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Aspergillus fumigatus: Potential Threat for Asthma patients

Tarfe Kishori S[1*] and Deshpande Sunita D[2]

[1]Department of Biotechnology, Smt. Chandibai Himathmal Mansukhani College, Ulhasnagar, District Thane, Maharashtra, India, Former PhD Scholar, Department of Microbiology, T. N. Medical College & B. Y. L. Nair Charitable Hospital, Mumbai Central, Mumbai, Maharashtra, India
[2]Former Professor, Department of Microbiology, Seth G. S. Medical College & KEM Hospital, Parel, Mumbai, Maharashtra, India
*Corresponding author email address : kishorkambli@gmail.com

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ABSTRACT

Asthma is a chronic inflammatory disease of the airways. In susceptible individuals it causes recurrent episodes of wheezing, breathlessness, chest tightness and cough. Inflammation can increase airway hyper responsiveness which can lead to infections by bacteria including atypical bacteria, fungi and viruses. Allergic Bronchopulmonary Aspergillosis (ABPA) is a disease of asthmatic subjects, is caused by hypersensitivity to Aspergillus antigens. It is life threatening disease associated with *Aspergillus fumigatus* colonization of the bronchial airway. A person with asthma who develops ABPA has difficulty in controlling their asthma despite using many medications. In the present study 140 patients with Asthma were analysed for presence of Aspergillus species in their sputum sample. To confirm their atopy total serum IgE was estimated. In order to determine their sensitisation for *A. fumigatus*, serum IgM for *A. fumigatus* was also tested. 4 patients (2.85%) showed presence of *A. fumigatus* in their sputum samples. All of them showed elevated levels of IgE which confirmed their allergic status. 17% patients showed elevated levels of *A. fumigatus* IgM antibodies which indicates sensitization of asthmatic subjects to *A. fumigatus*. Therefore it is recommended that while treating allergic patients presence of *A. fumigatus* in respiratory tract as well as presence of significant titres of antibodies against *A. fumigatus* should be considered. Focused treatment to irradicate *A. fumigatus* should be given so that *A. fumigatus* is cleared from respiratory tract.

Key words: Asthma, ABPA, *A. fumigatus*.

INTRODUCTION

The respiratory system is the most common site for infection by pathogenic microorganisms. These infections are common and usually mild, they are frequently taken for granted. These represent an immense disease burden on our society (Schaechter *et al.*, 1989) About 20% of the populations in western countries suffer from allergic diseases. These allergic diseases

include asthma, rhinitis, atopic dermatitis and food allergy. This prevalence is steadily rising. The rise correlates with improvement in living standards and better control of infectious disease in childhood.
Allergic diseases are environmental diseases. They are triggered by environmental allergens. They arise in an abnormal internal molecular and cytokine environment. This influences the decision of immune T cells to differentiate into allergy-promoting Th2 cells. (Geha 2000) There are varieties of triggering factors identified for asthma. Amongst these respiratory tract infections mainly by fungi, viruses and bacteria. Atypical bacteria like *M. pneumoniae* and *C. pneumoniae* are also associated. (Broide 2001) Fungi are causative factors that include asthma symptoms. Outdoor airborne fungi *Cladosporium*, *Alternaria*, Penicillium and Aspergillus and indoor fungi *Neurospora*, *Aspergillus* and *Eurotinum* are significant triggers of IgE formation. (Jindal 2006)

Allergic Bronchopulmonary Aspergillosis (ABPA) is an important allergic disorder. It is predominantly a disease of allergic subjects. ABPA is caused by hypersensitivity to Aspergillus antigen. Aspergillus species are ubiquitous occur worldwide. They are known to cause four distinct clinically and recognizable forms of hypersensitivity respiratory disorders. They are Allergic Bronchopulmonary Aspergillosis (ABPA), Allergic Aspergillus sinusitis, IgE mediated asthma and hypersentivity pneumonitis. ABPA is the most frequently recognized manifestation of allergic Aspergillosis. It is an indolent disease with a protracted course, occurs worldwide and is now seen as an important disease in India. (Gupta and Mangal 2006)

Microbial infections associated with allergic respiratory infections increase the severity and duration of the disease. They also may themselves act as an allergen. Therefore their treatment with appropriate antimicrobials is essential. Many studies have suggested that patients with such associated respiratory infections when treated with antibiotics can improve patient's ability to breath. Antibiotic treatment helps in fast recovery of patient, which can reduce corticosteroid consumption by patients (Nagayama and Tsubaki, 1999 Verghese 2001)

