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Original Research Article

EVALUATION OF GALACTOMANNAN ANTIGEN IN SERUM AND BRONCHO-ALVEOLAR LAVAGE (BAL) FOR DIAGNOSIS OF INVASIVE PULMONARY ASPERGILLOSIS IN A TERTIARY CARE HOSPITAL IN KOLKATA

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Abstract

Background: Invasive Pulmonary Aspergillosis (IPA) is a common and severe fungal infection in immunocompromised and critically ill patients. Galactomannan is a polysaccharide, secreted from hyphal cell wall of Aspergillus spp during invasion and found in body's fluids. Thus, detection of galactomannan in blood or body fluids makes the diagnosis of IPA early for prompt antifungal therapy and decrease mortality. The aim of this study is to evaluate galactomannan antigen in serum and BAL fluid for diagnosis of IPA in a tertiary care hospital in Kolkata. **Materials and Methods:** This cross-sectional study was performed in the Department of Microbiology of R. G. Kar Medical College in Kolkata from January to December 2022. Broncho-alveolar lavage (BAL) and serum samples were collected from 81 clinically suspected IPA cases as per guidelines of European Organisation for Research and Treatment of Cancer /Mycoses study Group (EORTC-MSG). Galactomannan antigen assay was performed by ELISA in duplicate. BAL fluids were processed in mycology laboratory as per standard protocol. **Result:** In this study male outnumbered female. Most affected age group was 31-50 years (59.26%). 16.05% had longer than 10 days of stay in ICU. Galactomannan positivity rate in BAL fluid was 53.09% and in serum 28.40%. Microscopy was positive in 28 (34.57%), whereas growth was seen in 15 (18.51%) cases. On follow up, sad demise occurred in 5 (6.17%) cases, whereas 76 (93.83%) cases were improved. **Conclusion:** Galactomannan assay was found a non-invasive adjunct for early diagnosis of Invasive Pulmonary Aspergillosis (IPA) for prompt antifungal therapy.

INTRODUCTION

Aspergillus spp. is a saprophytic filamentous fungus that is ubiquitously isolated in the environment and frequently associated with Invasive Pulmonary Aspergillosis (IPA) in immunocompromised and critically ill patients. It is a major threat for patients with hematologic malignancies and chemotherapy-induced neutropenia or hematopoietic stem cell transplantation (HSCT).[1] It is mainly caused by Aspergillus fumigatus followed by other species including Aspergillus niger and Aspergillus flavus and associated with high morbidity and mortality in immunocompromised patients.[2] As non-specificity of clinical or radiological findings makes the diagnosis of IPA more challenging, European Organisation for Research and Treatment of Cancer /Mycoses study Group (EORTC-MSG) sets the guidelines for diagnosis of "proven", "probable" & "possible" IPA is by histopathological examination from lung tissue as gold standard method along with

supportive microbiological, clinical and radiological evidences.[3,4] As per this guideline, diagnosis of IPA is based on following microbiological criteria along with other clinical and host factor criteria- Direct microscopic evaluation for Aspergillus species or positive culture for Aspergillus from sputum or BAL fluid or from sinus aspirate specimen; detection of Aspergillus antigen in plasma, serum, BAL fluid or CSF; detection of positive PCR in plasma, serum or BAL fluid in consecutive two or more occasions.[5] But due to procedural invasiveness and lack of obtaining sterile samples limits its feasibility especially in neutropenic, immunocompromised patients in critical care settings.[4] Although microbiological findings from body fluids may increase specificity, discrimination between invasiveness, colonization or contamination is not always possible.[6]

Galactomannan (GM) is a polysaccharide, secreted from hyphal cell wall of Aspergillus spp during invasion and found in different levels in body's fluids.[3] Although it is found in serum to a higher level in neutropenic patients than non-neutropenic, it has greater diagnostic importance in the broncho-alveolar lavage due to more fungal burden in bronchial tree.[7] Galactomannan assay in BAL fluid appears to be more sensitive (60%) and specific (82%) and higher cut-off (OD value >0.1) makes restraint from over diagnosis.[8]

Thus, detection of galactomannan antigen assay in blood or body fluids by ELISA makes the diagnosis of IPA early and accurate, to institute prompt antifungal therapy and decrease mortality.[2]

Although this assay is included in the guidelines of EORTC-MSG, but there are very limited studies found on galactomannan antigen in India especially in Eastern India. The aim of this study is to evaluate galactomannan antigen in serum and broncho-alveolar lavage fluid for diagnosis of IPA in a tertiary care hospital in Kolkata.

