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Microbiology Section

Clinico-Microbiological Profile of Chronic Pulmonary Aspergillosis from a Tertiary Care Centre in Southern India

KIRAN CHAWLA[1], KRANTHI KOSARAJU[2], SRIDEVI RAYASAM[3], CHIRANJAY MUKHOPADHYAY[4]

ABSTRACT

Background: Pulmonary aspergillosis is commonly seen in immunocompromised individuals. A significant rise has been seen in these cases in the past decade, owing to growing number of patients with impaired immune status.

Aim: This study includes the detailed clinical and microbiological profiles of all the culture positive cases of pulmonary aspergillosis, detected in three years, from Jan 2008–Dec 2010, at our tertiary care centre.

Methods: A hospital based observational and retrospective study was conducted to study the clinico-microbiological characteristics of patients with pulmonary aspergillosis. Respiratory specimens which showed repeated isolation of *Aspergillus* were included in the study. Demographic details, clinical findings and predisposing factors were noted down for all the patients. Treatment of patients with antifungal agents and their responses to treatment were also documented.

Results: There were 22 patients with male to female ratio of 1.2:1 and mean age of 52.5 years. The most common underlying lung disease was presence of bronchial asthma in 27.3% (6/22) cases. Many patients (40.9%; 9/22) were on steroid treatment. Cough with expectoration was the most common symptom observed in 72.7% (16/22) cases. Microbiologically, microscopy showed positivity for the presence of gram positive, acutely branched, fungal hyphae, suggestive of *Aspergillus*, in all the cases. *Aspergillus fumigatus* was the predominant species that was isolated in 40.9% (9/22) cases. All the diagnosed patients were given either oral itraconazole or intravenous amphotericin B. A clinical improvement was observed in 72.5% (16/22) cases, but 27.3% (6/22) patients died.

Conclusion: Pulmonary aspergillosis presents with non-specific clinical and radiological findings. An early suspicion and diagnosis is essential, especially in patients with underlying lung disease, to prevent dissemination and invasion.

Keywords: *Aspergillus, Pulmonary aspergillosis,* Chronic obstructive pulmonary disease

INTRODUCTION

Fungal infections have recently emerged as a world-wide health care problem, owing to extensive use of broad spectrum antibiotics, immunosuppressive agents and increasing population of terminally ill and debilitated patients [1]. *Aspergillus* infection is the commonest invasive fungal infection which involves respiratory tract. It can be ascribed to pervasive presence of the fungal spores in soil, water, decaying vegetation, indoor environment, including hospitals [2,3]. *Aspergillus* was first detected in 1729 and it received its name due to its resemblance to an aspergillum which was used to sprinkle holy water [4]. Man acquires the infection through inhalation of fungal spores, though infection has also been documented following exposure to water aerosols contaminated with *Aspergillus* spores and *Aspergillus fumigatus,* in particular, is the most important air borne saprophytic fungus [5]. Inhalation being the commonest mode of infection, lung is the most common site of involvement and pneumonia is the most important nosocomial infection caused by *Aspergillus* [6]. Depending on the host immunity, lung structure and degree of inoculums, the spectrum of pulmonary disease ranges from a noninvasive disease (colonization or aspergilloma), or an allergic bronchopulmonary aspergillosis (ABPA), to a semi-invasive (chronic necrotizing pneumonia) or an invasive infection (invasive pulmonary aspergillosis) [7]. Dissemination of infection from lungs can lead to systemic aspergillosis, that can be a major cause of morbidity and mortality in immunosuppressed persons [6].

Pulmonary aspergillosis is seen mostly in immunosuppressed individuals. The central cause is the impairment of phagocytic system and hence, patients with haematological disorders are particularly prone to develop it. Diabetes mellitus, alcohol abuse, renal and hepatic failure, etc. which have an effect on neutrophil proliferation, maturation, function and lifespan, are the important predisposing factors [4]. The incidence of *Aspergillus* infection is showing a rising trend, following solid organ or bone marrow transplantation, chemotherapeutic modalities like prolonged use of corticosteroids or other immunomodulating drugs and epidemic occurrence of human immunodeficiency virus (HIV) [8]. *Aspergillus fumigatus* is the commonest species causing infection, though other species like *Aspergillus flavus, Aspergillus niger* and *Aspergillus terreus* can also cause the disease less frequently [7].

In India, only few studies are available, which show the comprehensive profile of cases of pulmonary aspergillosis [1,6,9]. Herein, we are presenting the detailed profiles of all the pulmonary aspergillosis cases diagnosed in our institution from 2008–2010. We also studied the prevalence of various species of *Aspergillus* and the predisposing factors which cause the disease.