Allergy to fungi is a worldwide problem. Mould spores can be found in indoor or outdoor environments. Exposure to mould spores can occur by having contact with saprophytic species or by ingestion of edible mushrooms. Exposure to allergenic moulds may lead to IgE mediated rhinitis and asthma atopic dermatitis or rather infrequently to generalized reactions. Ratna Gupta et. al1999 & American College of Occupational and Environmental Medicine 2002)

Inhalation of fungal spores carrying allergens has been claimed as a risk factor for severe asthma. Some allergenic fungi are pathogenic for humans causing localized infections, e.g. *Candida*, *Malssezia*, *Trichophyton* or more severe respiratory diseases (e.g. Aspergillus) or systemic mycoses (American College of Occupational and Environmental Medicine 2002)

Allergy to spores of Basidiomycetes (e.g. Boletus, Coprinus, Pleoorotus, Psilocybes) has been reported. The relevance of their causative role in respiratory allergies has been documented. American College of Occupational and Environmental Medicine 2002)

Fungi are known to be causative factors that induce asthmatic symptoms. Outdoor airborne fungi including *Cladosporium*, *Alternaria*, *Penicillium* and *Aspergillus* are significant triggers of IgE formation. Indoor fungi *Aspergillus*, *Neurospora* and *Eurotium* are also found to be associated. In addition some practitioners believe that there is a strong fungal/ yeast component in the lung and/or gut microflora in individuals with asthma. American College of Occupational and Environmental Medicine 2002)

Bronchial asthma: In this there are sprouted conidia and mycelia in the bronchial lumen. Such patients have IgG as well as IgE antibodies. These fungal conidia are the common allergens in triggering bronchial asthma. The conidia of the following fungi are responsible – *Alternaria*, *Helminthosporium*, *Drechslera*, *Cladosporium*, *Penicillium* and *Aspergillus* species. There is seasonal variation which corresponds to the peak growth of these fungi. The childhood form of asthma reaches it's peak in early adolescence and gradually subsides. The other form of disease begins after forty and may become severe with increasing age. This type of disease has a more rapid and fatal course if status asthmatics develops. The patients have reaginic type of antibodies (IgE) to the allergic substrates. There may be an Arthus –type of reaction later on which may complicate the reaction and it is IgG mediated. (Chander 2002 ;Brown *et al*., 1979) The association between asthma and aspergilli was noted

as early as in 1925. Investigations have confirmed that Aspergillus spores are more common in the sputum of patients with asthma than with other lung disorders (Henderson, 1968 Henderson *et al*., 1968).

Kumar *et al*., (1989) studied 'Prevalence of aspergilli and antibodies to Aspergillus species in bronchial asthmatics using culture, and serological techniques like agar gel double diffusion, counter-immuno-electrophoresis and enzyme-linked immunosorbent assay and they found that 20 % asthmatic patients showed presence of *Aspergillus fumigatus* antibodies'. From this they concluded that bronchial asthmatic patients should be subjected to serological diagnosis of ABPA using highly sensitive technique like ELISA.

Allergic Bronchopulmonary Aspergillosis: Allergic Bronchopulmonary Aspergillosis (ABPA) is a disease of asthmatic subjects, is caused by hypersensitivity to Aspergillus antigens. It is life threatening disease associated with *A. fumigatus* colonization of the bronchial airway. It was described as a lung disease with defined clinical, serological, radiological and pathological features (Chander, 2002; Todd Sanford Division & John Bernard Henry 1989).

ABPA is also called as mucomembranous Aspergillus bronchitis. This form of aspergillosis may develop as an exaggeration of bronchial asthma. About 5% of patients with asthma and allergic rhinitis have features indicative of ABPA, namely IgG and IgE against *A. fumigatus*, eosinophilia and characteristic X-ray findings. The clinical symptoms are similar to those of asthma but are more chronic and severe and bronchial plugging occurs. Bronchoscopy reveals the fungus to be growing in patches of various sizes perpendicular to the bronchi. Fruiting heads and conidia are sometimes produced. The underlying mucus membrane is red and congested but not invaded. Coughing produces sputum in which the fungus may be cultured. Positive sputum culture is a constant finding and it remains during the length of the disease. A delayed type as well as immediate type skin reactions are demonstrated in these patients Chander 2002**;** Todd Sanford Division & John Bernard Henry (1989).

Causative agent *A. fumigatus* produces a powerful endotoxins and C-substance. The former may contribute to the disease. An absolute eosinophilia is present. Precipitins are demonstrated in the sera of almost all patients, thus both IgG and IgE are present in this disease. The IgE may be specific for the Aspergillus species involved or may be nonspecific. (hander 2002**;** Todd Sanford Division & John Bernard Henry (1989).

