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Serum Galactomannan Assay Positivity For Invasive Pulmonary Aspergillosis Among Chronic Obstructive Pulmonary Disease Patients In A Tertiary Care Hospital

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ABSTRACT:

Invasive Pulmonary Aspergillosis (IPA) is an important opportunistic infection in critically ill patients with various underlying risk factors and is associated with a high degree of morbidity and mortality. In recent years, it has been reported that the incidence of IPA has also increased in patients with chronic obstructive pulmonary disease (COPD).
Aims & Objectives: The purpose of the study was to determine the Serum Galactomannan Assay positivity rate for Invasive Pulmonary Aspergillosis in Chronic Obstructive Pulmonary Disease patients and to study co-morbid conditions and risk factors for invasive aspergillosis.
Materials & Methods: The study population included 56 patients admitted to PKTB hospital, Mysore with COPD/AECOPD. Serum Galactomannan antigen detection by ELISA was done in all subjects.
Results: Serum Galactomannan assay positivity was seen in 10(17.85%) of the study population. The clinical features seen in the study population were dyspnea (98.2%), chest pain (98.2%), cough with expectoration (83.9%), Haemoptysis (12.5%), fever (10.7%), at the time of presentation. Nonspecific Consolidation(46%) comprised the major X-Ray finding followed by Diffuse Patchy Infiltration (29%). Co-morbidities like Diabetes Mellitus (71.4%), Smoking (83.9%), prior administration of steroid (73.2%), past history of COPD (66.1%), Pulmonary Tuberculosis (10.71%) was seen in the study population.
Conclusion: Serum GM assay could increase the diagnostic sensitivity for IPA in COPD patients. Further studies with attention to the assessment of antifungal therapy, false-positive results and utility of the test are needed.

Keywords: Aspergillosis, Chronic obstructive pulmonary disease, Galactomannan, Invasive pulmonary aspergillosis

INTRODUCTION

Invasive pulmonary aspergillosis (IPA) is a life-threatening disease caused by the fungus *Aspergillus fumigatus* and other *Aspergillus* species and represents the leading cause of invasive fungal infection (IFI)-related morbidity and mortality in patients. Invasive pulmonary aspergillosis is frequently seen in patients with hematological malignancy, allogenic bone marrow transplantation, the late stage of HIV infection, solid organ transplantation and chronic granulomatosis. Acute leukemia was the most common underlying illness. Predisposing factors included chemotherapy, neutropenia and receipt of corticosteroids.
Chronic obstructive pulmonary disease is considered to be an important risk factor for aspergillosis. The portal of entry is mainly the respiratory tract, both lungs and sinuses. According to WHO estimates, 65 million people have moderate to severe COPD. More than 3 million people died of COPD in 2005 corresponding to 5% of all deaths globally and it is estimated to be the third leading cause of death by 2030. Crude estimates suggest there are 30 million COPD patients in India.

In COPD patients, ciliary activity is often impaired by tobacco smoke and multiple episodes of infection, as well as repeated epithelial damage. The impairment of the defence mechanisms of the airways facilitates the binding of conidia to the epithelial layer. Consequently, *Aspergillus spp*. first invade the bronchial mucosa, then the adjacent pulmonary parenchyma, and finally the vasculature inducing secondary pulmonary infarction. Thus in COPD patients ciliary activity impairment, immunosuppression due to alveolar macrophage and neutrophil inhibition in those treated with steroids and also receiving broad-spectrum antibiotics, play a role in the development of IPA. The use of systemic steroids in stable phase and the use of 3 or more antibiotics during hospitalization were risk factors for invasive
aspergillosis in COPD.
Early recognition and appropriate treatment of IPA in patients with COPD is crucial, as mortality rates greater than 90% have been reported, and early initiation of systemic antifungal therapy may lead to improved survival rates of up to 80%. Diagnosis of IPA in patients with underlying respiratory diseases is difficult, however, as clinical presentation is typically characterized by nonspecific symptoms such as dyspnea, cough or hemoptysis and early radiological signs are also non-specific.

