ORIGINAL ARTICLE

ISOLATION OF ASPERGILLUS SPECIES FROM SPUTUM SAMPLES: A STUDY CONDUCTED IN A TERTIARY CARE HOSPITAL, AHMEDABAD

Gaurishanker P Shrimali[1], Jaymin K Bhatt[2], Rakesh Rajat[3], Rohit V Parmar[4], Sunil Nayak[5], D Chandralekha[6]

Authors' Affiliation: [1]Assistant Professor, Microbiology Department, GMERS Medical College, Dharpur, Patan; [2]Assistant Professor, Pathology Department, UN Mehta Institute of Cardiology & Research Centre; [3]Assistant Professor, Microbiology Department, GMERS Medical College, Gandhinagar; [4]Assistant Professor, Preventive and Social Medicine Dept., GMERS Medical College, Gotri, Baroda; [5]Associate Professor, Preventive and Social Medicine Dept., GMERS Medical College, Dharpur, Patan; [6]Ex.Professor and Head, Microbiology Department, B.J.Medical College,Ahmedabad
Correspondence: Dr. Gaurishanker Shrimali, Email: drgaurishanker@yahoo.co.in

ABSTRACT

Introduction: In the last three decades, fungal infection has become important cause respiratory tract infection. The increase in frequency is mainly due to intensive cytotoxic therapy, greater use of broad spectrum antibiotics, corticosteroids and Immuno-suppresants.

Material and Method: Sputum Samples were collected from june 2005 to june 2006.The samples were subjected to direct microscopy using Gram staining, KOH wet mounts, India ink preparations depending on the type of specimen and the suspected infection in the patient. Fungal culture was done on Sabouraud dextrose agar, with and without chloramphenicol, Czapek`s solution agar and malt extract agar.

Result: This study was undertaken to determine isolation of Aspergillus species from sputum samples**.** 61% of sputum specimens were culture positive and 39% were negative among cases of chronic respiratory diseases. 29% of 100 samples are aspergillus spp. while 32% of positive culture are other than aspergillus spp. Highest number of male & female patients were from 31-40 years of age and then gradually decrease in number in 21-30 and 41-50 years of age group.

Conclusion: An increase in rate of infection occurs during a period of building construction, particularly in zones surrounding hospitals.

Key Words: Chronic Respiratory Disease. Pulmonary Aspergillosis, Immunosuppressant, fungus ball, Aspergilloma

INTRODUCTION

Fungal infection of the lung especially pulmonary aspergillosis is difficult to diagnose by simple laboratory procedure. Pre existing lung disease act as an important predisposing factor for pulmonary aspergillosis due to intensive cytotoxic therapy, greater use of broad spectrum antibiotics, corticosteroids and Immunosuppresants.[1]

Diagnosis of pulmonary aspergillosis is usually missed as test of their detection cannot be undertaken in routine diagnostic laboratories.[2]

Asergillus species are ubiquitous fungi, commonly occurring in soil, water, and decaying vegetation. Route of acquiring infection being inhalation of fungal spores. From lungs, dissemination takes place leading to systemic aspergillosis.[3]

Occurrence of aspergillus infection in association with chronic lung disease like pulmonary TB, lung abscess, bronchopneumonia, with residual lung cavity, asthma and lung malignancy has been documented.[3] Out of 185 species of genus aspergillus, only 20 can cause human infection. Fumigatus is the most common species found in human infection all over the world[3].

This study was undertaken to determine isolation of Aspergillus species from sputum samples**.**

MATERIAL AND METHOD

In present study, sputum sample were collected from 100 patients suffering from chronic respiratory diseases attending indoor and outdoor patients of tertiary health care centre, Ahmadabad.

This study was undertaken to determine isolation of Aspergillus species from sputum samples which were collected from June 2005 to June 2006 after taking approval of institutional ethical committee.

Microscopy, culture and identification[3]

Depending on the clinical symptoms, relevant clinical samples were collected with complete universal precautions and relevant methods were used for diagnosis and isolation, which included a battery of tests as per standard procedures. The samples were subjected to direct microscopy using Gram staining, KOH wet mounts, India ink preparations depending on the type of specimen and the suspected infection in the patient.

Morphological terms: Aspergillie produce conidia in a basipetal manner, which results in chains of asexual conidia where the youngest conidium is at the base and oldest at the tip of the chain. The conidiogenous cell is termed as a phialide.

The conidiophore is hyphae like structure that arise arise at its apex to form a swollen vesicle. The base of the conidiophore, where it originates from the perent vegetative hyphae is termed foot cell. The phialides may arise directly from the vesicle in a uniseriate and from metulae in biseriate[3].

