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ORIGINAL RESEARCH

Comparative Evaluation of Galactomannan Antigen Immunoassay with Fungal Smear and Fungal Culture in Patients with Suspected Pulmonary Aspergillosis

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ABSTRACT

Background: Pulmonary aspergillosis, caused by Aspergillus species, is a significant concern among patients with chronic respiratory diseases, particularly those with underlying immunosuppressive conditions. **Material and method:** This prospective observational study was conducted at Guru Nanak Dev Hospital, Amritsar, involving 150 suspected cases. Patients were categorized as "proven," "probable," and "possible" invasive aspergillosis (IA) based on clinical, radiological, and microbiological criteria per EORTC/MSG guidelines. **Results:** The mean age of patients was 55.5 ± 3.02 years, with a male predominance (64.66%, male-to-female ratio 1.8:1). The most frequent symptoms included dyspnea (61.3%), fever unresponsive to antibiotics (59.3%), and cough (56.6%). Radiological findings primarily showed consolidation (52%). Fungal smear positivity was observed in 52% of cases, with Aspergillus flavus being the most commonly isolated species (62.06%). The galactomannan ELISA assay had a positivity rate of 37.33%, with a statistically significant correlation to fungal culture and smear findings ($p < 0.001$).No cases were classified as "proven IA," while 37.33% were "probable IA," and 62.66% were "possible IA." **Conclusion:** The study underscores the importance of combined diagnostic approaches, including galactomannan ELISA, microscopy, and fungal culture, to ensure early and accurate detection, enabling timely management of pulmonary aspergillosis.

Keywords: Pulmonary aspergillosis, Aspergillus species, invasive aspergillosis, galactomannan ELISA, fungal culture.

INTRODUCTION

Aspergillus species are saprophytic, thermo tolerant fungi that areubiquitous in the air and environment.The genus *Aspergillus* was first defined by Micheli in 1729.He noted the pattern of conidial head of *Aspergillus* resembled to brush or perforated globe used for sprinkling holy water by the priests hence he named genus as *Aspergillus.*[1] Out of 185 species of genusAspergillus, about 20 can cause human infections. Most infections are caused by *Aspergillus fumigatus, Aspergillus flavus, Aspergillus terreus* and *Aspergillus niger*. Although humans inhale Aspergillus spores at the rateof hundreds per day, they rarely experience complications. However,under special circumstances likepre existing lung diseases (Bronchial asthma, Bronchiectasis, COPD, Pneumonia, Lung abscess, Tuberculosis, Malignancy, Pulmonary edema along with other conditions like diabetes mellitus, great use of broad spectrum antibiotics, corticosteroids, immune suppressants ,cytotoxic drugs.[2]

Aspergillus species can produce spectrumof diseases ranges from noninvasive disease, such as with colonization of the organism or the presence of a fungus ball (aspergilloma), or an allergic response responsible for the syndrome of allergic bronchopulmonary Aspergillosis (ABPA), to semi-invasive or invasive infections such as chronic necrotizing pneumonia and invasive pulmonary aspergillosis. [3-5]

Symptoms are usually nonspecific and mimic bronchopneumonia: prolonged fever unresponsive to

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antibiotics, cough, sputum production and dyspnoea. Patients may also present with pleuritic chest pain and haemoptysis. [6] *Aspergillus* infection may also disseminate haematogenouslyto other organs, including the brain, kidneys, heart, oesophagus and liver.[7]

Characteristic CT findings in angioinvasive aspergillosis consist of nodules surrounded by a halo of ground-glass attenuation ("halo sign") or pleura-based, wedge-shaped areas of consolidation.[8] Conventional methods (direct microscopyand fungal culture), serological methods(detection of antigen and antibodies), molecular methods(PCR) are available to diagnose IPA. The gold standard in the diagnosis of IPA is histopathological examination of lung tissue.[9] The most recent advances in the diagnosis of IPA is detecting Aspergillus galactomannan antigen and (1,3)-b-D-glucan (both are cellular wall constituents). Galactomannan (GM) is a polysaccharide cell wall component released by *Aspergillus* hyphae during growth detected by a single step immune enzymatic sandwich microplate assay.[10]

The treatment is difficult and depends upon the clinical type and host factors.Empiric therapy should be started as soon as there is clinical suspicion. Various antifungal drugs which are effective are Polyenes (Amphotericin B deoxycholate and its lipid-associated formulations), Triazoles (Voriconazole, Itraconazole, Posaconazole) and Echinocandins (Caspofungin and Micafungin).

