



PREVALENCE OF ASPERGILLUS SPECIES IN PATIENTS SUFFERING FROM CHRONIC RESPIRATORY ILLNESS.

Renu Khangarot*

Postgraduate, Department of Microbiology, JLN Medical College Ajmer, Rajasthan *Corresponding Author

Pushpanjali Verma

Senior Demonstrator, Department of Microbiology, JLN Medical College Ajmer, Rajasthan

Vijay Latha Rastogi

Senior Professor, Department of Microbiology, JLN Medical College Ajmer, Rajasthan

Neeraj Gupta

Senior Professor, Department of TBCD, JLN Medical College Ajmer, Rajasthan,

ABSTRACT

Aspergillus is a saprophytic filamentous and thermo tolerant fungi that can affect any organ system, but the respiratory tract is involved in more than 90% of affected patients. A retrospective analysis of 5-year (from Nov 2013 to Oct 2019) data on fungal etiology of chronic respiratory illness in patients admitted to respiratory medicine ward was done. Direct KOH mount and fungal culture was done on all 687 respiratory samples received in the laboratory using standard conventional mycological techniques. Fungal etiology was established in 67.39% of cases in which 16.15% cultures were positive for *Aspergillus*. The predominant species grown was *A.niger* (30.63%) followed by *A.flavus* (26.12%), *A.fumigatus* (19.81%) and *A.spp* (23.42%). The *Aspergillus* positive cultures were predominantly from the cases of multi drug resistant (MDR) tuberculosis (32%) followed by asthma (21%) and chronic obstructive pulmonary disease (14%). Our study concluded MDR patients were found to be more prone to *Aspergillus* infection as the patients were immunosuppressed due to diseases pathology and long-term treatment for pulmonary tuberculosis. Isolation of *Aspergillus* predominantly from MDR TB and asthma cases warrants thorough follow up of allergic bronchopulmonary aspergillosis for such cases to institute appropriate line of management.

KEYWORDS : *Aspergillus*, MDR patients, COPD, Asthma.

INTRODUCTION

Aspergillus species are saprophytic filamentous and thermo tolerant prevalent airborne fungal pathogens that survive and grow on organic debris. They are widespread in the environment and aerosolizes conidia, which are inhaled by the humans at the rate of hundreds per day without experiencing complications.⁽⁷⁾ *Aspergillus* can produce a spectrum of diseases, including allergic bronchopulmonary aspergillosis (ABPA), aspergilloma or fungus ball, chronic necrotizing aspergillosis and life-threatening invasive aspergillosis (IA).⁽⁷⁾ Although *Aspergillus* can affect any organ system, the respiratory tract is involved in more than 90% of affected patients. Inhalation of *Aspergillus* spores or conidia can give rise to various clinical conditions, depending on the host's immune system. *Aspergillus* species tends to infect open spaces such as pulmonary cavities caused by previous lung disorders.⁽⁸⁾ With the increasing number of immunocompromised patients suffering from different types of infections, invasive fungal aspergillosis is becoming an increased cause of morbidity and mortality in these patients.⁽¹⁶⁾ Hence, this study was done to know the prevalence of *Aspergillus* infections in patients suffering from chronic respiratory illness in our region.

MATERIALS AND METHODS

The study was conducted in the Department of Microbiology JLN medical college and hospital, Ajmer (Rajasthan) from Nov 2013 to Oct 2019. The number of patients admitted in the Respiratory Medicine and Chest department in 6 year was 22917 and clinically diagnosed patients with chronic respiratory illness were 687, in which 612 were sputum samples and 75 were BAL collected from patients for fungus culture.

Patients showing clinical or radiographic evidence were asked to provide early morning deep coughed sputum samples after a proper mouth wash with saline water within three consecutive days. Samples were collected in a properly labelled sterile wide-mouth container by taking all aseptic precautions. BAL was taken by using a fiberoptic

bronchoscope. The bronchoscope was advanced through mouth distally into a specific subsegmental bronchus, and if no specific segment, then middle lobe and/or lingula. Retrieved BAL fluids were transported to the microbiology department within 2 hrs. in sterile vials. Patients which do not show infection in radiography and sample containing more amount of saliva were excluded from further studies. Though BAL is an appropriate sample for study but due to invasive procedure, it is not easily available, therefore sputum samples were suitable for repeated cultures.

