



OUTCOME OF SPUTUM FUNGAL CULTURE IN CLINICALLY SUSPECTED CASES OF PULMONARY ASPERGILLOSIS.

Medical Microbiology

Zeenat Kausar	M. Sc. Medical Microbiology, Integral Institute Of Medical Science And Research, Integral University, Lucknow-226026
Sshrutkirti Gupta*	MBBS MD Microbiology, Integral Institute Of Medical Science And Research, Integral University, Lucknow-226026 *Corresponding Author
Akriti Sharma	M. Sc. Medical Microbiology, Integral Institute Of Medical Science And Research, Integral University, Lucknow-226026

ABSTRACT

Objective: To determine the percent positivity fungal culture in clinically suspected cases of pulmonary aspergillosis with reference to aspergillus species. **Methods:** This prospective and observational study was carried out at department of microbiology and Department of Respiratory Medicine, IIMSR, Lucknow. We collected sputum samples from the asthmatic patients to determine the culture positivity. **Results:** In my study total of 90 samples were included and processed, out of which only 15 were positive. In positive 15 samples the culture shows two different species, 6 were aspergillus positive and 9 were candida positive. The most affected age group was greater than 40 years. Out of 6 aspergillus positive patients who included in this study, 33% were males and 66% were females. In my study the diagnosis based distribution included the high occurrence of ABPA with bronchial asthma is 50%, the 33% of allergic rhinitis and 17% aspergilloma. **Conclusion:** Overall, the prevalence of clinically suspected cases of Allergic Bronchopulmonary Aspergillosis in asthmatic patients shows (6.6%). Sputum fungal cultures in patients with Allergic Bronchopulmonary Aspergillosis (ABPA) can provide valuable insights into the specific fungal species involved.

KEYWORDS

ABPA, Aspergillosis, Asthma

INTRODUCTION

Aspergillus species are common saprophytic fungus that are important to the world's recycling of carbon and nitrogen. Even while soil and decomposing plants are aspergilli's major biological niche, they also produce small, hydrophobic conidia that are easily dispersed into the air and can withstand a wide variety of environmental conditions. With around 200 species, of genus Aspergillus, Aspergillus fumigatus is the main human pathogenic species responsible for infections, followed by Aspergillus flavus and Aspergillus niger.^{1,2}

Aspergillus fumigatus exposure causes allergic bronchopulmonary aspergillosis (ABPA), a chronic pulmonary disease. Wheezing, a chronic cough are the major symptoms. Lung infiltration can be appreciated on chest X ray, ABPA is associated with bronchiectasis, asthma, cystic fibrosis (CF), few cases have also been reported in people with COPD and tuberculosis.^{3,4,5}

ABPA is a lung disease associated with the hyperactivity of immune system, and Aspergillus hypersensitivity is the initial step of its development.⁶

A. fumigatus is the most frequent Aspergillus species known to infect humans, followed by A. flavus, A. terreus, A. niger, and A. nidulans. An estimated 1 million to 2.8 million instances of allergic bronchopulmonary aspergillosis occur in people with fungal sensitivity and severe asthma. Fungal culture of respiratory samples is an insensitive method for the diagnosis of aspergillosis. Sputum fungal culture is a diagnostic tool that involves collecting and culturing samples of sputum, or respiratory mucus, to identify the presence of Aspergillus fungi.^{7,8}

MATERIALS AND METHODS

Study Design and Period: A prospective observational 6 months Study was conducted at Department of Microbiology and Department of Respiratory Medicine, Integral Institute of Medical Sciences and Research, Lucknow, UP.

Sample Size – 90

Inclusion Criteria

Patients who gave consent and with clinical suspicion of ABPA like respiratory symptom cough, wheezing, respiratory distress, decreased PFT, X-ray finding suggestive of parenchymal infiltrates

Exclusion Criteria

Patients who could not be kept off antihistamines and leukotriene antagonists for 1 week.

Laboratory Diagnosis

Sample Collection- Sputum samples were collected from patients suspected of having respiratory tract infection while visiting the Respiratory Medicine OPD. The samples were processed at the Microbiology laboratory, following standard protocols. To facilitate culture and identification, the sputum samples were collected in a wide-mouth, leak-proof, sterile container. Following the sputum collection, a potassium hydroxide (KOH) mount was performed.

KOH Mount- In the KOH mount process, direct microscopy of sputum in 10% KOH was conducted to observe the presence of fungal elements. Additionally, a culture for same sample was performed.

Culture- The sample was cultured on two pairs of SDA (Sabaured Dextrose Agar), in each pair one slant was with chloramphenicol and other was without it and after that one pair was incubated at 25°C for 2-3 weeks, and the other was incubated at 37°C for 2-3 weeks follow up of the sample was done and checked every alternate day for any fungal growth on slant. Aspergillus growth mostly occur within initial week from day of sample processing. However no growth i.e. final negative report was declared only after 3 weeks of incubation. In case of growth culture was observed and (LPCB) Lactophenol cotton blue mount was performed for the identification of species.