Typical patient of IPA has a history of using steroids during the stable phase of COPD and an exacerbation of dyspnea does not improve despite broad-spectrum antibiotics and steroids. Typical radiological signs for IPA (e.g., halo or air-crescent sign) are particularly rare in early stages of the disease in non-neutropenic patients.
Mycological culture—the previous gold standard for IPA diagnosis—is limited by low sensitivity and long turnaround time. Therefore, diagnostic tools based on fungal antigen detection have been developed within the last decade and have demonstrated their huge potential in IPA diagnosis.
Aspergillus antigen galactomannan has become an important and reliable tool for the early diagnosis of invasive aspergillosis.[7] Galactomannan (GM) is a circulating polysaccharide component of Aspergillus species that is released into the bloodstream and other body fluids by growing hyphae and germinating conidia.
The galactomannan assay is relatively specific for invasive aspergillosis. Many reviews highlight between-study heterogeneities but conclude that the detection of serum galactomannan can be used to define a case of IA in well-defined populations of at risk patients[8]. In studies carried out on COPD cases in different centres galactomannan antigen test sensitivity in the diagnosis of IPA was stated as 46.2% - 80% and specificity as 83.3 – 94.1%[2].

OBJECTIVES OF THE STUDY

- To determine the Serum Galactomannan Assay positivity rate for Invasive Pulmonary Aspergillosis in Chronic Obstructive Pulmonary Disease patients.
- To study co-morbid conditions and risk factors for invasive aspergillosis.
- To study the socio-demographic profile of study population.

METHODOLOGY

A prospective & cross sectional study conducted on 56 patients in the department of Microbiology, Mysore Medical college & Research Institute, Mysore for a period of one year from January 2017 to December 2017. The samples were obtained from PKTB hospital, Mysore.

Inclusion Criteria
Patients with Chronic Obstructive Pulmonary disease(COPD)/ Acute exacerbation of Chronic Obstructive pulmonary disease(AECOPD) willing to participate in the study.

Exclusion Criteria
Those patients with-
- Malignancies
- Solid organ transplantation
- Stem cell Transplantation
- Certain groups of antibiotics/antifungals which can cause false positive results

History Taking And Examination
An informed consent was taken from the confirmed cases of COPD patients admitted to the PKTB Hospital. Structured proforma was made to collect clinical data in the COPD patients which included documentation of name, age, sex, clinical information, systemic
complications and other co-morbid conditions.

Blood Sample Collection –
After selecting the site of venipuncture, a tourniquet was applied 3-4 inches above it. The area was wiped with 70% ethyl alcohol firmly with the cotton beginning in the center and continuing in an outward direction circularly for an area of 4 to 5cms in diameter and allowed to dry. About 4-5ml of venous blood was collected aseptically. It was centrifuged at 2500 rpm for 15-20 minutes to separate serum. The serum was collected in a dry sterile vial. Serum samples were stored in sealed sterile vials, unexposed to air. Unopened samples were stored at 2-8°C for up to 48 hours prior to testing. For longer storage, the serum was kept at –70°C.

Sample Size Estimation
During the data collection period of 1 year, around 400 COPD patients are expected to be treated as inpatients at PKTB hospital. Serum Galactomannan positivity among COPD is 3.9%[2]. Allowing 20% error and 95% Confidence Interval the sample size will be 52.

Statistical Methods
Descriptive statistics like frequency tables, mean, proportions is used. Analysis is done using R-Software.

RESULTS

Table-1 Age distribution of the patients in the study

Age Group	Subjects	Percentage
40-50y	7	12.5
51-60y	20	35.7
>61y	29	51.8
Total	56	100.0

Chi square: 13.107; p value < 0.05

The majority of patients belong to the age group of >61 years, which is statistically significant (P value < 0.05)

Table-2 Gender Distribution of the patients in the study

Sex		Subjects	Percentage
	Male	47	83.9
	Female	9	16.1
	Total	56	100.0

Chi square: 25.786; p value < 0.05

The majority of the study population contributed by males (84%) also had a significant P value of <0.05.

Table-3: Pattern of Clinical Features

Clinical feature	Present	Absent
Fever	6(10.7%)	50(89.3%)
Cough with expectoration	47(83.9%)	9(16.1%)
Dysnea	55(98.2%)	1(1.8%)
Chest pain	55(98.2%)	1(1.8%)
Haemoptysis	7(12.5%)	49(87.5%

Majority of the clinical subjects showed cough with expectoration, chest pain and dysnea as the predominant features on admission to the hospital. All of these clinical features had a statistically significant P value (<0.05).