Sensitization to *Aspergillus* conidia in asthmatic subjects when the thick secretions, which are usually present in the airways, trap the fungal spores. This generally develops in atopic subjects and is sustained by the continuous inhalation of Aspergillus antigens resulting in acute asthma. The reported 'frequency of skin test reactivity to Aspergillus antigens in patients with asthma has varied from 16-38% in different parts of the world. Schwarze and Gelfand (2002). Similarly the prevalence of ABPA also varies from 1-11% in patients with asthma. And from 25 to 28% in Aspergillus skin test positive asthmatic subjects. These variable prevalence rates probably reflect the lack of a single diagnostic criterion with a standardized test'. Maurya and Gunani 2005).

MATERIALS AND METHODS

Collection of respiratory specimens (Elmer *et al*. 1992; Isenberg 1992)
Senior clinician from Department of Medicine, Topiwala National Medical College & B. Y. L. Nair Charitable Hospital, Mumbai Central, Mumbai, Maharashtra, India selected subjects for study on the basis of symptoms, radiological & clinical findings. Minimum 3 consecutive sputum samples were studied for confirmation of results.

All universal safety precautions were taken while collection, transportation, handling and processing of specimens. All specimens were processed within one hour of collection. Fresh morning sputum specimens were collected with aseptic in a clean, sterile, leak-proof container. Each patient was advised to collect an early morning sputum sample after washing the mouth and gargling with tap water.

Processing of respiratory specimens Elmer *et al*., (1992 Isenberg 1992 ; Bailey and Scott's Diagnostic Microbiology 2002)**.** All clinical specimens were studied microscopically as well as macroscopically. In microscopic characters like color, appearance, presence or absence of blood in specimens were noted.

Pretreatment – NALC digestion was carried out for mucolysis of all sputum specimens.

Microscopy –
Wet mounts – Saline & KOH wet mounts of each specimen was carried out for detecting pus cells, RBCs and presence of organisms especially fungal elements.

Culture (Elmer *et al*., 1992) A loopful of each clinical specimen was inoculated or streaked on various culture media for isolation of pathogens. All aseptic precautions were taken while processing of samples.

Media used for isolation
Sabouraud's Dextrose agar with tetracycline, chloramphenicol and cycloheximide Sabouraud's Dextrose agar with and without antibiotic were incubated at 37+/-0.5°C and at 30+/-0.5ºC both.

Identification of *Aspergillus fumigatus*:
Microscopically hyphae are septate, hyaline. Conidial heads strongly columnar. Conidiophores smooth walled uncolored, upto 300 micrometer long but can be quite short, vesicles dome-shaped (20-30 micrometer in diameter), uniseriate with closely compacted phialides (5-10 X 2-3 micrometer) occurring only on the upper portion of the vesicle. Conidia smooth to finely rough subglobose, 2-3.5 micrometer in diameter. (Chander 2002)

Colonies are smoky gray-green with a slight yellow reverse. Old colonies (greater than a month) turn slate gray. Texture is woody to cottony to somewhat granular (Chander 2002).

Serological Tests
3-5 cc of whole blood was collected by venipuncture using a disposable 5.0 ml syringe and 21 gauze hypodermic needle in a sterile plain test tube taking all aseptic precautions. The sterile plain tube was incubated at 37ºC in a slanting position for 1-1.5 hours and later held at 4ºC for 1 hour. This method facilitates clotting of blood. The supernatant serum layer was separated and centrifuged at 2000 rpm to remove the cellular debris. The clear serum was preserved in the absence of preservatives in plastic storage vials after labeling it properly with patient's registration numbers and date at -20ºC until utilization. **(**Elmer *et al*., 1992)

Total IgE estimation – Total IgE estimation was done by Solid phase Enzyme Linked Immunoassay by IBL ELISA (Quantitative).

ELISA for determination of specific IgG and IgM
Aspergillus fumigatus IgM antibodies were estimated by Solid phase Enzyme Linked Immunoassay by IBL ELISA. (Quantitative).

RESULTS

Out of total respiratory samples studied *Aspergillus* was obtained in 2.87% of cases,

Serological investigations
Serological study was performed in 100 patients which included 4 patients whose samples showed growth growth of *Aspergillus fumigatus* & other patients whose samples which did not show any growth.

