

Current Scenario of Opportunistic Fungal Infections other than the Candida and Aspergillus Species, their correlation and Outcome with the CD4+T Cell Counts among Various Respiratory Samples in Immuno Competent and Immunocompromised Individuals in Aligarh Region, A North India Town

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ABSTRACT

Introduction: Invasive fungal infections other than the Candida species and Aspergillus species are becoming common in the nosocomial setting particularly among the immunocompromised patients but there is a hinderance in their accurate diagnosis. Aim of our study was to know the prevalence of rare fungal species and to know the patients outcome in our region. Aim of our study is to know the prevalence of rare fungal species and to know the patients outcome in our region.

Material and Methods: The present study was carried out on the patients attending outpatient department and inpatient department of T.B. and Respiratory Diseases, along with those attending antiretroviral treatment clinic and ICTC (Department of Microbiology), in J. N. Medical College, AMU during the period of January 2015 to October 2016.

Results: Majority of the cases i.e., 47 (31.3%) were between 31-40 years with a mean age of 32.5 years. Of 65 isolates, *Cryptococcus neoformans* and *Pneumocystis jirovecii* represented 2 (6.1%) isolates each, all of which were found in HIV positive cases. 1 (3.1%) isolate of *Mucor* from a diabetic patient and 1 (3.1%) isolate of *Penicillium marneffei* from an HIV positive patient as detected. *Cryptococcus* and *Pneumocystis jirovecii* were isolated from both patients with CD4 count < 200. and 114(±19.9) respectively.

Conclusions: Invasive fungal infections other than the candidia species and aspergillus species are becoming very common now a days.

Keywords: Cryptococcus Neoformans, Pneumocystis Jirovecii, Penicillium Marneffei, Pneumocystis Jirovecii, CD4 Cell

concerning due to the unique pathogenesis of the virus that lowers down the CD4+ T cell, signalling the emergence of opportunistic infections in the host.⁶ It is during the latency phase that CD4+ T cell counts begin to decrease and also inversion of the CD4+T/CD8+T cell ratios occurs. A CD4+ T cell count below 200cells/microliter and infection with at least one opportunistic infection such as *Pneumocystis jirovecii* defines clinical AIDS.⁷ Dissemination in cryptococcosis often occurs by the time pulmonary disease is diagnosed, and meningitis is found in 60% to 70% of patients with primary pulmonary symptoms.⁸ Cryptococcal meningitis occurs when the CD4 count is below 100/mm.⁹ The most commonly reported travel-related mycoses have been histoplasmosis and coccidioidomycosis, but cases of penicilliosis were also described among travelers returning from Southeast Asia.¹⁰

Few studies have compared the characteristics between different species in immunocompetent patients.¹¹ The data on the etiology and spectrum of fungal infections is scarce, particularly in Northern India. There is the need to undertake more studies on fungal etiological agents especially in a country with a growing population like ours where both the rural and urban masses are potentially at risk of developing infection.

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INTRODUCTION

As we know the most prevalent and well-known fungal pathogens is *Candida albicans* and *Aspergillus fumigatus*, a significant number of new emerging pathogens have been described.¹⁻³ Also significant are atypical fungi like *Pneumocystis jirovecii*, approximately 50% of HIV patients experience at least one bout of PCP (Pneumocystis pneumonia) during the course of the disease, Chronic lung disease and lung cancers are a leading cause of death in developed countries and are rising at alarming rates in developing countries.⁴ An estimated 4.7 million HIV-1-infected persons are living in Asia.⁵ The infection is very

Keeping the mentioned important facts and constraints in perspective, we undertook this study. Aim of our study was to know the prevalence of rare fungal species and to know the patients outcome in our region.

MATERIAL AND METHODS

The present study was carried out on the patients attending outpatient and inpatient unit of dept. of T.B. and Respiratory Diseases, along with those attending antiretroviral treatment clinic and ICTC (Department of Microbiology), in J. N. Medical College, AMU during the period of January 2015 to October 2016.

Selection of cases

Study group and design: Total 150 patients were divided into 2 subgroups:

- i) Immunocompetent – patients with clinical suspicion of lung carcinoma and chronic lung diseases like interstitial lung disease, chronic obstructive pulmonary disease etc.
- ii) Immunocompromised patients – Patients with compromised immunity i.e, with significant neutropenia <500 neutrophils/ μ l for longer than 10 days. These include AIDS, cancer and transplant patients who are taking corticosteroids, certain immunosuppressive drugs; and those with inherited diseases that affect the immune system (e.g., congenital agammaglobulinemia, congenital IgA deficiency). Cases were recruited from the outpatient departments, wards, Intensive Care Units (ICU), Antiretroviral treatment clinic, J. N. Medical College Hospital, A.M.U., Aligarh.

