.7)	18 (9.1)	
3–6 times a week (3)	17 (7.8)	16 (8.1)	
Once or twice a week (4)	54 (24.7)	53 (26.8)	
Not at all (5)	97 (44.3)	92 (46.5)	
ACT 3 – During the past 4 weeks, how often did your asthma symptoms wake you up at night or earlier than usual in the morning			
4 or more nights a week (1)	19 (8.7)	3 (1.5)	0.015
2–3 nights a week (2)	14 (6.4)	19 (9.6)	
Once a week (3)	12 (5.5)	9 (4.5)	
Once or twice (4)	45 (20.5)	38 (19.2)	
Not at all (5)	129 (58.9)	129 (65.2)	
ACT 4 – During the past 4 weeks, how often have you used your rescue medication			
3 or more times a day (1)	11 (5)	2 (1)	0.12
1 or 2 times a day (2)	20 (9.1)	14 (7.1)	
2 or 3 times a week (3)	36 (16.4)	35 (17.7)	
Once a week or less (4)	19 (8.7)	24 (12.1)	
Not at all (5)	133 (60.7)	123 (62.1)	
ACT 5 – How would you rate your asthma control during the past 4 weeks			
Not controlled at all (1)	1 (0.5)	0	0.16
Poorly controlled (2)	28 (12.8)	13 (6.6)	
Somewhat controlled (3)	42 (19.2)	42 (21.2)	
Well controlled (4)	59 (26.9)	49 (24.7)	
Completely controlled (5)	89 (40.6)	94 (47.5)	

study suggests good quality of the antigen used.³⁸ Fungal sensitisation could also be diagnosed by measurement of fungus-specific IgE levels. Two studies have compared skin testing with specific IgE levels. One study found that specific IgE levels performed better than skin prick test (SPT), whereas the other study found that intradermal skin testing was superior to specific IgE levels.^{39,40} Although theoretically both intradermal

tests and SPTs should perform in a similar manner, it has been shown that intradermal tests are generally more sensitive than SPTs.^{18,41,42} The intradermal test is also believed to be associated with a higher complication rate than SPT. However, in our experience of more than 5000 intradermal tests, we have not encountered any complication.^{16,17,19,37,38,43,44} Ideally both intradermal testing and specific IgE levels should be used for the

diagnosis of fungal sensitisation. However, we generally use only the intradermal test because of the increased cost of specific IgE levels.

Another important issue with diagnosis of fungal sensitisation is the accuracy of the diagnosis. As there is no gold standard for the diagnosis of fungal sensitisation, there is no method to assess the accuracy of diagnosis. Most centres including ours use crude antigen for the diagnosis of fungal sensitisation. These antigens frequently cross-react with other antigens and may overdiagnose the prevalence of fungal sensitisation in asthma. For example, we currently use a crude antigen prepared from *A. fumigatus*, and it is possible that this antigen mix causes overdiagnosis of *Aspergillus* sensitisation in asthmatic patients. However, this situation is desirable because we primarily use this antigen as a screening test for ABPA and a positive test is followed by other diagnostic workup for ABPA.

Exposure to environmental fungal spores has been associated with worsening asthma symptoms, inferior lung function, hospital admissions and asthma-related deaths.^{12,45–49} This is understandable as a foreign particle is being inhaled in the lung. However, the mechanism of fungal sensitisation leading to worsening of asthma control remains unclear. Whether severe asthma is actually caused by fungal sensitisation or is just an association remains unknown. The greater degree of mould sensitisation in the severe asthma group could also be a manifestation of generalised increase in atopy among this group. However, as we did not measure sensitisation to other antigens, the present study cannot answer this question. Demonstration of effectiveness of antifungal therapy for asthma could also implicate fungal exposure in the pathogenesis of asthma.

What are the clinical implications of diagnosing fungal sensitisation in asthma? In our setting, the most important connotation is that a diagnosis of *Aspergillus* sensitisation is followed by a complete workup for ABPA as the prevalence of ABPA in *Aspergillus* sensitised asthmatic patients is approximately 40%.¹⁸ Two studies have also utilised azoles in patients with severe asthma with fungal sensitisation (SAFS). In one study, the authors used fluconazole in *Trichophyton*-sensitive severe asthmatic patients, and demonstrated reduced bronchial hyperresponsiveness to inhaled *Trichophyton*, decreased steroid requirement and increase in peak expiratory flow (PEF) with fluconazole therapy.⁵⁰ Denning *et al.* [2] used itraconazole in patients with SAFS and found that it was associated with modest improvements in QoL, rhinitis score, PEF and total IgE. The only

caveat is that azoles themselves have direct and profound immunological effects.^{51–57} Hence, it is not clear whether it was the anti-inflammatory or the antifungal action of azoles that led to improvement in patients with SAFS.

Finally, our study has several limitations. This is a cross-sectional study and the patients with asthma were non-systematically included in the study. Hence, the proportion of 50% of *Aspergillus*-sensitised asthmatic patients does not reflect the prevalence of *Aspergillus* sensitisation in asthma, which is approximately 38% as reported in our previous investigations.^{16,17,19} One can also question the validity of the diagnosis of bronchial asthma in our study as bronchodilator reversibility was not essential for diagnosis. However, the diagnosis of asthma was made on a history of recurrent or episodic attacks of chest tightness, breathlessness and cough (especially nocturnal) with documented wheeze on auscultation of the chest and obstructive defect on spirometry. In contrast, patients with chronic obstructive pulmonary disease (COPD) usually have progressive dyspnoea on exertion with a generalised decrease in breath sounds. Moreover, the majority of the patients were in the younger age group with a mean age of 35 years, and only one patient smoked tobacco. Hence, COPD patients were unlikely to have been included in the study population. Another major limitation is the fact that we did not measure sensitisation to other environmental allergens such as house dust mite and other fungi. However, the study was planned to evaluate the clinical significance of *Aspergillus* sensitisation in patients with asthma given the high prevalence of ABPA in our locale.¹⁸ The strengths of the study are the large sample size and assessment of asthma severity by different criteria including the use of a QoL questionnaire.

Conclusions

The results of this study suggest a weak association between *Aspergillus* sensitisation and severity of asthma. Whether this relationship is causal or not remains to be seen.

Conflicts of interest

None.

Financial disclosure

None.

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