Original Article

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Microbiology Section

Opportunistic Fungal Infections in Immunocompromised Patients of a Tertiary Care Centre, Amritsar, India

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ABSTRACT

Introduction: The incidence of opportunistic fungal infections has increased considerably in recent years. The clinical manifestations of these infections are non specific, and laboratory methods generally cannot diagnose infection at an initial phase.So,it is necessary to understand these infections for the treatment of immunocompromised patients adequately.

Aim: To study the prevalence of common opportunistic fungal infections in immunocompromised patients in northern India.

Materials and Methods: A prospective cross-sectional observational prevalence study was conducted in the Department of Microbiology, Sri Guru Ram Das Institute of Medical Sciences and Research, Amritsar, Punjab, India, from March 2020 to August 2021, on 185 immunocompromised patients. Follow-up was done for a period of six month. Weekly blood samples were drawn and presence of any fungal colonisation was identified with methods such as growth in sabouraud dextrose agar and Indian ink staining. Statistical tests used were prevalence and positive prevalence rate to estimate the most common opportunistic infection in immunocompromised individuals.

Results: Out of 185 patients, oropharyngeal candidasis was found to be most common (143, 77.3%) in which opportunistic fungal infections caused by *Candida* spp. were found in 67 (46.8%) patients representing the most common causing agents followed by *Pneumocystis jiroveci* (12.5%) and *Cryptococcus meningitis* (38.2%).

Conclusion: Oropharyngeal candidiasis was found to be most common opportunistic fungal infection. This study would help to extend the awareness to clinicians to come up with the right diagnosis and earlier treatment of those infections with the right management of the patients especially in resource limited regions in India.

Keywords: *Candida* spp., Cryptococcus, Immunocompetent, Mycosis

INTRODUCTION

The incidence of fungal infections is increasing in immunosuppressed patients, such as patients who have received hematopoietic stem cell and solid organ transplantation, patients who have used steroids for a long time, and Human Immunodeficiency Virus (HIV) patients. In fact, certain trends have emerged in these fungal infections, such as an increase in the incidence of Invasive Fungal Infections (IFI), an increase in non *Candida albicans* strains that cause invasive disseminated diseases, and the emergence of less susceptible fungal strains, resistant to broad-spectrum antifungal agents, due to the excessive and unreasonable use of these drugs. Therefore, it is very important for clinicians to determine the patient population who may be at risk of these IFI and the risk factors for these infections [1].

The risk factors for IFI have been extensively evaluated and are known to significantly affect the incidence of IFI, affecting the performance of the diagnostic test. Herbrecht R et al., describe host factors in patients as high risk patients, moderate and low risk for opportunistic fungal infections [2].

Laboratory diagnosis of invasive fungal infection involves one or more of three methods, including isolation of fungi by culture, serological detection of antibodies, antigens or fungal metabolites, and histopathological evidence of invasion [3]. Unfortunately, diagnosing infections with invasive fungi is still difficult, and sometimes they can only be confirmed by biopsy. So by this far time, most patients are too sick to undergo a biopsy. Pathogenic fungi may take atleast two to three weeks or more to grow in the medium. The detection of galactomannan antigen in serum by enzyme immunoassay has become an important auxiliary method for the diagnosis of invasive aspergillosis and other dangerous infections [4].

Clinicians should be familiar with the possible clinical manifestations of opportunistic fungal infections in patients with suppressed immune function. Fever is the most common first sign of infection, followed by signs and symptoms that may indicate the involvement of specific organ systems [5].

Opportunistic fungal infections affecting the skin or musculoskeletal system are generally associated with disseminated disease. Head and neck infections can be self-limiting, such as oral yeast infections or thrush, or they can be fatal quickly, such as more serious eye or sinus infections, which can spread to the brain. Gastrointestinal infections usually affect the oesophagus and rarely the small intestine and colon. Cardiovascular disease is usually the result of a disseminated infection. Urogenital diseases can be the result of a disseminated disease, usually in the form of renal parenchymal disease or in the form of cystitis and pyelonephritis due to indwelling catheters. Direct transmission or spread of infected facial sinuses can lead to central nervous system infections. Currently, a sharp increase in fungal infections has been observed in patients with Acquired Immunodeficiency Syndrome (AIDS), mainly candidiasis and cryptococcosis. This situation presents a unique challenge for clinicians when considering the increasing number of patients who may acquire opportunistic fungal infections [5].