MATERIAL AND METHODS

The present observational and retrospective hospital based study included all cases of pulmonary aspergillosis which were microbiologically proven in time period of three years, from Jan, 2008–Dec, 2010 at a tertiary care centre in southern India.

All culture positive patients and those fulfilling at least two of following three clinical criteria were included in the study: 1) Predisposing illnesses like tuberculosis, chronic obstructive pulmonary disease

(COPD), diabetes mellitus, etc., 2) Long term steroid therapy, 3) Radiological features suggestive of *Aspergillosis.*

To rule out the chances of contamination, samples that showed repeated isolation of *Aspergillus* (minimum 2 sputum samples or 1 sputum + 1 bronchoalveolar lavage [BAL]) were included in the study. Cases were classified according to revised definitions given by EORTC/MSG consensus group [10], as follows:

A. Proven Invasive Aspergillosis: Presence of host factors + Clinical criteria + Positive culture + Detection of Aspergillus antigen

B. Probable Invasive Aspergillosis: Presence of host factor + clinical criteria + Positive culture

C. Possible Invasive Aspergillosis: Presence of host factor + clinical criterion but mycological criterion absent [10].

Processing of Samples

Gram staining was done for all respiratory samples like sputum, BAL or endotracheal aspirates (ETA) and the slides were microscopically checked for the presence of gram positive septate, acutely branched fungal hyphae, which were suggestive of *Aspergillus*. Though gram staining is not a classical staining method for identification of filamentous fungi, it was done here as a part of routine screening of respiratory specimens.

All these samples were cultured on Sabouraud's dextrose agar (SDA) along with culturing on routine Mc Conkey's, sheep blood and chocolate agar and these media were incubated aerobically at 37°C for 24-48 hours. Isolated colonies, suggestive of *Aspergillus* were speciated following standard microbiological procedures [11].

For clinico-microbiological correlation, demographic details, clinical findings and predisposing factors were noted down for all the patients. Treatment of patients with antifungal agents and their responses to treatment were also documented.

RESULTS

There were 22 patients (M:F = 1.2:1) with proven diagnosis of bronchopulmonary aspergillosis during the study period, within the age 19–81 years (mean = 52.5 years) [Table/Fig-1]. Fever and respiratory symptoms like cough and expectoration were present in 50% and 72.7% cases of proven bronchopulmonary aspergillosis respectively. The main predisposing factors identified in our study were chronic lung disease, including COPD and bronchial asthma (50%), smoking (13.6%), diabetes mellitus and tuberculosis (each 9.1%). Some patients, however, had no apparent predisposing factor. Three (13.6%) patients required ventilator care. Chest radiography revealed consolidation/opacity in 50% of the patients, followed by findings suggestive of adult respiratory distress syndrome in 22.7% of patients. Chest X-ray was normal in 18.2% of patients with bronchopulmonary aspergillosis. *Aspergillus fumigatus* was the most

Parameter	Number (%)
Age in years	
20-40	7 (31.8)
41-60	6 (27.3)
61-80	9 (40.9)
Clinical findings	
Fever	11(50)
Cough/Expectoration	16(72.7)
Wt. loss	2(9.1)
Dyspnea	10(45.4)
Predisposing factors	
COPD	5(22.7)
Bronchial asthma	6(27.3)
Steroid R_x	9(40.9)
Post TB	2(9.1)
DM	2(9.1)
Chronic smoking	3(13.6)
Others	4(18.2)
Chest X-ray findings	
Consolidation/opacity	11(50)
Cavity	1(4.5)
Pleural effusion	1(4.5)
ARDS pattern	5(22.7)
Normal	4(18.2)
Outcome	
Improved	16(72.7)
Expired	6(27.3)
Culture findings	
A.fumigatus	9(40.9)
A.flavus	6(27.3)
A.niger	2(9.1)
A.versicolor	1(4.5)
A.terreus	1(4.5)
A.species	3(13.6)

[Table/Fig-1]: Clinical, Demographic and Microbiological profile of pulmonary aspergillosis cases