MATERIALS AND METHODS

This cross-sectional and observational study was carried out in the Department of Microbiology of R. G. Kar Medical College & Hospital in Kolkata from January 2022 to December 2022. This study was approved by Institutional Ethics Committee. A total 81 clinically suspected IPA cases were selected as per guidelines of European Organisation for Research and Treatment of Cancer /Mycoses study Group (EORTC-MSG). Symptoms or signs suggestive of invasive pulmonary aspergillosis (IPA) are fever refractory to at least 3 days of appropriate antibiotic therapy, recrudescent fever after a period of defervescence of at least 48 hours while still on antibiotics, pleuritic chest pain or rub, dyspnoea, haemoptysis, or worsening respiratory insufficiency in spite of appropriate antibiotic therapy and ventilatory support [9]. Demographic and thorough clinical history were taken. Broncho-alveolar lavage (BAL) fluid was collected from 48 patients and duplicate serum samples were collected from 33 patients. Evaluation of galactomannan antigen was performed in duplicate from all these samples by Enzyme linked immunosorbent assay (ELISA). OD index for positivity was considered for serum >1.0 and for BAL fluid >1.0 [5]. All BAL fluids were processed in mycology laboratory for KOH (potassium hydroxide) mount and fungal culture as per standard laboratory protocol.

Statistical analysis was performed using software Graph Pad Prism 7. All materials for microscopy and culture were purchased from Hi-Media Pvt. Ltd. Mumbai. Galactomannan ELISA kit (Platelia Aspergillus Ag) was purchased from Bio-Rad Laboratories, Hercules, California, USA.

RESULTS

A total 81 clinical suspected IPA cases were selected. Out of which 74.07% were male and 25.93% were female with male: female ratio was 2.86:1 [Figure 1]. Most of the cases belongs to the age group of 31-50 years (59.26%) [Table 1]. 40.74% cases had history of ICU stay, out of which 16.05% had longer than 10 days [Figure 2].

48 samples of BAL fluid and 33 serum samples were collected. Out of 48 (59.26%) BAL samples, 43 (53.09%) were positive in galactomannan assay. Fungal hyphae were seen in KOH microscopy [Image 1] in 28 (34.57%), whereas fungal culture [Image 2] was found to be positive in 15 (18.51%) cases. [Table 2]. Out of 33 (40.74%) serum samples, 23 (28.40%) were found galactomannan positive in duplicate. On follow up, sad demise occurred in only 5 (6.17%) cases, whereas 76 (93.83%) cases were improved. [Table 3].

Table 1: showing age wise distribution of cases (n=81)

Age (Years)	Number	Percentage (%)
<10	6	7.41
11-20	6	7.41
21-30	3	3.70
31-50	48	59.26
51-60	12	14.81
>60	6	7.41

Table 2: showing distribution of KOH, culture and galactomannan antigen assay in BAL fluid

	Number	Percentage (%)
Total BAL samples collected	48	59.26
Fungal hyphae seen in KOH mount	28	34.57
Fungal culture positive	15	18.51

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Galactomannan positive	43	53.09

Table 3: showing distribution of cases according to status after treatment and follow up (n=81)

Status after treatment	Number	Percentage (%)
Improved	76	93.83
Expired	5	6.17
On follow up	70/76	92.11
Lost to follow up	6/76	7.89

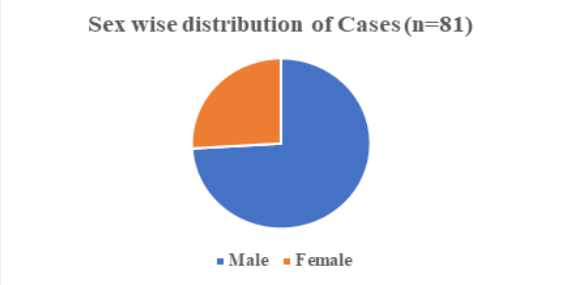

Figure 1: showing sex wise distribution of cases (n=81)

