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ASPERGILLUS COLONIZATION: A MARKER OF EXACERBATION IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE

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ABSTRACT

Introduction: Patients with exacerbation of Chronic Obstructive Pulmonary Disease (COPD) are becoming one of the main risk groups for development of Invasive Pulmonary Aspergillosis (IPA). **Aims and Objectives:** To isolate *Aspergillus* species from COPD patients having lower respiratory tract infections and to determine its role as an exacerbation factor. **Material and Methods:** Respiratory samples were collected from patients having COPD with or without exacerbations and cultured on Sabouraud Dextrose Agar (SDA) for isolation of *Aspergillus* spp. which were confirmed by standard mycological methods. Whole blood samples were also collected and screened for the presence of specific anti - *Aspergillus* antibodies. **Results:** Of the total 150 LRTI patients, 30% (45) had COPD. 56% (22/45) of these COPD patients were unstable and hospitalised while 45% (20/45) were stable and did not require hospitalisation. 37% (17/45) *Aspergillus* spp. were isolated from the COPD patients majority of them being unstable. **Conclusion:** Colonization by *Aspergillus* spp. increased exacerbation in COPD patients and worsened their clinical condition.

KEYWORDS: *Aspergillus flavus, Aspergillus fumigatus.* Chronic Obstructive Pulmonary Disease (COPD), Exacerbations, Invasive Pulmonary Aspergillosis (IPA).

INTRODUCTION

Isolation of *Aspergillus* from lower respiratory tract samples may represent a non significant colonization in majority of conditions as it may indicate a long term carriage with no harmful consequences or a progressing invasive disease as a result of unknown incubation period.[1,2] When inhaled, *Aspergillus* spores may result in colonization, allergic manifestations or may progress into an invasive infection depending on host immunity.[3]

Recent reports have documented expansion in patients with COPD which constitute a less immunodeficient group that are becoming susceptible to invasive aspergillosis specially on steroid therapy, along with patients receiving immunosuppressive therapy (not resulting in neutrophil impairment), patients with cancer under treatment or without treatment and patients with liver cirrhosis.[4-9] COPD patients have lung immune imbalance and immune dysfunction which are of concern as a result of non specific clinical and radiological features as compared to patients with haematological malignancies which show specific findings and form a classic risk group of development of IPA.[10]

Impaired defense mechanisms in COPD increases susceptibility of lung to various microorganisms such as viruses and bacteria resulting in COPD exacerbations. Role of fungal agent as an exacerbation factor is unknown.[11] COPD exacerbations are demonstrated as an incident resulting in, worsening the patient's respiratory clinical conditions which are uncommon variations and resulting in change in therapy.[12] COPD exacerbations remarkably corresponds to morbidity, mortality and health care cost thus, plays a significant role.[13]

Patients with severe chronic obstructive pulmonary disease who are receiving broad spectrum antibiotics and corticosteroids are becoming one of the main risk groups for invasive pulmonary aspergillosis.[14,15] Unfortunately, the probability of IPA in patients with COPD is almost unknown.[4]

Therefore, the aim of our study was to determine *Aspergillus* colonization in COPD patients as an exacerbation factor.

Place of The Study

This cross sectional 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 150 patients visiting Department of TB and Chest, showing symptoms associated with lower respiratory tract infections were selected for the study and a written consent was obtained from them. The study was ethically approved by Institutional Ethical Committee. Patients who refused to participate in the study and patients with active tuberculosis, atypical mycobacterial infections and those who constitute classic risk group for IPA such as HIV reactive, immunocompromised patients and those with malignancies were excluded from the study.

METHODOLOGY

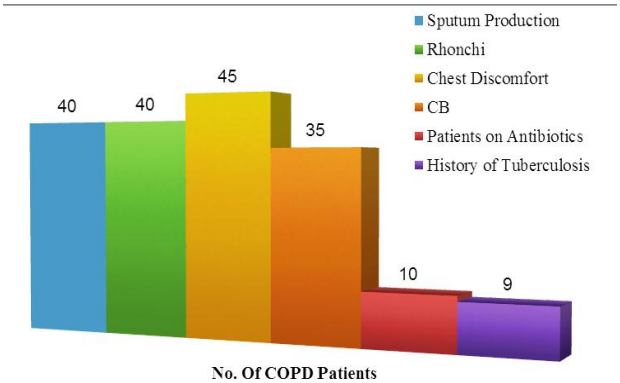
Sputum and whole blood samples were obtained from patients diagnosed with Lower Respiratory Tract Infections (LRTI). Microscopic examination of the sputum samples was done by 10% KOH mount for observing fungal elements. Sputum samples were homogenised by adding N-acetyl L-Cystine in M/50 Trisodium citrate and diluting double the amount with phosphate buffer and cultured on Sabouraud's Dextrose Agar (SDA) slant in tubes supplemented with cycloheximide following homogenisation. SDA tubes were incubated for 3- 4 days at 25-26° C for isolating *Aspergillus* spp. which were identified and confirmed based upon macroscopic and microscopic morphological characteristics following standard mycological procedures. Serum was separated from whole blood samples and screened for the presence of specific Anti–*Aspergillus* antibodies using commercial Kits (OMEGA DIAGNOSTICS SCOTLAND). Statistical analysis was performed with SPSS 11.0 (SPSS Inc., CHICAGO, IL, USA) and Chi square test.