Determination of total IgE
Total serum IgE levels were found to be elevated in case of all patients. In the present study total serum IgE was estimated in selected asthma patients by ELISA, as total IgE levels provide the evidence in support of atopy. Table 2 shows the incidence of *Aspergillus fumigatus* IgM antibodies in the cases studied. Amongst all the cases studied 17% cases were positive for *Aspegillus fumigatus* IgM

Table 1 Incidence of *Aspergillus fumigatus* in respiratory samples tested

Total number of samples tested	Samples showing growth of Aspergillus isolates	Differentiation of isolates
		Aspergillus fumigatus
140 (100)	4 (2.85)	4 (2.85)

Table 2. Determination of prevalence of *Aspegillus fumigatus* IgM antibodies in patients studied

Total number of samples tested	No. of patients with *Aspegillus fumigatus* IgM positive
100	17

DISCUSSION

Several species and genera have been reported to cause fungal allergy. Epidemiological, environmental and clinical research was focused on relevant species like *Alternaria*, *Aspergillus*, *Cladosporium* and *Penicillium*. Some studies reported the clinical relevance of *Candida*, *Trichiphyton* and *Malssezia* in either respiratory or skin allergic diseases. Allergy to spores of *Basidiomycetes* (e.g. *Boletus*, *Coprinus*, *Pleorotus*, *Psilocybes*) has been reported and the relevance of their causative role in respiratory allergy has been documented. (Mari *et al*., 2003)

Sensitivity to fungal allergens has also been found to be a risk factor for severe life-threatening asthma. A New Zealand study of patients admitted to Hopsital intensive care unit revealed that patients admitted to the ICU had a significantly greater incidence of reactivity to *Alternaria tenuis*, *Cladosporium cladosporoides*, *Helminthosporium maydis* or *Epicoccum nigrum*. Fungal cultures were performed from bronchial secretions of 13 asthma patients and from the skin of 91 patients with atopic dermatitis.

Allergic Bronchopulmonary Aspergillosis (ABPA) is the most frequently recognized manifestation of allergic aspergillosis. It is an indolent disease with a protracted course, occurs worldwide and is now seen as an important emerging disease in India. (Bailey and Scott's Diagnostic Microbiology 2002)

Kumar *et al*. (1989) studied '330 asthmatic subjects for prevalence of *Aspergilli* and antibodies to different *Aspergillus species.* Out of total patients tested 6.06% yielded *Aspergillus* species in sputum specimen repeatedly. Precipitins against Aspergillus species were detected in 7.88% of cases by DD and CIEP. ELISA showed 20% of cases to be having antibodies to *Aspergillus fumigatus*.'

In the present study, the asthmatic subjects presented no features of ABPA. All subjects proven by history, clinical examination, pulmonary function studies and routine chest x-rays revealed no abnormalities or features suggestive of hyperinflation.
As the presence of asthma is the usual feature of ABPA, some of the asthmatics may become potential candidates for ABPA after the onset of asthma and hence require long term follow-up. With the employment of highly sensitive technique like ELISA, antibodies to *Aspergillus* may be detected in more number of cases in this specific group of patients.

When the patients are admitted in hospital, during the course of the disease it is quite possible that presence of Aspergilli species in the respiratory tract may give rise to allergic manifestations. Therefore it becomes necessary to detect the presence of Aspergilli in respiratory secretions of patients who are at high risk of developing such infections. (patients on steroid therapy, immunocompromised patients)

Henderson *et al.* (1968) reported that, of their 46 asthmatic patients, 11% had definite allergic aspergillosis. (John *et al*.,1993)

All these studies give a strong support to the importance of fungal sensitization as a important risk factor for the increasing severity of asthma. Exposure to environmental molds may play a role in asthma – related mortality. (sensitization to aspergillus antigens)

CONCLUSION

In this study 04 asthma patients out of 140 showed growth of *Aspergillus* species, All patients with *Aspergillus fumigatus* culture positive showed raised IgE which confirmed their allergic status. Total 100 patients were tested for *Aspergillus fumigatus* IgM . Total 17 patients including above 4 showed elevated IgM for *Aspergillus fumigatus* which indicates recent sensitization to *Aspergillus fumigatus*. They also gave history of atopy.

It is possible that asthma patients are sensitized to *Aspergillus fumigatus* or any other *Aspergillus species* during the regular course of the disease. Later these patients can further develop Allergic Bronchopulmonary Aspergillosis (ABPA) which is difficult to manage.

Therefore it is recommended that while treating allergic patients presence of *Aspergillus fumigatus* in respiratory tract as well as presence of significant titres of antibodies against *Aspergillus fumigatus* should be considered and focused treatment to irradicate *Aspergillus fumigatus* should be given so that *Aspergillus fumigatus* is cleared from respiratory tract otherwise as it is a well known fact that steroid

therapy suppresses the immune response. Therefore focused treatment after confirmation of probable presence of *Aspergillus fumigatus* should be given.

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Conflict of Interest

The author declares that there is no conflict of interest.

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