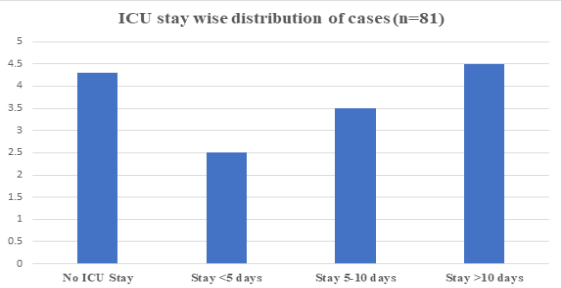

Figure 2: showing ICU stay wise distribution of cases (n=81)

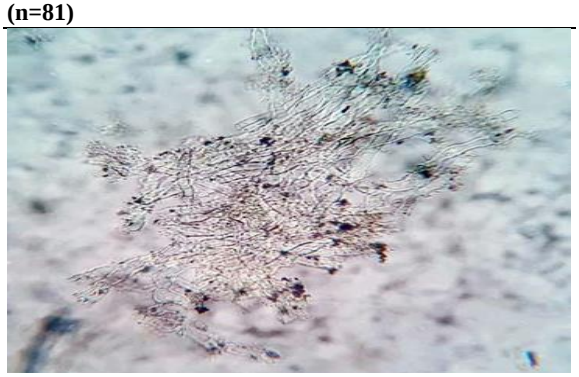

Image 1: showing dichotomous branching of fungal hyphae on 10% KOH (Potassium hydroxide) mount [x400]

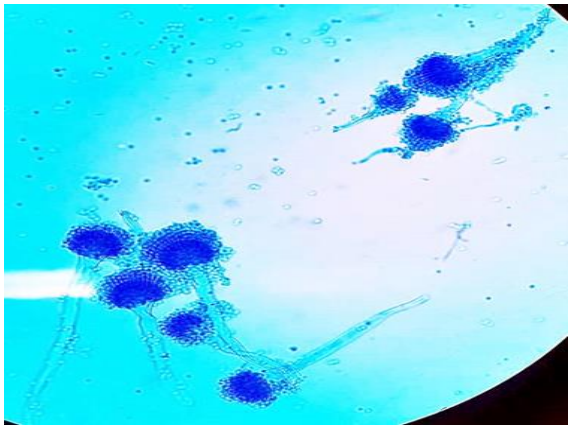

Image 2 showing LPCB (lactophenol Cotton blue) mounting of Aspergillus species from fungal culture [x400]

DISCUSSION

Invasive pulmonary aspergillosis (IPA) is an opportunistic fungal infection of lower respiratory tract with high morbidity and mortality, thus demanding rapid diagnosis and prompt and appropriate antifungal therapy.[9] But due to limited feasibility of appropriate samples, microbiological diagnosis could not be possible always, thus making the galactomannan assay as an adjunct to clinical and radiological findings more attractive and important.[3] In this study, male was outnumbered than female. Male preponderance was also noted in Malhotra S et al (75%),[2] Savio J et al,[4] (72%) and Sun KS et al (63.14%).[11] In the present study, majority of the cases belong to 31-50 years of age group. 33(40.74%) cases required admission in intensive care unit, out of which 13(16.05%) stayed in ICU for more than 10 days. Similar findings were found in Savio J et al,[4] and Teering S et al.[12] 93.83% cases were improved after treatment. Death reported in 6.17% only. Similar findings were reported by Savio J et al.[4]

The galactomannan positivity in BAL fluid was 53.09%, whereas its positivity in KOH was 34.57% and in fungal culture was 18.51%. The galactomannan positivity in serum samples became 28.40%. Malhotra S et al,[2] also showed low culture positivity (18%) in comparison to galactomannan positivity (56%). Similar findings were also noted in Krishtina affolter et al,[13] [BAL galactomannan positivity 50%, Fungal culture positivity 12%], Wei Zhou et al,[14] [BAL galactomannan positivity 75%, Fungal culture positivity 9%]. Although Jorien D Hasse et al,[15] found higher galactomannan positivity in BAL fluid (86%), thus showing usefulness of galactomannan assay for the diagnosis of Invasive Pulmonary Aspergillosis (IPA).

CONCLUSION

Invasive Pulmonary Aspergillosis (IPA) is an emerging life-threatening condition, requiring early diagnosis and prompt institution of appropriate antifungal therapy.[4] Galactomannan assay in BAL fluid was found a non-invasive and useful adjunct in relation to serum galactomannan assay for early diagnosis of IPA to reduce mortality.[16,17]

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