MATERIAL & METHODS

A prospective observational study was carried out in Microbiology department in collaboration with Chest & TB hospital and other wards of Guru Nanak Dev Hospital, Amritsar. A total of 150 cases of chronic respiratory diseases clinically suspected of Pulmonary Aspergillosis were included. Details of patients including epidemiological data, provisional clinical diagnosis, risk factors, treatment history, Hemogram, radiological, and microbiological evaluation (Galactomannan assay, fungal smear/culture) were recorded.

Relevant respiratory samples(sputum, broncho alveolar lavage, tracheal aspirate) were collected in sterile, leak-proof and screw-capped container under all aspetic precautions and five ml blood was collected by venipuncture in sterile plain vials taking all aseptic precautions and serum was separated and stored in serum storage vials and kept at -70°C until analysis. Sputum samples were analyzed by direct microscopy(using 10% KOH) to visualize the presence of narrow septate hyaline hyphae with acute angle branching followed by culture on Sabouraud's Dextrose Agar incubated at 25°C and 37°C for a maximum period of 4 weeks. The growth obtained was identified on the basis of colony morphology, pigment production and Lactophenol Cotton Blue (LCB) preparation. The serum has been subjected for the testing of Galactomannan antigen.

The patients were categorized into "proven," "probable," and "possible," cases based on risk factors, microbiological and clinical criteria, as per Invasive Fungal Infections Co-operative Group (IFICG) of the European Organization for Research and Treatment of Cancer and Mycoses study group case definitions (EORTC/MSG).[11-13]

RESULTS

In the present study, out of total 150 patients of suspected pulmonary aspergillosis, maximum number of suspected patients of pulmonary aspergillosis 48/ 150 (32%)

belonged to age group 51-60 years, predominately belonging to male sex. The male to female ratio was 1.8:1 and the mean age group affected was 55.5 years with standard deviation of 3.02 (55.5±3.02).

Majority of the patients were from wards 85(56.66%) followed by OPD 47 (31.33%) and 18 (12%) patients were admitted in ICU.The most common presenting complaint was dyspnoea (61.3%) followed by fever unresponsive to antibiotics (59.3%), cough (56.6%), chest pain (27.3%),weight loss (26%) and hemoptysis (21.33%).Most common chest X-ray finding was consolidation / fluffy air spaces (52%) followed by pleural effusion (17.3%), reticulonodular opacification (12.6%) and cavitatory lesions (12%).

Galactomannan ELISA assay positivity among suspected patients of pulmonary aspergillosis was 37.33%. Out of 150 samples, 78 (52%) showed positive fungal smear findings and 29(40.84%) showed fungal growth of *Aspergillus* species. Out of 29 *Aspergillus* species, the most common species isolated was *A. flavus* (62.06%) followed by *A. fumigatus* (24.13%), *A.niger*(10.34%), *A.terreus* (3.44%). (Figure1)

11.33% of patients were positive both by galactomannan assay and fungal smear. 18% of patients were positive both by galactomannan assay and fungal culture. (Figure 2) The relationship between fungal smear/culture and GM assay was found to be highly significant statistically.(P < 0.001)All 150 patients were categorized as Proven IA (0%), Probable IA (37.33%) and Possible IA (62.66%) respectively.(Figure 3)

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Figure 1: Distribution of *Aspergillus* species (n=29)

***Aspergillus* species**	**Total (%)**
Aspergillus flavus	18 (62.06)
Aspergillus fumigatus	7(24.13)
Aspergillus niger	3 (10.34)
Aspergillus terreus	1 (3.44)

Figure 2: Correlation of Galactomannan assay with fungal smear in suspected patients of pulmonary aspergillosis (n=150)

Galactomannan assay	**Fungal Smear Positive (n=21)**	**Fungal Smear Negative (n=129)**	**Fungal Culture Positive(n=29)**	**Fungal Culture Negative(n=121)**
GM assay Positive (n=56)	17 (11.33%)	39 (26%)	27 (18%)	29 (19.33%)
GM assay Negative (n=94)	4(2.66%)	90(60%)	2 (1.33%)	92 (61.33%)