Fungal culture was done on Sabouraud dextrose agar, with and without chloramphenicol, Czapek`s solution agar and malt extract agar. Specimens were streaked in duplicate; one set of inoculated slants was incubated at 25 °C and the other at 37 °C, and they were examined every other day for growth up to 4–6 weeks before discarding as negative.

Fungal growth was identified by colony morphology, Gram staining, lacto phenol cotton blue preparation and slide culture as per standard recommended procedures.

RESULTS

In present study, sputum sample were collected from 100 patients suffering from chronic respiratory diseases to isolate the Aspergillus species from sputum samples.

Samples were classified according to various age groups, sex, and detection of Aspergillus fungi among male and female patients.

Table 1: Age and Sex wise distribution of patients.

Age	Male	Female	Total
11-20	5	1	6
21-30	11	2	13
31-40	29	10	39
41-50	18	5	23
51-60	6	1	7
61-70	5	0	5
>71	5	2	7
Total	79	21	100

Table–1 Shows that there were 79% male and 21% are female.

Table–1 Shows that among male patients, highest number of patients were from 31-40 years of age and then gradually decrease in number in 21-30 and 41-50 years of age group. Among female patients, highest numbers of patients were from 31-40 years of age and then gradually decrease in number in other age group.

Table 2: Fungal growth in sputum culture

Sputum Culture	Patients (%)
Positive	61 (61.0)
Negative	39 (39.0)
Total	**100 (100)**

Result of fungal growth in sputum culture is shown in table 2. 61% of sputum specimens were culture positive and 39% were negative among cases of chronic respiratory diseases.

Table 3: Isolation of aspergillus species in sputum culture

Species	Positive
Aspergillus fumigates	18
Aspergillus niger	07
Aspergillus flavus	03
Aspergillus nidulans	01
Other fungus	32
Total	**61**

Table 3 shows 29% of 100 samples are aspergillus spp. while 32% of positive culture are other than aspergillus spp. Out of 61 positive culture 18 culture shows Aspergillus fumigates, 7 are Aspergillus niger, 3 culture shows Aspergillus flavus and 1 case show Aspergillus nidulans.

DISCUSSION

In the present study sputum samples were collected from 100 patients suffering from chronic respiratory diseases attending indoor and outdoor department, tertiary care centre and 20 healthy persons were taken as control group.

Out of total 100 patients, 21% were female and 79% were males. A good number of patients belong to age group of 21-40 years.

In one study[4] carried out between June 1, 1989 and May 31, 1991, it was observed that prevalence of Aspergillus species was 15.1% in sputum specimens.

In another study carried out by Pursell K J[5], It was found that 4.8% had been colonised with Aspergillus species based on sputum positive culture.

According to Grover et al[6] (1973) incidence was 28.3% and according to Jain at al [7](1982) incidence was 22.2%. Present study in suggests that the incidence of aspergillus spp. is 29%.

CONCLUSION

From this study we conclude that the infection rate of Aspergillus spp. is very high in our setup at tertiary care

hospital. This infection rate is increasing when compared with past studies.

REFERENCES

1. Kusne S, Torre-Cisneros J, Mañez R, Irish W, Martin M, Fung J, Simmons RL, Starzl TE. Factors associated with invasive lung aspergillosis and the significance of positive Aspergillus culture after liver transplantation. J Infect Dis. 1992 ;166(6):1379-83.
2. Shahid M, Malik A, Bhargava R. Prevalence of Aspergillosis in chronic lung diseases. Indian J Med Microbiol. 2001;19(4):201-5.
3. Jagdish Chander. Textbook of Medical Microbiology, 2nd edition. NewDelhi; 2002. pp. 272-283.
4. Daleine G, Salmon D, Lucet JF, Longuet P, Bouchaud O, Chochillon C, Le Bras J. Frequency of bronchopulmonary isolation of Aspergillus species in patients infected with human immunodeficiency virus: pathogenic role?. Pathol Biol (Paris). 1993 Mar;41(3):237-41.
5. Pursell KJ, Paredes J. The incidence of colonisation and invasive disease in a population of AIDS patients. International conference AIDS. 1990; June 22 6(1): p 237.
6. Grover S, Chaubey BS, Nawaz M, Sonsati MK. Pulmonary aspergillosis. J Assoc Physicians India. 1977 Aug;25(8):541-5.
7. Jain SK, Tiwari SK, Pandey RC. Mycological flora in sputum of chronic bronchitis. Indian J Pathol Microbiol. 1981 Oct;24(4):209-12.