Various studies have been carried out in India to analyse the spectrum of opportunistic infections [5,6]. Among the fungal infections, oral candidiasis has been reported as the most common opportunistic infection in many studies [6,7]. Although *Candida albicans* have been found to be the most commonly isolated organism, some studies have shown that species such as *C. krusei*, *C. tropicalis*, and *C. galbrata* are more prevalent than *C. albicans* [7,8]. Opportunistic fungal infections are mainly caused by low virulent fungi. These are usually non pathogenic in immunocompetent individuals but may

cause severe infections in patients with suppressed immunity. Some organisms such as *Cryptococcus neoformans* or dermatophytes with known pathogenic status may cause atypical presentations like meningitis in immunocompromised individuals compared to immunocompetent individuals [9,10].

In recent years, the incidence of IFIs has increased significantly due to the increase in patients with tourists, transplant patients, cancer patients and others receiving immunosuppressive treatment. IFI is more frequent in patients undergoing transplantation or for the treatment of malignant tumours, in which the most intensive regimens lead to profound immunosuppression for long periods of time. The prevalence of IFI in the kidneys and in the liver transplant varies from 5% to 50% [11]. Among the patients with neutropenia, these infections are the main cause of morbidity and mortality [12] among the beneficiaries of the transplant of solid organs candida and aspergillus cause more than 80% of fungal infections. However, since the clinical manifestations of these infections are non specific, and laboratory methods generally cannot diagnose infection at an initial phase, it is necessary to understand these infections for the treatment of immunocompromised patients adequately. The aim of this study was to analyses the prevalence and scope of common opportunistic fungal infections in immunocompromised patients in northern India.

MATERIALS AND METHODS

This study was a prospective, cross-sectional and observational study, carried out on 185 immunocompromised patients from March 2020 to August 2021 in the Department of Microbiology, Sri Guru Ram Das Institute of Medical Sciences and Research, Amritsar. After obtaining approval from the Institutional Ethics Committee/Review Board (ref. no. Path/1045/2020 dated 09/02/2020) and informed consent from the patients/guardians.

Inclusion criteria: All clinically suspected cases of fungal infections with HIV seropositive status and those with various malignancies like breast cancer (53 patients), leukaemias/lymphomas (17 patients) and lung cancer (8 patients) on chemotherapy were included in the study.

Exclusion criteria: Those clinically suspected cases of fungal infection subjects with fungal infections but no known cause of immunosuppression were excluded from the study.

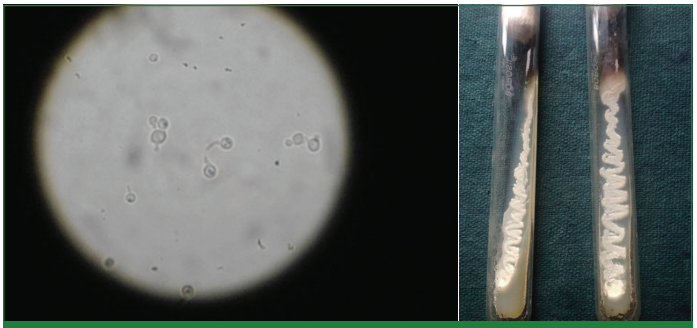
Procedure

Total of 185 immunocompromised patients were included in the study and were followed for a period of six months. After admission, all patients were assessed for fungal colonisation. This was done by providing oral swabs as well as vaginal and rectal tissue swabs. Urine samples were collected in the culture medium and centrifuged at 2500 Revolutions per minute (rpm) for 10 minutes, and then cultured on Sabouraud-Dextrose Agar. In the absence of local or systemic symptoms or signs of infection, the presence of fungus in one or more monitored cultures was defined as colonisation [13].

There were no clinical symptoms of the disease. At this stage, the number of fungal organisms was very small, there was no fungal transmission in the body, and no other organ systems were affected. The infection time was measured in terms of days following the immunodeficiency condition, that is, when the patients underwent chemotherapy or was diagnosed with HIV. During the follow-up period, whenever the patient developed fever after 96 hours of antibiotic treatment, or showed radiographic or mycological evidence of fungal infection, physicians suspected a fungal infection. Clinical specimens consisting of urine, Cerebrospinal Fluid (CSF), bronchoalveolar lavage fluid, sputum, pleura, and abdominal puncture fluid from these patients were analysed for fungal infection [Table/Fig-1]. All samples were grown on Sabouraud-dextrose agar and subjected to direct microscopic examination. Blood samples were inoculated in BACTEC medium through the header. Simple random sampling was done in patients who presented with symptoms of fungal infection.

Oral swabs were taken from 143 patients suspected of oropharyngeal candidiasis, samples of induced sputum in eight patients suspected of *pneumocystis pneumoniae* and CSF samples from 34 patients suspected of cryptococcal meningitis.

