

COLONIZATION OF ASPERGILLUS SPECIES IN ELDERLY PATIENTS WITH LOWER RESPIRATORY TRACT INFECTIONS IN A TERTIARY CARE HOSPITALAlosha Sharma¹, Dakshina Bisht^{2*}, Shukla Das³, Ritu Agarwal⁴ and V.K.Arora⁵¹Ph.D Student, Department of Microbiology, Santosh Medical College, Ghaziabad.²Professor and Head Department of Microbiology, Santosh Medical College, Ghaziabad.³Director Professor, Department of Microbiology UCMS GTBH, New Delhi.⁴Associate Professor Department of Microbiology, Santosh Medical College, Ghaziabad.⁵Superannuated Vice Chancellor and Ex Professor Department of TB and Chest, Santosh Medical College, Ghaziabad.***Corresponding Author: Dakshina Bisht**

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ABSTRACT

Introduction: Isolation of *Aspergillus* species from lower respiratory tract samples is the first indication of IPA (Invasive Pulmonary Aspergillosis) which depends on the underlying condition of the patients. Elderly patients having lower respiratory tract infections are among the risk group of developing invasive disease by *Aspergillus* spp due to impaired lung conditions. **Aims and Objectives:** To isolate *Aspergillus* species from elderly patients having lower respiratory tract infection. **Material and Methods:** Sputum samples and whole blood samples were collected from elderly patients. Sputum samples were cultured on Sabouraud's dextrose agar and blood samples were screened for detecting IgE, IgG and IgM specific for *Aspergillus*. Fungal isolates were confirmed by conventional methods. **Results:** From the 81 elderly patients, 11 (13 %) *Aspergillus* spp. were isolated. Of these 4 (5%) patients had raised IgG specific for *Aspergillus fumigatus* and were clinically categorized as having probable IPA. However, 7 (8%) patients who yielded *Aspergillus* spp. and negative for serum IgG specific for *A.fumigatus* were categorized as having possible IPA. **Conclusion:** Early identification and detection of colonisation in critically ill elderly patients having LRTI is important and treatment should be considered if *Aspergillus* spp are isolated in their pulmonary secretions.

KEYWORDS: Invasive pulmonary aspergillosis (IPA), *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, Chronic necrotizing aspergillosis (CNA).

INTRODUCTION

Aspergillus is a saprophytic filamentous fungus widespread in the environment. Inhalation of *Aspergillus* spores or conidia can give rise to various clinical conditions, depending essentially on the host's immunological status. In more than 90% of affected patients the respiratory tract is the main site of involvement.^[1]

The significance of isolation of *Aspergillus* spp. from respiratory cultures has been studied extensively in immunocompromised hosts who develop invasive pulmonary aspergillosis.^[2-4] It poses a major threat in patients with depressed immune system.^[5] On the other hand, little is known about the significance of isolation of *Aspergillus* from respiratory specimens of apparently immunocompetent or those have impaired lung conditions. In immunocompetent patients, it almost always represents colonisation with no clinical consequences. It has been postulated that isolation of an

Aspergillus species from respiratory samples in critically ill patients (even when immunocompetent) should not be routinely discarded as colonization, but in elderly patients (commonly having underlying diseases) isolation is usually interpreted as colonization.^[6,7]

Therefore, diagnosis of invasive pulmonary aspergillosis on the basis of an *Aspergillus* positive culture from tracheal aspirates remains most difficult in patients with intermediate risk, or in patients without currently recognized risk factors.^[8] Confirmation of infection obliges the demonstration of histopathological evidence that is not feasible in this type of patients.^[9] In elderly patients having LRTI clinical signs and symptoms for acquisition of invasive fungal disease are non specific so a combination of host risk factors, clinical symptoms, radiological, microbiological criteria and serological tests are needed to rule out invasive disease in them. Very few studies have been conducted in which emphasis on *Aspergillus* isolation and its consequences from this risk group is been given on elderly patients

who also constitute a risk group for invasion by *Aspergillus* due to their impaired lung functions. Keeping this in mind present study was undertaken to observe significance of *Aspergillus* isolation in elderly patients having lower respiratory tract infections.

Place of the study

The study was conducted in Department of Microbiology in Collaboration with Department of TB and Chest Santosh Medical College and Hospital Ghaziabad.

Study Population

A total number of 81 patients visiting Department of TB and Chest, having acute episode of cough for 21 days as the cardinal symptoms, symptoms associated with lower respiratory tract infections such as sputum production, dyspnoea, wheeze, chest discomfort/pain and patients whose chest radiography /CT scan showing symptoms of lower respiratory tract infections were selected for the study after taking written consent from them. Institutional ethical clearance was obtained.