P<0.001; Highly significant

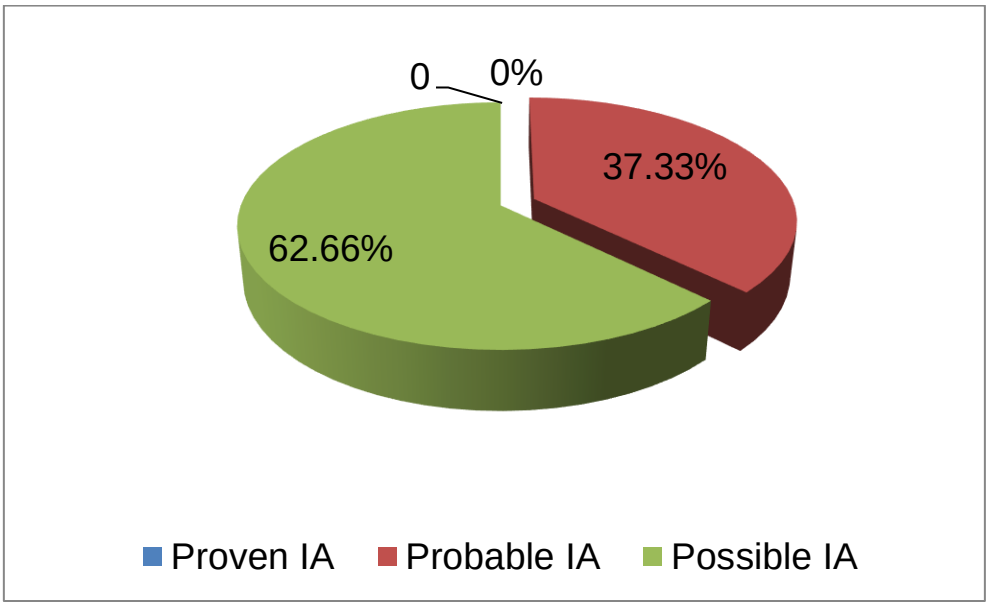

Figure3 Distribution of patients as per EORTC/MSG (n=56)

DISCUSSION

Fungal diseases kill more than 1.5 million and affect over a billion people.

Aspergillus infection is the commonest invasive fungal infection which mainly involves respiratory tract. In the present study of 150 cases of suspected pulmonary aspergillosis, the mean age of the patients was 55.5 years with standard deviation of 3.02 (55.5±3.02) which was more common in males (64.66%) as compared to females (35.33%). ChawlaK et al conducted a study onClinico Microbiological Profile of Chronic Pulmonary Aspergillosis from a Tertiary Care Centre in Southern India found that with male to female ratio of1.2:1 and mean age of 52.5 years. This may be explained by the fact that males are maximally exposed to fungal spores particularly working with decaying vegetation like moldy hay in agriculture and due to health seeking behavior among male patients than female patients.

In the present study, 56.66% of patients were admitted in wards followed by OPD and ICU patients. The possible reason for this finding could be as patients admitted with respiratory symptoms were shifted to pulmonary wards.Bongomin F et al have reported increased prevalence of invasive aspergillosis in indoor patients which is in concordance with our findings. This can be explained by the presence of multiple risk factors and severe illness makes patients admitted to the hospitals particularly vulnerable to these infections. Overuse of use steroids, antibiotics, antifungals and immunosuppressive drugs could be the other possible additional factors for IPA in indoor patients.

Chest X-ray was the most common radiological investigation done in the present study. The most common chest X-ray finding was consolidation (52%) followed by pleural effusion (17.3%), reticulonodular opacification (12.6%) and cavitatory lesions (12%). These findings were consistent with study done by Linna Huang et al, which also reported consolidation as most common chest X-ray finding.

The most common fungal isolate was *Candida* species (47.88%) followed by *Aspergillus* species (40.8%), *Alternaria* (5.63%), *Mucor* (4.22%) and *Penicillium* species (1.40%). A study conducted by Alfia et al in the department of Microbiology, Rohtak, India

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showed similar results. The most common species of *Aspergillus*, isolated was *A. flavus* (62.06%)followed by *A. fumigatus* (24.13%), *A.niger*(10.34%), *A.terreus*(3.44%). The findings of this study match with the study done by Agarwal R et al in Chandigarh, India who studied high prevelance of *Aspergillus flavus* in the North India, followed by *A. fumigatus*.

In the present study, Galactomannan assay positivity among suspected patients of pulmonary aspergillosis was 37.33 %. Mohindra R et al conducted a study on the evaluation of serum galactomannan enzyme immunoassay and found the incidence of GM positivity was 47.75%.In contrast, Maertens et al reported the positive GM-ELISA in only 8.9%.

In our study, 150 cases were categorized as proven IPA (0%), probable IPA (52.7%), possible IA (41.4%) and no IA (5.9%) as per EORTC/MSG criteria.] A similar study was conducted by Kaur S, et al and categorized the patients as Proven IA (0%), Probable IA (52.7%), Possible IA (41.4%) and No IA (5.9%).

CONCLUSION

The incidence of infection with Aspergillus species continues to rise with the increasing immunosuppressed patient population with recent history of chronic lung diseases. Delay in diagnosis can lead to increased severity of the disease. Microscopy is an important technique which can be used for making an early provisional diagnosis , that can later be substantiated with other diagnostic modalities. Galactomannan ELISA should be performed in conjunction with fungal smear and culture, so that early diagnosis and timely treatment of pulmonary aspergillosis can be done.

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