All Samples were subjected to direct microscopy using 10% KOH mount and gram's staining to observe the presence of fungal elements then inoculated onto SDA with Chloramphenicol (0.05 mg/mL) and incubated at 22°C and 37°C respectively. Identification of fungi grown was done based on macroscopic and microscopic observation using standard conventional mycological techniques. Macroscopic identification was based on growth rate, colony morphology, colour and texture while microscopic observation was done based on appearance of conidiophores, vesicle and conidia in LPCB mount. All materials and culture media were obtained from HiMedia laboratories Pvt. Ltd., Mumbai, India.

RESULTS

In this study total patients with chronic respiratory illness are 687 in which fungal etiology was established in 67.39% of cases. Out of these positive cases 16.15% were positive for *Aspergillus spp.* (55.85% were pure growth while 44.14% were mixed growth). Predominant species was *A. niger* 30.63% followed by *A. flavus* 26.12%, *A. fumigatus* 19.81% and *A. spp* 23.42% figure-1. *Aspergillus* positive cultures were predominantly from the cases of MDR tuberculosis 32%, asthma 21% and COPD 14% table 1.

TABLE-1 Samples from different clinical conditions and their correlation of KOH with culture positivity-

Type	Total samples	Total <i>Aspergillus</i> positive	Total KOH

MDR TB	275	35(32%)	28(36.8%)
Asthma	116	23(21%)	17(22.4%)
COPD	85	15(13.51%)	09(11.8%)
Bronchitis	63	5(4.50%)	02(2.63%)
Bronchiectasis	20	3(2.70%)	01(1.31%)
Others	128	30(27.02%)	19(25%)
	687	111	76

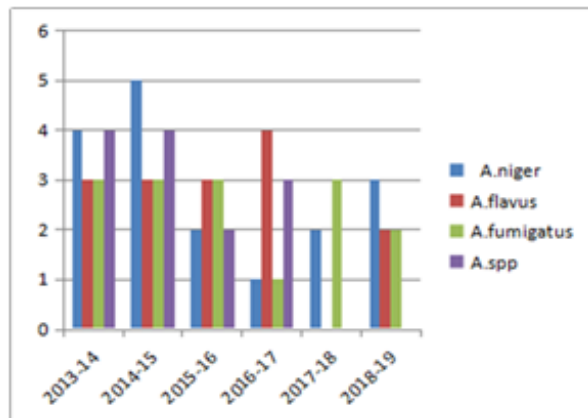


Figure 1: Year wise distribution of Aspergillus isolates

DISCUSSION

Many fungal pulmonary infections usually go unnoticed because there exists a commensal relationship between fungus and TB infections which does not show specific clinical manifestation. Raised incidences of respiratory tract infections due to fungal agents in immunocompetent individuals causes high rates of morbidity and mortality which is a major cause of concern.⁽⁶⁾ Therefore, this study was conducted to know the prevalence of Aspergillus in respiratory samples with its correlation to KOH positivity for proper diagnosis of the opportunistic fungal pathogens.

In our study the prevalence of Aspergillus infection was 16.15% and the predominant species was *A. niger* (31%) followed by *A. flavus* (26.12%), meanwhile other studies showed that among the species *A. fumigatus* and *A. flavus* were the most common pathogens and the most frequency of infections with possible IPA was related to *A. fumigatus* with a combined prevalence of 57.6%.⁽⁸⁾ This is because of the factors responsible for the growth of *Aspergillus spp*, such as characteristics of the soil & plant debris, hospital host factors, microbiological factors, different geographical and climatic conditions.

This study also correlated direct microscopy along with culture aided in the identification of *Aspergillus spp.*, as an etiological agent.⁽⁶⁾ showed that MDR patients were found to be more prone to Aspergillus infection i.e., 32% with 36% positivity for KOH followed by asthma i.e., 21% with 22.36% positivity for KOH.