Identification of Species

LPCB mount- From the positive culture, LPCB mount was made and was observed under high power (40X).

Aspergillus flavus- The colonies on SDA are velvety, yellow to green or brown. On LPCB mount the conidiophores are of variable length, rough and spiny. The phialides are single and double cover entire vesicles.

Aspergillus fumigatus - The colonies on SDA are velvety and powdery at first and turning into smoky green. On LPCB mount the conidiophores are smooth, phialides are single, usually cover upper half of vesicles.

Aspergillus niger- The colonies on SDA are typically black or dark green. On LPCB mount the conidiophores are single row uniseriate or irregularly branched.

Data Analysis and Interpretation

Data was analyzed using SPSS Software, Microsoft excel

RESULTS

Total 90 samples from patient were cultured and were included in this study, of which 49(54%) sample were from male and 41(46%) from female patients. Out of a total sample of 90 sample, 43 samples were from patient more than 40 years of age.

Out of all 90 samples 64 (71%) samples were taken from the clinically

proven case of bronchial asthma, 8 (9%) from allergic rhinitis, 6 (7%) of COPD, 11% of PTB and 1% of LRTI (table 1)

Out of all samples 15 (17%) were showed positive culture growth. Out of 15 positive samples, 40% (6) were male and 60% (9) were female. However when culture growth was compared to KOH mount result it was found out of 15 positive sample for fungal culture only 10 sample showed fungal filament on KOH mount rest 5 were negative on KOH mount.

When all the sample that showed culture positive were correlated clinically to respective patient it was found that (FIG 1) ...79 PATIENT WERE IMMUNOCOMPROMISED. AND OUT OF 79 IMMUNOCOMPROMISED PATIENT 13 SHOWS POSITIVE GROWTH FOR FUNGAL CULTURE AND REST 2 POSITIVE CULTURES WERE FROM OLD AGE PATIENT.

Table 1-

DIAGNOSIS	NO. OF PATIENTS	POSITIVE FOR KOH MOUNT	POSITIVE FOR FUNGAL CULTURE	TOTAL NO OF ISOLATES IDENTIFIED	ISOLATE (IT WILL INCLUDE ALL 15 ISOLATES NAME)	FUNGAL INFECTION DUE TO ASPERGILLUS SPECIES
BRONCHIAL ASTHMA	64 (71%)	5/64	7/64	7/64(10%)	ASPERGILLUS FUMIGATUS(2), ASPERGILLUS NIGER (1) MUCOR(1), CANDIDA SPECIES (3)	3/64
ALLERGIC RHINITIS	8 (9%)	2/8	4/8	4/8 (50%)	ASPERGILLUS FUMIGATUS (2) ,CANDIDA(1) MUCOR (10)	2/8
COPD	6 (7%)	0/6	1/6	1/6(16%)	CANDIDA SPECIES (1)	0
PTB	10 (11%)	2/10	2/10	2/10(20%)	CANDIDA SPECIES (2)	0
LRTI	2(2%)	1/10	1/2	1/2(50)	ASPERGILLUS FUMIGATUS (1)	1/2

Table 1 estimates that case positivity was 50% in cases of allergic rhinitis and lower respiratory tract infection followed by pulmonary TB (18%), COPD (16%), and bronchial asthma (10%) . Speciation was done for all positive sample. Out of the 15 isolates candida accounts for 46%, followed by,aspergillus species 40 % (6/15),and mucor 13%. Of 6 aspergillus species isolate, 3 were from the case of bronchial asthma, 2 from allergic rhinitis and 1 from case of lower respiratory tract infection (LRTI).

Overall case positivity for aspergillus species was (6/90) 6.67%. Most common isolated species of aspergillus was aspergillus flavus followed by niger

DISCUSSION

In this study, the prevalence of aspergillus infection is 6/90 (6.6%). Compared to other studies, it was observed that the prevalence rate of BHANKHUR et al. (2019)⁹ is 35/50 (70%), while SHARMA JK et al. (2020)¹⁰ is 5/100 (5%) and SAXENA P et al. (2021)¹¹ is 106/543 (19.5%). It shows the rate of positive prevalence in this study is less than in the other studies..

In our study, the positivity percentage in males was 2/6 (33%), and in females it was 4/6 (66%), whereas in the study of BHANKHUR et al. (2019)⁹, the positivity percentage in males was 17/35 (48%), and in females it was 18/35 (51%).. As compared with another study (SHARMA JK et al., 2020)¹⁰, the positivity of males and females was 3/100 (3%) in males and 2/100 (2%). The prevalence of males and females in my study showed that females had 66% positivity, which is somehow similar to BHANKHUR et al.'s (2019)⁹, where the female suffering from fungal infection was found to be higher than the male. The study by Nath et al. (12) reported no significant gender prediction toward fungal infection

According to SAXENA P et al. (2021)¹¹, the highest prevalence of aspergillus infection was seen in the younger than 66-year-old age group, and my study shows that the high prevalence of ABPA is in the greater than 40-year-old age group.