Collection of specimens

Expectorated Sputum- Early morning sputum samples were taken. Before collecting sampling patients were asked to rinse their mouth with normal tap. Two consecutive samples were considered positive.

Induced sputum – 8% saline was used to nebulise the patient for 15 minutes for induction of sputum.

Bronchoalveolar lavage- Bronchoalveolar lavage fluid was collected in a clean sterile vial by fiberoptic bronchoscopy after taking written informed consent. Five 20mL sterile saline aliquots at room temperature were infused and manually aspirated with a 20-mL volume syringe. The first aliquot was discarded, and the others were pooled and sent to the laboratory soon after taking. Pulse oximetry, electrocardiogram, and ventilatory parameters were monitored throughout the procedure.

Endotracheal aspirates- The catheter was introduced after donning sterile gloves, via the endotracheal tube for at least 30 cm, aspirate was suctioned and directly collected into sterile containers. The samples were transported immediately to the Microbiology Laboratory.

Pleural fluid - The proposed site for aspiration was directly over a palpable intercostal space and above the level of the diaphragm (no lower than 8th intercostal space). An 18G cannula was attached to a syringe and the needle was advanced along in the same plane as the local anaesthetic

was injected. On collection of 5ml pleural fluid, the needle was removed leaving behind the cannula in place, sealed to prevent entry of air. The catheter was removed at end expiration and the cannula was removed during the breath hold.

Blood – Total 5 ml of venous blood was collected taking all aseptic precautions in sterile plain vial. The skin site was disinfected before withdrawing blood. If the patient had an existing IV line, blood was withdrawn below the existing IV line.

All of the respiratory samples were subjected to the following tests:

- a) Microscopy
 - Gram staining
 - KOH mount
 - Giemsa stain
 - Calcofluor white stain
 - India ink
- b) Culture
 - Media used
 - Sabouraud dextrose agar (SDA) [Emmon's modification]
 - SDA with antibiotics
 - BHI Agar/ broth Biphasic medium for blood culture
 - BHI Agar with penicillin
 - Staib agar
 - CHROMagar
 - Czapek Dox Agar
- c) Antigen detection

Characterization of fungal isolates obtained from culture of various clinical specimens

All culture media were examined after initial inoculation and incubation, for fungal growth daily during the first week and on alternate days thereafter up to 3 weeks. The isolates were identified on macroscopic and microscopic morphological characteristics using standard techniques described in Medical mycology.¹²

Antigen detection in respiratory samples and serum

Latex Agglutination for the detection of Cryptococcal Antigen in Serum and Respiratory samples: Cryptococcal Antigen Latex Agglutination System (CALAS®) [Meridian Bioscience, Europe] was used to detect Cryptococcal Antigen in Serum and Respiratory samples.

RESULTS

Majority of the patients i.e., 47 (31.3%) were between 31-40 years with a mean age of 32.5 years. The male to female ratio was 1.8:1.

Amongst the total 150 patients, the immunocompetent patients comprised of 70 cases whilst the immunocompromised patients comprised of 80 cases. Most of the of immunocompetent cases were those presenting with lung mass, i.e., carcinoma (26.6%) and secondaries in lung (8%), whilst HIV positive patients constituted the main bulk ie (40%) of immunocompromised cases. 65 (43.3%) samples were positive for fungal elements on culture. Immunocompromised patients showed a

Fungal isolate		No. of cases (n=65)	Mean CD4 Count (cells μl^{-1}) (SD)	CD4 range (cells μl^{-1})
Cryptococcus	Immunocompetent	0	0	0
	Immunocompromised	2	88 (± 22.2)	72-104
Penicillium	Immunocompetent	0	0	0
	Immunocompromised	1	68	-
Mucor	Immunocompetent	1	520	-
	Immunocompromised	0	0	0
Pneumocystis	Immunocompetent	0	0	0
	Immunocompromised	2	114 (± 19.9)	101-128

Table-1: Distribution of isolates other than the Candida spp. and Aspergillus spp. in relation to CD4 counts

Cases	No. of cases	Deceased	Survived	Unknown
Cryptococcosis	2	1	1	0
Penicillosis	1	0	1	0
Mucormycosis	1	1	0	0
Pneumocystosis	2	1	1	0
Total(%)	65(100)	18(27.7)	37(56.9)	10(15)

Table-2: Clinical outcomes of the patients during follow up with relation to fungal infection (n= 65)

Duration	No. of cases with increase in counts (%)	No. of cases with stable counts (%)	No. of cases with fall in counts (%)	No. of cases lost to follow up (%)
Follow up-1 (6 months)	19(31.6)	32(53.3)	8(13.3)	1(1.6)
Follow up-2 (12 months)	21(35)	33 (55)	5(8.3)	1(1.6)

Table-3: Clinical outcomes of the patients during follow up with relation to CD4 counts (n=60)

higher rate of detection of 26.7% as compared to 16.7% in immunocompetent patients which was statistically insignificant.