Two swabs were taken from the oral cavity of each suspected case patient with oropharyngeal candidiasis [Table/Fig-2]. Gram staining was then done on one smear prepared from one swab and then examined microscopically for determination of gram positive budding yeast-like cells with or without pseudohyphae. The other swab was inoculated into three Sabouraud Dextrose Agar (SDA) slopes. To differentiate *C. albicans* and *C. dubliniensis*, the slopes was incubated at 37°C, 42°C, and 45°C, respectively. Strains of *C. dubliniensis* may grow at 42°C, but neither of the species can grow at 45°C. Then, the genus *Candida* was preliminarily identified based on colony morphology. By inoculating them in serum and incubating them at 37°C for 23 hours, a bacterial tube test was performed on the cultured Candida samples to confirm Candida *albicans*. Also, the slide culture technique and sugar assimilation tests were performed. Slide culture technique was done on corn meal agar to demonstrate chlamydospore formation differentiating various species of Candida. Sugar assimilation tests were done by using various disks of sugars including glucose, maltose, sucrose, lactose, and galactose [14].



[Table/Fig-1]: *Candida albicans* in Germ Tube test.
[Table/Fig-2]: Growth of *Candida* on Sabouraud's Dextrose Agar (SDA) medium. (Images from left to right).

CSF culture: From each suspected patient with cryptococcal meningitis. CSF samples were taken. These CSF samples were then inoculated on two SDA slopes and incubated under aerobic conditions at 25°C and 37°C for 23 days. Direct wet smears were prepared and gram-stained sand were then examined under a microscope. The colonies were examined for their morphology after incubation. Colonies of *C. neoformans* were moist in texture, white cream coloured and mucoid and could grow equally well at 25°C and 37°C. Gram stained smear was prepared from these colonies and examined for oval or round yeast cells.

An India ink preparation was then made to demonstrate the capsule. Christensen's urea agar hydrolysis test was also performed. *C. neoformans* were differentiated from *C. albidus*, and *C. laurentii* by sugar assimilation tests using sugar disks of galactose, maltose, sucrose, lactose, cellobiose and xylose [15]. One induced sputum sample was collected from each suspected case of pneumonia to demonstrate the presence of *Pneumocystis jirovecii* cysts and trophozoites. Smears were prepared and stained by Giemsa and toluidine blue O. These were examined for the presence of *P. jirovecii* trophozoites and cysts, respectively. Cysts were found to be 4-7 µm in diameter, non budding and had round, ovoid, or collapsed crescent forms. The trophozoites were seen as pleomorphic tiny bodies, 2-5 µm in size and were found in clusters with basophilic cytoplasm and reddish-purple nuclei in eosinophilic mass. Ziehl-Neelsen stain was also performed, to rule out infection with *Mycobacterium tuberculosis*.

STATISTICAL ANALYSIS

It was a prevalence study so total positive cases and prevalence rates were calculated as number and percentages.

RESULTS

The present study was carried out in 185 immunocompromised patients. The various malignancies included were breast cancer (53 patients), leukaemias/ lymphomas (17 patients) and lung cancer (8 patients) on patients receiving chemotherapy. Opportunistic fungal infections caused by *Candida* spp. were found in 67 (46.8%) patients, representing the most common causing agents. Cryptococcal meningitis (C.neoformans) was positive in 13 (38.2) and pneumocystis pneumonia (P.jirovecii) was positive in 1 (12.5) patients. The distribution of various opportunistic fungal infections is presented in [Table/Fig-3]. Demographic profile of various patients with opportunistic infections is presented [Table/Fig-4].

Opportunistic infections (Sample)#	Number of cases^	Total positive~	Positivity (%)	Prevalence (%)*
Oropharyngeal candidiasis (Oral swab)	143	67	46.8	36.21
Cryptococcal meningitis	34	13	38.2	7.02
Pneumocystis pneumonia (Induced sputum)	8	1	12.5	0.54

[Table/Fig-3]: Various opportunistic fungal infections (N=185).
*: Calculated out of total number of cases; ^: Represent number of clinical suspected cases;
#: Sample taken for diagnosis of infection; ~: Represent number of microbiologically confirmed cases

Infections	Age group (Years)					
	0-15	16-30	31-45	46-60	61-75	Total
Oropharyngeal candidiasis						
Male	1 (2.3%)	13 (29.5%)	24 (54.5%)	4 (9%)	2 (4.55%)	44
Female	0	9 (39.1%)	12 (52.1%)	1 (4.3%)	1 (4.3%)	23
Cryptococcal meningitis						
Male	0	2 (28.5%)	5 (71.4%)	0	0	7
Female	0	2 (33.3%)	4 (66.6%)	0	0	6
Pneumocystis pneumonia						
Male	0	0	1 (100%)	0	0	1
Female	0	0	0	0	0	0

[Table/Fig-4]: Demographic profile of various opportunistic infections.