The diagnosis criteria included in my study show the high prevalence of asthmatic patients; the positive cases included bronchial asthma patients were 50%, and the above-mentioned studies show the

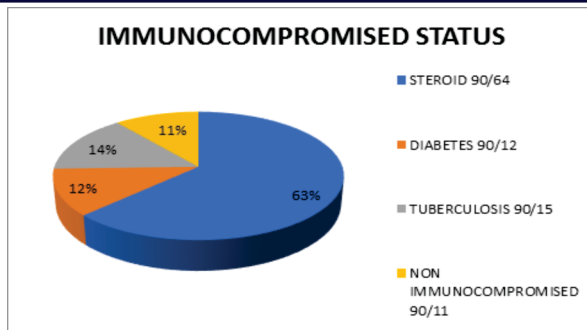


Fig 1- Distribution According To Immunocompromised Status Of Patient

prevalence of ABPA in bronchial asthma in BHANKHUR et al.'s (2019)⁹ is 70%, in SHARMA JK et al.'s (2020)¹⁰ is 5%, and in SAXENA P et al.'s (2021)¹¹ is 19.5%. Overall, we found a high occurrence of ABPA in patients with bronchial asthma. This research emphasizes that many asthma patients have ABPA. Therefore, it shows that the assessment of all these patients for ABPA followed standard guidelines.

When the study was compared with other studies, it was observed that the prevalence of positive cases of aspergillus differs, and its prevalence among males and females is quite similar to other studies. The range of age group prevalence is above 40 years, and it was observed that the clinical presentation association of ABPA with bronchial asthma was the same.

CONCLUSION

In my study the prevalence of clinically suspected cases of Allergic Bronchopulmonary Aspergillosis in asthmatic patients shows (6.6%). Sputum fungal cultures in patients with Allergic Bronchopulmonary Aspergillosis (ABPA) can provide valuable insights into the specific fungal species involved.

We strongly recommend fungal culture as a standard procedure in microbiology investigations. Among the six Aspergillus-positive patients included in this study, the females were mostly affected than the males. The distribution of diagnoses revealed a high occurrence of ABPA with bronchial asthma (50%).

REFERENCES

- Gugnani, H. C. 2003. Ecology and taxonomy of pathogenic aspergilli. *Front.Biosci.* 8:s346-s357.
- Denning, D. W. 1998. Invasive aspergillosis. *Clin. Infect. Dis.* 26:781-803.
- R. Agarwal, Allergic bronchopulmonary aspergillosis. *Chest* 135 (3) (2009) 805-826.
- A. Shah, C. Panjabi, Allergic bronchopulmonary aspergillosis: a perplexing clinical entity. *Allergy Asthma Immunol Res* 8 (4) (2016) 282-297.
- E. Mir, A. Shah, Allergic bronchopulmonary aspergillosis in a patient with chronic obstructive pulmonary disease. *Prim. Care Respir. J.* 21 (1) (2012) 111-114.
- Agarwal R, Singh N, Gupta D. Pulmonary hypertension as A presenting manifestation of allergic bronchopulmonary Aspergillosis. *Indian J Chest Dis Allied Sci* 2009;51:37-40.
- Ben-Ami, R.; Lewis, R.E.; Kontoyiannis, D.P. Enemy of the (Immunosuppressed) State: An Update on the Pathogenesis of Aspergillus fumigatus Infection. *Br. J. Haematol.* 2010, 150, 406-417. [CrossRef]
- El-Baba, F.; Gao, Y.; Soubani, A.O. Pulmonary Aspergillosis: What the Generalist Needs to Know. *Am. J. Med.* 2020, 133, 668-674. [CrossRef] [PubMed]
- Bhankhur D, Singla N, Aggarwal D, Chander J. Prevalence of allergic

- bronchopulmonary aspergillosis among patients with severe bronchial asthma in a tertiary care hospital in Northern India. *Indian J Pathol Microbiol.* 2019;62:111–3.
10. Sharma JK, Jain S, Joshi V, Khippal N. Clinical significance of fungal sensitization in bronchial asthma: A prospective cross-sectional study at a tertiary care center. *Indian J Allergy Immunol.* 2020;34:98–102.
 11. Saxena P, Choudhary H, Muthu V, Sehgal IS, Dhoooria S, Prasad KT, et al. Which are the optimal criteria for the diagnosis of allergic bronchopulmonary aspergillosis? A latent class analysis. *J Allergy Clin Immunol Pract.* 2021;9:328–35.e1.
 12. Nath A, Khan A, Hashim Z, Patra JK. Prevalence of *Aspergillus* hypersensitivity and allergic bronchopulmonary aspergillosis in patients with bronchial asthma at a tertiary care center in North India. *Lung India* 2017;34:150-4.