Of 65 isolates, 33 (50.7%) were yeasts and 32 (49.2%) were molds. Among the yeast isolates, 21 (63.6 %) and 12 (36.3%) were collected from immunocompromised and immunocompetent patients respectively. 14 (42.4%) isolates were of *C. albicans*; The remaining 19 (57.5%) isolates of Candida were Non Albicans Candida. *Cryptococcus neoformans* and *Pneumocystis jirovecii* represented 2 (6.1%) isolates each, all of which were found in HIV positive cases. Amongst the mold isolates, 30 (93.7%) were found to be Aspergillus species. 1 (3.1%) isolate of *Mucor* from a diabetic patient and 1 (3.1%) isolate of *Penicillium marneffei* from an HIV positive patient were detected. (Table-1, 2 and 3)

DISCUSSION

Pulmonary cryptococcosis was seen in 2 (1.3%) patients, both of which were HIV positive, one of which was also a case of MDR TB. Similar observations of 1.6% and 1.2% were reported by Wadhwa A. et al., and Khan PA, et al., respectively.^{13,14} Also, in a study conducted by Banerjee et al., at AIIMS, 23% of HIV positive patients presented with non-meningitis manifestations especially with pyrexia of unknown origin.¹⁵ Lungran P et al., showed a high prevalence of cryptococcosis in HIV positive patients (87.5%), which is reasoned by high prevalence of HIV infection in Manipur.^{16,17} Worldwide, *C. neoformans* is a leading opportunistic pathogen

in patients with HIV and represents the third most common cause of IFIs in solid-organ transplant recipients.¹⁸⁻²⁰ (Reports have suggested that HIV-negative patients (30-70%) suffer from pulmonary cryptococcosis more commonly as compared to AIDS patients (2%) who suffer from disseminated cryptococcal disease.^{21,18,22} In our study, however, not a single positive cases of pulmonary cryptococcosis were observed amongst the immunocompetent.

PJP is one of the most frequent opportunistic infections among patients with HIV, with a mortality of about 10% to 20%.²³ *Pneumocystis jirovecii* was found positive in 2.5% (2 out of 80) of immunocompromised patients, both of which were from children. Mucus is notorious as an aggravating factor of respiratory illnesses, the documentation of Pneumocystis-related mucus pathology in infant lungs warrants continued research to elucidate whether Pneumocystis plays a role in the increased respiratory morbidity of infants characteristic of this age group.²⁴ North Indian studies with similar prevalence rates of 1.8% and 1.3% have been evidenced by Vajpayee M et al., and Khan PA, et al., respectively.^{25,14} Causes of low prevalence may be due to lack of diagnosis or prevalence of more virulent conditions, like tuberculosis, leading to pulmonary disease before PCP could manifest.²⁶ However, Wadhwa A et al., (2007) found PJP in 8.3% of patients.¹³

PCP has been rarely reported in immunocompetent subjects.²⁷ In them, the infection presents with fulminant respiratory failure, along with fever and dry cough; this is in contrast to an indolent course in the immunocompromised

population.²⁸ However, no cases were reported amongst the immunocompetent patients in our study. 1(3.1%) isolate of *Mucor* was found in a diabetic individual. In a series from India, approximately 12% of cases occurred in immunocompetent individuals.²⁹ Single-center data from India also show an increase in mucormycosis over time.³⁰

Penicillium marneffi also contributed to 1(3.1%) isolate from an HIV positive patient. The disease has been reported among HIV-infected persons in Thailand, Myanmar (Burma), Vietnam, Cambodia, Malaysia, northeastern India, Hong Kong, Taiwan, and southern China.³¹ Also, cases have been documented in immunocompetent hosts in China and death is reported in immunocompetent children.^{32,33} Our study revealed no such cases.

Cryptococcus was isolated from both patients with CD4 count < 200. Wadhwa et al. showed the CD4 counts of five positive patients were <200 cells/microl., and three among these five had a CD4 count <100 cells/microl.¹³ Other studies from Pune and Delhi show comparable results of 114.0 and 135 respectively by Ghate M et al., and Sharma SK et al.,^{34,35} PJP was seen in HIV positive cases with a mean of CD4 as 114(±19.9). Studies by Vajpayee M et al., also reported CD4 counts of 150.²⁵ Penicilliosis presents primarily as a disseminated disease in HIV-infected patients with CD4+ T-cell count <100 cells/μL (Le T et al., 2011).³⁶ 1 HIV positive patient with CD4 count of 68 was seen in our study. Pulmonary mucormycosis has been increasingly documented though rare, in diabetic patients. Our study revealed 1 such patient with a CD4 count of 520.