Features	Kurhade et al., [6]	Shahid M et al., [9]	David M Denning et al., [18]	PR Gupta et al., [19]	Hae-Seong Nam et al., [20]	Present study
Region	Nagpur, North India	Aligarh, Uttar Pradesh	Manchester, United Kingdom	Jaipur, India	Seoul, Korea	Southern India
No. of Patients Studied	20	13	18	98	43	22
Patient group studied	Chronic pulmonary infections	Chronic lung disease	Chronic pulmonary aspergillosis	Pulmonary Aspergilloma	Chronic necrotizing pulmonary aspergillosis	Chronic pulmonary infections
Age group of the patients	Not mentioned	45 – 60 years	20 – 77 years; median age – 59 yrs	Mean age – 38.0±5.8 years	Median age – 60 years	19-81 years; mean age 52.5 years
Gender	Not mentioned	Predominantly Males	Predominantly Males	Predominantly Males	Predominantly Males	Predominantly Males
Duration of illness	Not mentioned	Prolonged – more than one year	Several months to > 12 years	≥2 years	Not mentioned	Prolonged – usually more than 2 weeks to > one year
History of chronic lung disease and use of broad spectrum antibiotics and/or steroids	Seen in 13 (65%) cases	All the cases	Seen in 12 cases (66.66%)	All cases	Seen in all cases	Present in 35% of cases
Commonest species isolated	*Aspergillus fumigatus*	*Aspergillus fumigatus*	*Aspergillus fumigatus*	*Aspergillus fumigatus*	*Aspergillus fumigatus*	*Aspergillus fumigatus*
Sample for isolation	Sputum only	BAL only	Sputum Predominantly	Sputum	Respiratory specimens	Predominately Sputum followed by BAL and ET Aspirate
Predominant Radiographic abnormality detected	Not mentioned	Not mentioned	Left upper lobe cavity	Cavity and ball-like leisons	Cavities	ARDS like picture in 26.66%
Prognosis	Not mentioned	Not mentioned	Improved (71%)	Not studied	Clinical Improvement in 58%	Improved (72.22%)

[Table/Fig-2]: Comparison of Present study with Recent Case series of Broncho-Pulmonary Aspergillosis from different parts of world

common species isolated, accounting for 40.9% of cases, followed by *A. flavus*, *A. niger*, *A. tereus* and *A. versicolor* in decreasing order. Mortality was seen in 6/22 cases (27.3%). Comparison of present study with few studies done in recent past has been shown in [Table/Fig–2]. All our cases belonged to the category of probable invasive aspergillosis as per standard definitions.

DISCUSSION

The presence of underlying chronic lung disease (bronchial asthma, bronchiectasis or COPD) and prolonged use of broad spectrum antibiotics and steroids has been documented in association with *Aspergillus* infection, as the infection is attributed to alteration of pulmonary architecture and/or local immunity and is implicated in the causation of semi-invasive or chronic necrotizing disease [4,6]. Moreover, prolonged use of broad spectrum antibiotics in chronic lung disease patients may stimulate the growth and virulence of infecting fungi by destruction of competing bacterial flora [6]. Smith NL and Denning DW, in their study, have documented the presence of tuberculosis, followed by ABPA and COPD in cases of chronic pulmonary aspergillosis [12].

Clinical diagnosis of aspergillosis is often difficult to make, as clinical signs and symptoms are not specific and as they are consistent with bronchopneumonia – fever unresponsive to antibiotics, cough, sputum production and dyspnoea [4]. A majority of our patients (80%) presented with variable symptoms mimicking bacterial pneumonia, making clinical diagnosis difficult. A high index of suspicion is crucial in identification of these cases, more so, in those patients who show an atypical presentation. Presence of haemoptysis and/or pleuritic chest pain may indicate angio-invasion, which was not found in our patients [7].

Radiological findings are also of little diagnostic help, especially in early stages of disease, because of the presence of non-specific changes. Usual findings include rounded opacities, pleural-bases infiltrates and cavitations. Typical 'halo sign' and 'crescent sign' which can be seen in invasive disease are less specific and they can be found in other fungal and bacterial infections [2,7]. However, chest radiography done in our patients revealed consolidation/opacity in 50% cases, followed by ARDS in 22.7% cases and cavity and pleural effusions in 4.5% each. No radiological abnormality was observed in 18.2% of them, which could be due to minute changes in early stage of the disease, which may not have been picked up by X-rays and this limits the role of radiography in diagnosis of pulmonary aspergillosis [13]. Radiology, however, has a role in assisting the diagnosis, especially in high risk patients [7]. However, chest CT scan, especially when combined with high resolution images (HRCT), helps in early diagnosis and improved prognosis of patients [2].