Table-4: Co-morbidities among the study population

Co-morbidities	Present	Absent
Diabetes mellitus	40 (71.4%)	16 (28.6%)
Smoking	47(83.9%)	9(16.1%)
Administration of steroids	41(73.2%)	15(26.8)
Past history of COPD	37(66.1%)	19 (33.9%)
Tuberculosis	6(10.71%)	50(89.28%)

Diabetes Mellitus was seen in 71.4%, Smoking in 83.9%, Administration of steroid in 73.2%, past history of COPD in 66.1%, Pulmonary Tuberculosis in 10.71% of the study population. All of these co-morbidities had a statistically significant P value of <0.05.
Among the study population Nonspecific
Consolidation (46%) comprised the major X-Ray finding followed by Diffuse Patchy Infiltration (29%).
Sera with an index ≥ 0.50 are considered to be positive for galactomannan antigen. Serum Galactomannan was positive in 10 of the patients which comprised to 17.85% of the study population which was statistically significant (p value <0.05)**.**

Table-5: Distribution of Serum Galactomannan values among different age groups

	Value	**Age**			**Total**
		40-50	51-60	>61	
Serum Galactomannan	<0.49	7(15.2%)	20(43.5%)	19(41.3%)	46(100.0%)
	0.5-1	0	0	0	0
	1-1.5	0	0	6(100%)	6(100%)
	>1.5	0	0	4(100%)	4(100%)
Total		7(12.5%)	20(35.7%)	29(51.8%)	56(100%)
Cramer's V – 0.318; p value < 0.05					

Serum Galactomannan positivity was seen only in the age group of more than 61 years, which is statistically significant (P value < 0.05).

Table-6: Distribution of serum Galactomannan values according to gender

	Value	**Sex**		**Total**
		Male	**Female**	
Serum Galactomannan	<0.49	39(84.8%)	7(15.2%)	46(100%)
	0.5-1	0	0	0
	1-1.5	4(66.7%)	2(33.3%)	6(100%)
	>1.5	4(100%)	0	4(100%)
Total		47(83.9%)	9(16.1%)	56(100%)
Cramer's V – 0.194; p value > 0.05				

Among the study population Serum Galactomannan positivity was seen in 8 Males and 2 females, thereby suggesting male Preponderance among the positive study population. Here p value was not statistically significant.

Among the 10 patients who showed a positive galactomannan by ELISA 8 presented with cough with expectoration. P value not significant.
All the 10 patients who showed a positive galactomannan antigen test had a history of dyspnea at the time of presentation. P value not significant.

When the Serum galactomannan test was compared with clinical symptoms, positivity was seen in those who presented with the symptoms of cough with expectoration and dyspnea.

Table-7: Comparison of Clinical Features with Galactomannan Positivity

Clinical Features	Present	Serum galactomannan positivity
Chest pain+ Dysnoea+ Cough with expectoration	46(82.14%)	8(80%)
Dysnoea + Chest Pain	9(16.07%)	2(20%)
Fever + Cough with expectoration	1(1.7%)	0
Total	56(100%)	10(100%)

In the present study, 82.14% of the patients had symptoms of chest pain, dysnoea and cough with expectoration. This group contributed to 80% of Serum galactomannan positivity. Dysnoea and chest pain was seen in 16.07% of the patients which contributed to 20% of serum galactomannan positivity. One patient had fever and cough with expectoration wherein the serum galactomannan was negative.

Diabetes Mellitus was present in all the subjects who had a positive galactomannan by ELISA test. There was no statistical significance between Diabetes Mellitus and Serum Galactomannan value (p value > 0.05).
When the smoking history was taken into account which is a very important co-morbid condition for the development of COPD, positive galactomannan was seen in 8 of the patients with history of smoking and 2 of the patients without the history of smoking.

All the subjects who gave a positive galactomannan value had a past history of COPD. Only 2 patients with Tuberculosis gave a positive galactomannan by ELISA.
All the subjects with the positive Galactomannan test had a history of administration of steroids prior to the present hospital admission.
There was no statistical significance between serum Galactomannan values and steroid administration. (p value > 0.05)

Table 8: Comparison of comorbidities with serum galactomannan positivity

Co-morbidities	Present	Serum Galactomannan positivity
Diabetes mellitus + Smoking +Steroid	32(57%)	8(80%)
Diabetes mellitus + past history of COPD + steroid	4(7.1%)	1(10%)
Past history of COPD + Diabetes mellitus	1(1.7%)	1(10%)
Past history of COPD + Diabetes mellitus + Smoking	1(1.7%)	0
Smoking + Steroid	3(5.35%)	0
Tuberculosis + Smoking	1(1.7%)	0

When the Co-morbidities were considered Diabetes mellitus, smoking and steroid administration were present in 57% of the study population which contributed to 80% of the galactomannan positivity. Diabetes mellitus, past history of COPD and steroid administration was present in 7.1% of the study population which comprised

10% of the galactomannan positivity. Past history of COPD and diabetes mellitus was present in 1.7% of the population which contributed to 10% of the galactomannan positivity. The patients presenting with only smoking and steroid administration did not show any positive galactomannan value. The study population with only Tuberculosis and history of smoking also did not give a positive galactomannan by ELISA.