We observed the presence of septate fungal hyphae on direct microscopy of sputum samples of 12.41% of LRTI patients. This explains that fungus positivity was higher in the treated (chronic) group than in the fresh untreated cases of tuberculosis, correlating with a study by Kurhade et al. which observed 16.2% of Aspergillus spp., from cases with chronic pulmonary infections,⁽⁷⁾ while retrospective study of 8 years by Barberan et al. found 56.6% Lower respiratory tract infections patients with single culture positive for *Aspergillus spp.*, which was higher as compared to other studies by Vandewoude et al., Bouza et al., and Soubani et al.^(18,3,20,1) These observations demonstrate the diagnostic importance of direct microscopy of sputum samples. However, it remains unclear if the presence of septate fungal hyphae in direct microscopy demonstrates invasion or colonization.

Therefore, the clinical and radiological features of all patients were recorded, to differentiate the possibility whether species were part of their flora i.e., colonization or infection. Infected patients had clinical or radiographic evidence which included characteristic findings of Aspergillus infection such as the halo sign initially, later the crescent sign and appearance of walking pneumonia. Pulmonary diseases such as aspergilloma (fungal ball), characterised by saprophytic growth of the fungus in pre-existing tuberculous cavities is also a major finding in X rays. These findings prevent delay in the diagnosis related to therapeutic failure and progression of the disease, ultimately leading to complications and death.

The weakening of the immune system, and the prolonged anti-tubercular therapy, duration of diseases, chronicity of lesion and associated illness, becomes an effective predisposing factor for the beginning of co-infection by the fungal agents in active pulmonary tuberculosis. Apart from that if tests are not conducted, pulmonary mycoses can be easily misdiagnosed and mistreated as pulmonary tuberculosis, which leads to inappropriate therapy or none at all. Therefore, it is needed that all fungi detected in clinical specimens from patients especially immune-compromised and suffering from pulmonary tuberculosis should be carefully identified and physicians evaluate the clinical significance of these isolates such as coinfection of tuberculosis with opportunistic fungi. There is presence of comparable clinical signs of tuberculosis and pulmonary fungal co-infection, therefore cases in which direct smear and culture of samples for TB are negative, the specimens were evaluated for opportunistic fungi.⁽⁶⁾

Fluconazole (FLU), Voriconazole (VRC), Itraconazole and Amphotericin B (AMB) are the appropriate treatment for most of the fungal infections. Itraconazole can penetrate the walls of the cavity and even inside the fungal balls. Hence, azoles are considered as an important therapeutic option in patients with aspergilloma. Voriconazole has better pharmacokinetics and tolerability than Itraconazole and is currently preferred over Itraconazole in management of aspergillosis. However, Voriconazole is significantly expensive and is rarely purchased by the patients.

Serological tests of galactomannan and β -D glucan glucose can be detected several days before the presence of clinical signs, an abnormal chest radiograph, or positive culture may allow earlier confirmation of the diagnosis, and serial determination of its values may be useful in assessing the evolution of infection during treatment.⁽¹⁴⁾ Using the recent advanced molecular technique polymerase chain reaction (PCR) is another way for early diagnosis of mycosis infection, which can aid in the successful treatment of established Aspergillus infection consequently preventing its progression to fibrotic stage, increasing the survival rate and avoiding pulmonary disability.⁽²⁰⁾

CONCLUSION

Our study reveals *Aspergillus niger* as the most common species found in the respiratory tract samples. MDR patients were found to be more prone to Aspergillus infection because of their immunocompromised state due to long term treatment for pulmonary tuberculosis with broad-spectrum antibiotics and corticosteroids. Isolation of Aspergillus predominantly from MDR TB and asthma cases warrants thorough follow up for ABPA in such cases, to institute appropriate lines of management. Rapid diagnostic test like galactomannan enzyme immunoassay, β -d glucan and PCR in conjunction with other diagnostic test like culture histopathologic examination of biopsy specimen and radiographic examination aid in early diagnosis of invasive aspergillosis and better management of diseases in high-risk patients.^(11,14) With raised incidences and difficulty to diagnose fungal agents in respiratory tract infections early, sensitive assays for accurate diagnosis of the fungal infections are warranted.

Therefore, appropriate early diagnostic strategies are needed to aid in prompt management, prevent invasion and subsequent serious complications by the fungi.

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