In the current prospective study, response to treatment and mortality during follow up were assessed with relation to fungal infection and CD4 counts. Factors which influenced the clinical outcome of patients were also analysed. Out of the 65 cases with diagnosed fungal infection, an overall mortality rate of 27.7% was seen. However, because people who develop IFI are typically already sick with other medical conditions, it can be difficult to determine the proportion of deaths directly attributable to the fungal infection. Certain clinical factors such as low serum albumin, need for mechanical ventilation, and development of a pneumothorax were predictive of mortality.³⁷ Tuberculosis and fungal co-infection poses a major therapeutic dilemma in resource-poor settings as rifampicin is a potent P450 inducer and markedly reduces itraconazole concentrations.³⁸

The (crude) mortality among cases of cryptococcosis (2) and pneumocystosis (2) showed a mortality rate of 50%. Selik RM et al., found that the epidemic of human immunodeficiency virus (HIV) had unexpectedly increased mortality due to the opportunistic mycoses pneumocystosis, cryptococcosis, and histoplasmosis.³⁹

100% mortality rate was observed in pulmonary mucormycosis. The single case reported in our study died. There are increasing reports of breakthrough mucormycosis in the setting of antifungal prophylaxis or treatment (eg, voriconazole, echinocandins) that is effective against most fungi (eg, *Aspergillus*) but not mucormycosis. Surviving mucormycosis requires rapid diagnosis and

aggressive coordinated medical and surgical therapy. *Penicillium marneffi* can cause a fatal systemic mycosis in immunosuppressed patients and is one of the common causes of mortality in people living with Human Immunodeficiency Virus (HIV) in South-East Asia as reported by Vanittanakom N et al., and; Cuong DD et al.^{40,41} The single patient of penicilliosis in our study, however, survived.

10 patients were lost to follow up. Some of these patients were either referred to higher centres and some did not return for further therapy.

On the whole, 56.9% cases survived. Thus, the clinical outcomes of these patients remained poor even if diagnosis is confirmed and routine antifungal treatment therapy has been started. Reports by Pinner RW et al., Armstrong GL et al., Kao AS et al., have suggested that mortality due to invasive mycoses has increased presently.⁴²⁻⁴⁴

The CD4 count is the most important laboratory indicator of immune function in HIV-infected patients. It is also the strongest predictor of disease progression and survival according to several studies from clinical trials and cohort studies by Mellors JW et al., and Egger M et al.^{45,46} The CD4 count is used to evaluate the patient's immunologic response to ART. It is also used to check whether prophylaxis for OIs can be discontinued.⁴⁷ On determining the respective CD4 counts of all the HIV positive patients, the first follow up at 6 months showed that 32(53.3%) of patients continued to have stable counts, the counts improved in 19(31.6%), and 8(13.3%) patients demonstrated a fall in blood counts. After 12 months of continued treatment, 33(55%) of the cases had stable counts and 21(35%) showed an increase in counts. Fall in counts was observed in 5(8.3%) patients. 1(1.6%) patient each was lost to follow up at 6 months and 12 months. For most patients on therapy, an adequate response is defined as an increase in CD4 count in the range of 50 to 150 cells/mm³ during the first year of ART, generally with an accelerated response in the first 3 months of treatment. Subsequent increases average approximately 50 to 100 cells/mm³ per year until a steady state level is reached.⁴⁸ This compares well with our study where majority of the patients showed stable counts after an initial rise. Also, a subgroup of patients experience a clinical deterioration as a consequence of rapid and dysregulated restoration of antigen specific immune responses during the treatment i.e., Immune reconstitution inflammatory syndrome (IRIS) reported by French MA et al.⁴⁹ IRIS is associated with invasive mycoses and could be a factor responsible for rise in counts. Babu KS et al., reported that out of 139 subjects, 67(48.2%) showed >300 cells/cmm, just 2 of which had counts > 300 cells/cmm before initiating ART.⁵⁰ Recovery of neutropenia may be a factor associated with response to treatment.

Patients who initiate ARV having low CD4 cell count present higher morbidity and mortality and have poorer treatment response.⁵¹ The unrestricted use of ART caused an increase in prevalence of drug-resistant HIV strains which could explain the fall in counts in few patients. In many countries in Asia, second-line combination antiretroviral treatment (cART) is not widely accessible.⁵²⁻⁵⁴ CD4 count declines can occur in

a small percentage of virologically suppressed patients and may be associated with adverse clinical outcomes such as cardiovascular disease, malignancy, and death.⁵⁵

CONCLUSION

Invasive fungal infections other than the *Candida* species and *Aspergillus* species like *Cryptococcus neoformans*, *Pneumocystis jirovecii* *Mucor* and, *Penicillium marneffei* etc. are becoming common in the nosocomial settings particularly among the immunocompromised individual but lack of diagnosing modalities and expertise in resource poor countries are a hinderance in their accurate and timely diagnosis and treatment.

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