DISCUSSION

COPD patients, especially those that are critically ill and require admission to an ICU, are increasingly recognized as an emerging population at risk for opportunistic invasive pulmonary aspergillosis. COPD patients frequently experience acute exacerbations of their underlying illness, and IPA may be a possible cause of these acute respiratory incidents.

Diagnosis of IPA in COPD remains difficult because its signs and symptoms are non-specific and may be masked by steroid treatment. These patients have a very high mortality rate, in spite of sufficient support and antifungal treatment. Therefore, early diagnosis seems crucial for improving their prognosis.
The majority of the Patients belonged to the age group of >61 years (51.8%) and the male to female ratio was 5.2. COPD is more common in men, because of the comparably high levels of tobacco smoking prevalent, than that seen in women.

Table-9: Comparison of Mean Age and Male to Female Ratio of the Study Population

Sl.No	Study	Mean Age of the study Population	Male to Female Ratio
1	J. Guinea et al.,2009[9]	68.62	4.3
2	Nuri Tutar et al.,2013[2]	65.1±9.7	4.5
3	Xiao-Bin Zhang et al.,2013[10]	66.36±9.66	4.05
4	Present Study	60.93±9.73	5.2

The clinical Features in the present study dyspnea (98.2%), chest pain (98.2%), cough with expectoration (83.9%), Haemoptysis (12.5%), fever (10.7%) is comparable with a study conducted by Nuri Tutar et al.[2] where the prevalence of dynoea was seen in 100% of the study population. Fever was seen in 45.4%, Cough in 90.9%, Sputum Production in 63.6% and haemoptysis in 18.1%. Infiltration (63.6%) followed by Nodule (27.2%) and Cavity formation(27.2%) was the predominant chest X-Ray
findings whereas in the present study Nonspecific Consolidation (46%) comprised the major X-Ray finding followed by Diffuse Patchy Infiltration (29%).
J.Guinea et al.[9] in their study reported fever in 31.8% and Dyspnea in 75.3%. Majority of the chest X-Ray showed bilateral infiltrates (27.2%) followed by worsening radiological findings in 19.7%.

In a study conducted by P. Bulpa et al.[5] Dysnoea was seen in 79%, fever in 38.5%, chest pain and
haemoptysis were not common. Nonspecific Consolidation was the major Radiological feature seen in 64% of the patients.

In a study Conducted by Xu et al.[11] Fever was present in 18.8%, dyspnea in 73.3%, Sputum production in 61.1%. Chest Radiograph showed Infiltrates (34.6%) followed by nodules(11.5%) and consolidation(7.7%).
In COPD patients where IPA had developed, dyspnea was reported as the most frequent symptom and cough, wheezing, increase in sputum amount and fever may also be seen. The most frequent radiologic finding was infiltration that is non specific and may correspond to a COPD exacerbation caused by bacterial pulmonary infection, frequently encountered especially in chest disease clinics.

In COPD patients, because the structures and defense functions of the airways and lung parenchyma are damaged by underlying respiratory diseases, Aspergillus may colonize in these sites. During the early period of invasive aspergillosis, infection may be limited to the tracheobronchial region, presenting as Aspergillus tracheal- bronchitis. With corticosteroids and broad-spectrum antibiotics therapy, the infection could spread to the distal airways and lung parenchyma, presenting as IPA.

Co-morbidities were more common in the case group, suggesting that COPD patients with underlying conditions may be more susceptible to Aspergillus. An underlying disease might also be partly responsible for more severe clinical conditions.