Our study group included patients of all socioeconomic backgrounds. All patients belonged to age group of above 60 years. Patients with active tuberculosis, atypical mycobacterial infections, malignancies, immunocompromised who were unwilling to participate in the study or refused to sign the consent were excluded from the study.

Methodology

Early morning sputum samples and whole blood samples were collected from patients and direct microscopy in 10% KOH (Potassium hydroxide) was done to observe the presence of fungal element. Sputum samples were homogenised and cultured on Sabouraud's Dextrose Agar (SDA) supplemented with cycloheximide and were incubated for 3- 4 days at 25 -26° C for isolation of *Aspergillus* species. Homogenisation of sputum samples was done by adding N-acetyl L-cystine in M/50 Trisodium citrate and diluting double the amount with phosphate buffer. Identification and confirmation of the *Aspergillus* spp was done on the basis of microscopic and macroscopic morphological characteristics following standard mycological procedures such as colony characteristics, lactophenol cotton blue preparation and slide culture. Whole blood samples were also collected and serum was used for detecting Anti -*Aspergillus* IgG, IgM and IgE antibodies using Kit (Omega Diagnostics) based identification methods. The statistical analysis was done by using SPSS (Statistical Package for Social Sciences) software.

Elderly patients who yielded *Aspergillus* spp were categorized according to the criteria proposed by EORTC/MSG (European Organization for Research on Treatment of Cancer/Mycoses Study Group):

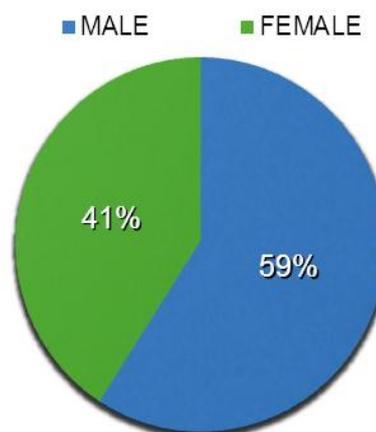
Probable IPA- Host factors and Minor Clinical Criteria: Symptoms of LRTI (Cough chest pain,

Haemoptysis or dyspnoea) and Repeated Positive *Aspergillus* microscopy or culture from BAL or sputum.

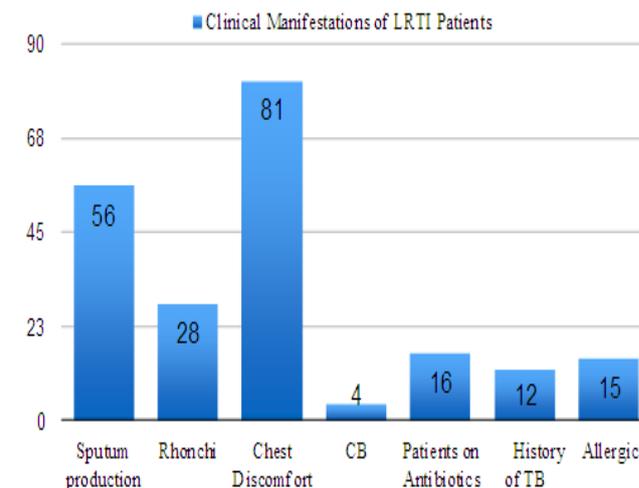
Possible IPA- Host factors and Minor Clinical Criteria: Symptoms of LRTI (Cough,chest pain, Haemoptysis or dyspnoea) and Positive *Aspergillus* microscopy or culture from BAL or sputum.^[10]

RESULTS

Of the 81 elderly patients selected who were suffering from LRTI, 48(59%) were males and 33 (41%) were females. Only 12 (14%) had past history of Tuberculosis, while 16 (19%) were on antibiotics and 15 (18%) had allergic bronchitis.



Graph 1: Gender Wise Distribution of Elderly Patients.



CB - Central Bronchiectasis

Graph 2: Distribution of Elderly Patients based on Clinical Manifestations.

Majority of the patients had complaints of chest discomfort and sputum production while only a very few had past history of tuberculosis.

Direct microscopy of sputum samples collected from all the patients showed presence of fungal hyphae in 11% (9/81) elderly LRTI patients who were also confirmed culture positive for *Aspergillus* spp. However, sputum samples of 2 patients were KOH negative but culture positive. The remaining, 70 (86.4%) patients were all KOH as well as culture negative.

Table 1: Microscopy and Culture Positivity of Sputum Samples In Elderly Patients Having LRTI.

N= 81 Microscopy (KOH Mount)	Culture		
	Positive	Negative	Total
Positive	9 (11%)	0	9 (11%)
Negative	2 (2%)	70 (86%)	72 (89%)
Total	11 (13%)	70 (86%)	81

A total of 13% (11/81) *Aspergillus* spp were isolated from these patients of which 7 % (6/81) were identified as *A.fumigatus* and 6 % (5/81) as *A.flavus*. Among these 36% (4/11) patients had raised IgG specific for *A. fumigatus* and the rest were found to be negative.