Histopathological examination of lung tissue being an invasive procedure, though it is confirmatory for diagnosis, cannot be done in all cases. Histopathological confirmations were also lacking in present study. Recently, detection of *Aspergillus* antigens in body fluids is being considered as another mode of diagnosis of aspergillosis. Nucleic acid detection of *Aspergillus* can also help in early diagnosis, but it has a disadvantage, that it cannot differentiate between colonization and infection [8,14,15]. PCR detection is also limited to few laboratories and it cannot be considered as a routine diagnostic test.

Diagnosis of pulmonary aspergillosis requires repeated isolation of fungus from the airways with appropriate clinical and/or radiological features and/or a positive tissue diagnosis [4]. We isolated *Aspergillus* from at least 2 sputum samples in all these patients. In three patients, where BAL fluid could possibly be obtained, we substantiated our diagnosis by isolation of *Aspergillus* from BAL fluid. Significance of isolation of *Aspergillus* from sputum samples depends upon the immune status of the hosts. In immunocompetent persons, isolation is always considered as colonization and it needs no treatment [6]. On other hand, in immunodeficient hosts, isolation of *Aspergillus* is predictive of invasive disease and it demands initiation of antifungal treatment [1,6]. All the cases included in present study were immunocompromised in one form or other.

Aspergillus can be identified in the direct specimens microscopically on detecting the presence of hyaline, regular, slender, septate hyphae with dichotomous branching (~45º) [4]. In our study, microscopy helped us to obtain positive results in all the cases, which were later confirmed by culture. Based on our study, we emphasize the importance of microscopy in making an early provisional diagnosis of bronchopulmonary aspergillosis, which can be later confirmed by culture and other diagnostic modalities.

In the present study, *A. fumigatus* was responsible for almost half of proven cases of pulmonary aspergillosis. Similar observation was seen in studies done by Kurhade *et al* [6] and Shahid *et al* [9]. *A. fumigatus* is responsible for a majority of the invasive diseases, followed by *A. flavus, A. tereus* and *A. niger* [5,7]. One interesting feature associated with *A. niger* infection is formation of calcium oxalate crystals, which can be demonstrated in tissue or fluid. Presence of such crystals, though not demonstrable in our study, is usually known to indicate fungal infection [4].

According to the standard definitions, none of our cases were identified as 'proven' invasive pulmonary aspergillosis. Earlier studies have also reported an alarming rate of 64% patients with proven invasive aspergillosis at autopsy, who did not meet these standard criteria before death [16]. Use of these standard definitions for making clinical decisions for individual patients may carry a risk of undertreatment. Hence, failure in meeting these criteria clinically, which is a major limitation, doesn't prove absence of invasive disease. It may be due to lack of sufficient evidence in support of invasiveness [10].

Treatment of pulmonary aspergillosis depends on the type and stage of disease. Depending on the involvement of lung and spread of disease, antifungal treatment or surgery can be considered [2]. All patients diagnosed with pulmonary aspergillosis in our study received specific antifungal therapy (either oral Itraconazole or intravenous Amphotericin B). A majority of our patients (72.7%) responded favourably, but remaining 27.3% of cases showed no improvement and they expired. In a study done by Jhun et al., 99% cases of chronic pulmonary aspergillosis were treated with itraconazole and mortality was reported in 14% cases [17]. Death could be attributed to underlying co-morbid conditions or advanced stage of disease. Underlying co-morbidities in our study included diabetes mellitus, chronic obstructive lung disease, tuberculosis and patients receiving steroids and broad spectrum antibiotics for longer durations.

To conclude, pulmonary aspergillosis is a devastating disease with varied clinical presentations and nonspecific radiological findings. A high index of suspicion is necessary, especially in those with chronic lung disease and in those who are on prolonged steroid or broad spectrum antibiotic therapy. Microscopy is an important technique which can be used for making an early provisional diagnosis, which can later be substantiated with other diagnostic modalities.

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PARTICULARS OF CONTRIBUTORS:

1. Additional Professor, Department of Microbiology, Kasturba Medical College, Manipal University, Manipal, Karnataka, India.
2. Assistant Professor, Department of Microbiology, Kasturba Medical College, Manipal University, Manipal, Karnataka, India.
3. Postgraduate, Department of Microbiology, Kasturba Medical College, Manipal University, Manipal, Karnataka, India.
4. Professor and Head, Department of Microbiology, Kasturba Medical College, Manipal University, Manipal, Karnataka, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Kiran Chawla, Additional Professor, Department of Microbiology, Kasturba Medical College, Manipal, Karnataka, India-576104
Phone: 9980220484; Fax: 91 0820 2571927
E-mail: arunkiranchawla@yahoo.com

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