COPD patients who yielded *Aspergillus* spp. were diagnosed of having Aspergilloma based on radiological, microbiological and serological characteristics and were classified into probable IPA and possible IPA based on criteria proposed by EORTC/MSG (European Organization for Research on Treatment of Cancer/Mycoses Study Group).[16] Aspergilloma was diagnosed in a COPD patient culture positive for *Aspergillus*, when fungal ball was detected in pre cavity lesion on chest X ray examination and when serum of the patient was positive for the presence of antibodies specific for *Aspergillus*.[17]

RESULTS

Of the 150 patients who were suffering from Lower Respiratory Tract Infections, 45 (30 %) were clinically diagnosed with COPD. Of these majority 76% (34/45) were males as compared to 24% (11/45) females.

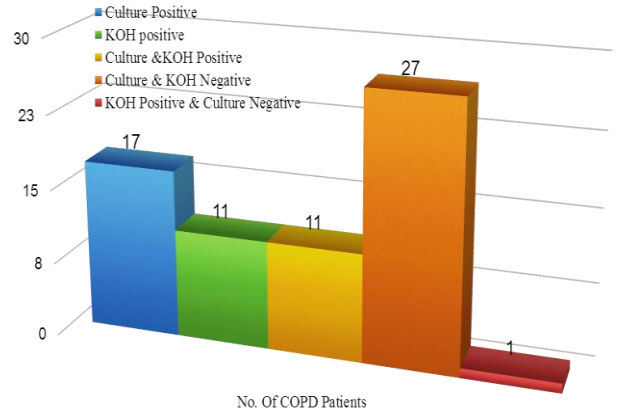
Graph 1: Distribution of COPD Patients Based on Past Clinical Presentation.



CB - Central Bronchiectasis

All the 45 COPD patients complained of chest discomfort and difficulty in breathing along with history of smoking for more than 5 years. Of these 25 (55%) COPD unstable patients were admitted to the hospital while 20 (44%) patients were stable and did not require hospitalisation.

Graph 2: Microscopy and Culture Positivity of Sputum Samples In COPD Patients Having LRTI .



Direct microscopy of sputum samples collected from all the 45 COPD patients showed presence of fungal hyphae in 11 (24%) patients who were also confirmed culture positive for *Aspergillus* spp. However, sputum samples of 6 (13%) patients were KOH negative but culture positive. The remaining, 27 (60%) patients were all KOH as well as culture negative.

Among the 45 COPD patients having LRTI a total number of 17 (37%) *Aspergillus* spp. were isolated of which 11 (24%) were identified as *A. fumigatus*, 5 (11%) as *A. flavus* and only 1 as *A.niger*. Among the 25 COPD

unstable patients who were hospitalised 15 (60%) were culture positive. Of the 20 patients who were stable and non hospitalised only 2 (10%) were culture positive.

On screening the serum samples of COPD patients it was observed that 7/45 (15%) patients had raised IgG specific for *A.fumigatus*. Of these 5 patients were also observed to be unstable and represented multiple nodular opacities on chest X ray examination, while, 2 patients were stable and had clear chest radiographs. None of the patients were found to be positive for IgE and IgM specific for *A. fumigatus.*

Table 1: Clinical Examination, *Aspergillus* Colonization and Serology of COPD Patients Culture positive for *Aspergillus* spp.

COPD (n = 45)								
COPD Patients With *Aspergillus* Isolation = 17								
Chest X Ray				**Clinical Examination**				**Serological Examination**
	Fungus Ball in pre cavity lesions	Multiple opacities	Suspected Central Bronchiectasis (CB)	Rhonchi	Chest discomfort	Previously on Antibiotic	History of TB	Raised IgG for Aspergillus
n=17	2	5	15	16	17	2	5	7

Unstable patients who were negative for exacerbations but culture positive for *Aspergillus* spp in their first visit, were followed and it was observed that their clinical condition worsened. Among 25 unstable COPD patients, 64% (16/25) had exacerbation of COPD in their first visit and yielded 9 *Aspergillus* spp . 36% (9/25) of unstable patients were negative for COPD exacerbation, however, yielded 6 *Aspergillus* spp. in their first visit, resulted in development of exacerbations which worsened with time. This suggests that isolation of *Aspergillus* spp. increased exacerbation in unstable patients.

DISCUSSION

The development of COPD is multifactorial and the risk factors of COPD include genetic and environmental factors. The interplay of these factors is important in the development of COPD. This disease irreversibly and progressively limits airflow which results in defective immune response of lung to various particles.[10] Invasive IPA in immunocompetent patients which constitute a risk group such as COPD have been increasingly documented.