Among elderly patients 4% (3/81) amongst the culture negative for *Aspergillus* had raised IgE specific for *A.fumigatus*. However, none of the patients were found to be positive for IgM specific for *A. fumigatus*.

Table 2: Distribution of *Aspergillus* Species Isolated from LRTI Patients and Their Serological diagnosis.

<i>Aspergillus</i> Species Isolated	No. of <i>Aspergillus</i> Species	No. Of Patients Showing Raised Serum IgG along with <i>Aspergillus</i> isolation
<i>A.fumigatus</i>	6	3
<i>A.flavus</i>	5	1
Total	11	4

DISCUSSION

The isolation of *Aspergillus* spp from LRTI samples is associated with colonization in high percentage of cases, with an unclear significance, as it may represent a temporary passage, a long - term benign carriage or a sign preceding invasive disease since incubation period is unknown.^[11,12]

Direct microscopy by KOH mount showed the presence of septate fungal hyphae in sputum samples of 11 percent patients who were also culture positive for *Aspergillus* spp. Our results are similar to a study by Khurade et al where 16 percent (20/123) were culture and KOH positive for *Aspergillus*. In another study by Mwaura et

al examination of the sputum revealed that 37.8% (65/172) samples positive for fungal elements, while 62.2 percent (107/172) samples were negative.^[13] These observations confirm the diagnostic importance of direct microscopy of sputum samples for identification of *Aspergillus* infection.

7 percent (6/81) *A.fumigatus* and 6 percent (5/81) *A.flavus* were identified as the common culture isolates. In a retrospective study by Tashiro et al, *A. fumigatus* was the most commonly isolated species from respiratory samples, followed by *A. niger*, *A. versicolor*, *A. terreus*, *A. flavus*, *A. nidulans* and *A. sydowii*. In contrast to our results analysis of their laboratory records and clinical disease revealed that the isolation of a particular *Aspergillus* spp. does not confirm it as an etiologic agent for disease in patients.^[14]

However, according to D. Denning et al, in clinical guidelines for diagnosis and management of aspergillosis, mentions that the presence of *A. fumigatus* in sputum is not diagnostic because of the ubiquitous nature of the fungus and the different pathologies attributable to the fungus.^[15] But the presence of *A. fumigatus* in a bronchoscopic specimen is a more common indication of infection compared to colonization.^[16]

Of the patients who yielded *Aspergillus* spp. only 5% (4/81) of patients had raised IgG specific for *A. fumigatus* who were diagnosed as having probable IPA while 8% of patients were diagnosed to be having Possible IPA with sputum culture positive but negative for IgG specific for *A. fumigatus*. Among 18% patients with allergic bronchial asthma only 3% patients showed raised IgE specific for *Aspergillus* along with raised absolute eosinophil count, suffering from central bronchiectasis. However, sputum of none of the allergic patients was culture positive for *Aspergillus*.

A semi invasive form of aspergillosis which is chronic necrotizing pulmonary aspergillosis (CNA), usually affects middle aged and elderly patients with altered local lung defences.^[17] According to Denning et al, majority of patients with chronic necrotising aspergillosis have positive serum IgG antibodies to *A.fumigatus* but it varies over time and may be negative at some point during the course of disease.^[18] However, raised serum IgE and IgG antibodies against *Aspergillus* along with initiate acute presentation of asthma, peripheral eosinophilia, elevated total serum IgE and pulmonary infiltrates are suggestive of acute stage ABPA (Allergic bronchopulmonary aspergillosis)^[19].

The presence of anti- *Aspergillus* antibodies differentiates between infected and colonized patients with a positive predictive value of 100% for detecting infection.^[15] Almost all the patients in our study who had raised IgG along with *Aspergillus* isolation were unstable with various clinical manifestations. However, clinical

conditions of rest of the patients who yielded *Aspergillus* but negative for anti- *Aspergillus* antibodies were stable. As we were not able to perform histopathological investigations, we could not categorise patients into proven invasive aspergillosis. Our observation shows that colonisation by *Aspergillus* plays a significant role in gradually worsening the clinical conditions in such risk group patients if not diagnosed early.

CONCLUSION

Aspergillus spp isolated from sputum samples of elderly patients correlated well with our clinical, serological and microbiological findings and confirmed the role of *Aspergillus* in elderly patients with impaired lung conditions who are at risk of developing respiratory *Aspergillus* invasion on long term colonization. Hence, early diagnosis of *Aspergillus* colonization is important in these patients. Also isolation of *Aspergillus* spp in respiratory samples of these patients should not be discarded as contaminant as these patients are needed to follow up for monitoring repeated *Aspergillus* isolation along with screening of their serum for Anti - *Aspergillus* antibodies to prevent worsening of their respiratory illness.

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