Table-10: Comparison of Steroid Administration in various studies

Sl.No	Study	Steroid Administration
1	P. Bulpa et al.,2007[5]	77%
2	J. Guinea et al.,2009[9]	64.4%
3	H. Xu et al., 2012[11]	66.7%
4	Nuri Tutar et al.,2013[2]	90.9%
5	Xiao-Bin Zhang et al.,2013[10]	63.6%
6	Present study	73.2%

IPA is more frequent in patients receiving high cumulative doses of corticosteroids and in those with imaging evidence of progressive lung disease after admission. In one retrospective study, it was shown that steroid use of over 700 mg in total within the last three months in COPD patients increased the risk of IPA.
Steroids enhance the growth of some Aspergillus (especially *A. fumigatus*) and decrease alveolar macrophage antifungal activity by inhibiting reactive oxidant intermediates (ROI) production and, therefore, favour the emergence of IPA.

In the Present Study the positive Serum Galactomannan values (>0.5) were observed in only those who received Corticosteroid therapy prior to hospital admission. Not only oral but also inhaled steroids might promote IPA in COPD patients. High doses of inhaled steroids therefore have potential systemic effects and could depress the adrenal function.

Table-11: Showing the comparison of Serum Galactomannan assay positivity for IPA in COPD with other studies

Sl.No	Studies	Serum Galactomannan Positivity
1	P. Bulpa et al.,2007[5]	48%
2	J. Guinea et al.,2009[9]	42.4%
3	H. Xu et al., 2012[11]	47.4%
4	Nuri Tutar et al.,2013[2]	40.65%
5	Present study	17.85%

The present study showed a positive serum Galactomannan in only 17.85% of the study population. The slightly lower positivity could be because these are only possible cases with clinical and host factors without any cultural evidence. Other studies quoted above are proven possible cases of IPA. Consecutive serum Galactomannan test and BALF-GM could not be performed on the study population which could be a reason for decrease positivity. It could also be possible that the geographical prevalence of IPA in COPD in this region could be slightly lower.
In studies carried out on COPD cases in different centers, GM antigen test sensitivity in the diagnosis of IPA was stated as 46.2-80% and specificity as 83.3-94.1%. In addition, it has been indicated that GM positivity in COPD patients with IPA development in intensive care and the isolation of *Aspergillus* species in respiratory samples may be significant in terms of mortality.
Few studies showed that a single GM assay had a sensitivity of only 44 – 60% for patients with proven or probable IPA. It has been hypothesized that neutrophils are capable of clearing GM from the blood by mannose-binding receptors on the cells' surfaces. Since COPD patients are not neutropenic, the clearance of GM might explain the low sensitivity of serum GM tests in the diagnosis of IPA.
It may not be necessary or economically feasible to do serial GM tests for all hospitalized COPD patients. However, for those that require ICU admission, it is reasonable to do consecutive GM tests since they are at high risk of IPA.
COPD as an underlying condition for IPA may be under detected or under-reported, because clinical manifestations of the condition are non-specific and because the severity of the underlying condition can mask lung invasion by Aspergillus. He *et al*. stated that the mortality rate for critically ill COPD patients with positive serum GM results was as high as 54.6 – 72.7%. It was even higher, i.e., from 72.7 – 83.3%, when *Aspergillus*

was isolated from the respiratory tract samples in association with single or consecutive positive GM results. Thus, a prompt diagnosis and treatment are important for improving the outcomes.

CONCLUSION

The overall epidemiological data suggest that the incidence of invasive aspergillosis in non-haematological patients is underestimated, particularly in COPD patients. Also the occurrence of pulmonary infiltrates in COPD patients receiving long-term and/or high-dose regimens of glucocorticoids should raise the question of invasive aspergillosis.

Diagnosing invasive aspergillosis with confidence is usually difficult. Confirmation of the diagnosis requires demonstration of hyphae in tissue and/or culture of *Aspergillus* species from sterile specimens. Cultures for fungi and cytopathological examination of respiratory specimens often yield negative results and lack sensitivity for detecting the fungus in an early stage of the infection.
Because of the limitations of the aforementioned diagnostic methods, a nonculture method, based on the detection of *Aspergillus* antigen galactomannan, has been developed. The detection of galactomannan in clinical specimens is firmly established as the test of choice for any laboratory providing diagnostic services for patients at risk of IA. Serum detection remains a key approach and amenable to serial measurements whilst BAL material appears to have a higher sensitivity but is often harder to obtain.

Prompt treatment remains the critical prognostic factor, and the recent expansion of the antifungal armamentarium is a cause for optimism. However, this serves only to emphasise the need to improve prophylactic measures, diagnostic procedures and therapeutic approaches.

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