In the present study males were affected more than females among COPD patients with LRTI. This higher predilection of LRTI in males may be attributed to their increased exposure to the outside environment. Humphrey et al, also reported high prevalence of LRTI and pneumonia in males as compared to females.[18] A study by Vijay et al, which focused on prevalence of LRTI in patients presenting productive cough, found males to be at a high risk of having LRTI as well as COPD due to smoking, tobacco and alcohol consumption that causes decrease mucocilliary defenses, airway collapse and respiratory muscle fatigue high risk.[19]

55% of hospitalised COPD patients had exacerbations and worsened clinical conditions were observed unstable with severe chest discomfort and dyspnoea compared to rest 44% OPD apparently stable cases not resulting in hospitalisation. It was found that among 25 of the admitted unstable patients 8% (2/25) patients were previously on antibiotics. While, of the 20 stable patients 10% (2/20) had past history of pulmonary tuberculosis and were not on antibiotics. Muquim et al, in a similar study also observed that use of broad spectrum antibiotics before hospitalization in COPD cases was associated with development of IPA.[20] He H et al also found that use of two or three antibiotics for more than 10 days was a risk factor for IPA in patients with COPD.[21] Similar observations were observed in a study by Tutar et al.[22]

In our study direct microscopy of sputum samples revealed presence of septate fungal hyphae in 24% patients with COPD who were also culture positive for *Aspergillus* Spp. Culture along with direct microscopy was found to be useful for *Aspergillus* detection (P<.00001). Among the 37% *Aspergillus* spp. isolated in our study from COPD patients having LRTI, *A. fumigatus* was the predominant species isolated. Our study results are similar to the study by Guinea et al, according to whom *A. fumigatus* was involved in 83% of cases of IPA.[23] 60% (15/22) *Aspergillus* species were isolated from the unstable patients who needed treatment and hospitalization and had exacerbation while 10% (2/20) were isolated from patients which were stable. Among the patients from whom *Aspergillus* spp. were isolated, two patients had past history of tuberculosis and their X ray finding showed formation of fungus ball in past cavitary lesion in lung. Both of them also had raised IgG specific for *A. fumigatus* and both of them yielded *A. flavus* and were diagnosed to be suffering from Aspergilloma. One of the unstable patients had

developed invasive pulmonary disease with isolation of *A. flavus* and raised IgG specific for *A. fumigatus.* The other unstable patients from which *Aspergillus* spp. were repeatedly isolated were categorised as possible IPA. It was also found that all of them had low socio- economic background having varied professions and living in homes which were poorly ventilated. In contrast to our study, O. S Zmeili et al, found that among 13 patients of IPA the only risk factor found was corticosteroid treatment for development of COPD.[16]

In the present study we found that colonization of *Aspergillus* spp. in COPD patients was the predisposing condition for the development of COPD exacerbation and development of IPA. In contrast a study by Guinea et al, found COPD as the predisposing condition for development of IPA in their hospitalised patients.[23] In our study patients who had exacerbations of COPD and were stable but yielded *Aspergillus* spp. were followed to assess their stability and it was found that exacerbations increased in them along with frequent complains of chest discomfort, chest pain, sputum production and isolation of *Aspergillus* spp. Also patients who were unstable and culture positive for *Aspergillus* spp. but did not have exacerbation of COPD earlier but later on when followed developed exacerbations. This shows that colonization of lungs by *Aspergillus* spp. of stable as well as unstable COPD patients having LRTI further impaired the lung functions and progressed towards increased COPD exacerbation and worsened the conditions especially in elderly patients . Our study results are in contrast to a study by Bafadhel et al, in which clinical significance of positive culture in COPD did not change exacerbations and lung functions therefore remained uncertain.[24]

As no lung biopsies was done in our study therefore, we classify the cases of IPA only as probable and possible IPA. Which is in contrast to a study by Bulpa et al, who categorized the LRTI patients into proven, probable and possible IPA.[1] Although the clinical manifestations and imaging findings of patients with COPD and IPA are non–specific, the two conditions share common features and predisposing factors. It has been postulated that isolation of an *Aspergillus* spp. 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.[7,9] Confirmation of infection usually needs the demonstration of histopathological evidence that is not feasible in this type of patients.[4]

CONCLUSION

Present study helped to find a direct and evident co-relation between the exposure, colonization and isolation of *Aspergillus* spp. from COPD patients and *Aspergillus* colonization in such hospitalised unstable elderly chronic smoker COPD patients being a reason of exacerbation in them.

Early noninvasive diagnosis becomes crucial to initiate timely lifesaving therapies, given the invasive nature of the fungus and the mortality associated with it.[25] Studies evaluating the outcome of elderly COPD patients with *Aspergillus* spp. isolation are required and the impact of early isolation along with an adequate anti fungal therapy in this high risk population has to be assessed for preventive therapy.

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