Oropahryngeal candiasis was found to be most common in males of the age of 31-45 years (24, 54.5%) while females of the same age group showed a lesser incidence. Cryptococcal meningitis and pneumocystis pneumonia also showed a similar pattern.

In the present study, various opportunistic fungal infections showed to be associated with the absolute Cluster of Differentiation (CD) 4+ counts; the CD4+ counts for the patients suffering from oropharyngeal candidiasis, pneumocystis pneumonia, cryptococcal meningitis was ≤200 cells/μL.

DISCUSSION

Opportunistic fungal infections remain an important cause of morbidity and mortality in immunocompromised patients. The most important parameter that determines the success of treatment of these infections in immunosuppressed patients is the speed at which a diagnosis is made and treatment is initiated [16]. Although HIV is the causative agent of AIDS, the main cause of morbidity and mortality in these patients is opportunistic infections due to weakened humoural and cell-mediated immunity [17]. Opportunistic infections range from viral, fungal, bacterial and parasitic infections [18]. In the present study, the most prevalent Candida species obtained from oral thrush lesions was *C. albicans*. An increasing trend of non albicans Candida infections has also been reported by others [19], and this trend has been favoured by the extensive use of fluconazole [20]. In 45 of 65 isolates of *Candida* species, pseudohyphae were found, which have long been known as a sign of fungal infection [21]. pneumonia due to *P. jirovecii* is one of the common opportunistic fungal infection. The main reason for the low incidence of pneumocystis in India is inaccessibility of high quality diagnostic laboratories and low yield specimens, as bronchoalveolar lavage is not performed in most hospitals [22]. Cryptococcosis has been found to be the most common systemic fungal infection among AIDS patients (7.02%). Its incidence is on the rise with the rapid spread of the AIDS [23]. Similar occurrences have been reported by Sharma SK et al., (4%), Mulla SA et al., (3.7%) and Mbanya D et al., (2.9%) [24-26]. CNS cryptococcosis is one among the foremost important risk factors related to HIV infection contributing to a really high degree of morbidity and mortality among immunocompromised patients [9]. Hence, surveillance of mycosis in immunocompromised especially HIV infected individuals and adopting an appropriate treatment at early stages is necessary [26]. In this study, various opportunistic fungal infections showed to be correlated with the absolute CD4+ counts; the CD4+ counts for the patients suffered from oropharyngeal candidiasis, pneumocystis pneumonia, cryptococcal meningitis was ≤200 cells/μL. This finding is also supported by other published literature [17]. In this study, oropharyngeal candidiasis was the most common opportunistic fungal infection.

Prevention of opportunistic infections by specific measures such as good personal hygiene, early and regular medical examination of suspected individuals with opportunistic infections, prompt diagnosis, and appropriate antifungal prophylaxis and/or treatment are necessary to decrease the morbidity and mortality associated with these infections. Hence, knowledge of spectrum of opportunistic fungal infections and its correlation with CD4+ counts may help clinicians in early diagnosis and prompt treatment of opportunistic fungal infections in immunocompromised patients more efficiently, which in turn may increase their longevity.

Limitation(s)

Immunocompromised patients although present a large population, the study included mainly the patients who reported to get their HIV status checked or were already on treatment for HIV.

CONCLUSION(S)

Oropharyngeal candidiasis was found to be most common opportunistic fungal infection in this setting. This study would help to extend the awareness to clinicians to come up with the right diagnosis and earlier treatment of those infections with the right management of the patients especially in resource limited regions in India.

REFERENCES

[1] Martin GS, Mannino DM, Eaton S, Moss M. The epidemiology of sepsis in the United States from 1979 through 2000. N Engl J Med. 2003;348(16):1546-54.
[2] Herbrecht R, Bories P, Moulin JC, Ledoux MP, Letscher-Bru V. Risk stratification for invasive aspergillosis in immunocompromised patients. Ann NY Acad Sci. 2012;1272:23-30. Doi: 10.1111/j.1749-6632.2012.06829.x.
[3] Hamilton AJ. Serodiagnosis of histoplasmosis, paracoccidioidomycosis, and penicilliosis marneffei: current status and future trends. Med Mycol. 1998;36(6):351-64.
[4] Stynen D, Sarfati J, Goris A, Prevost MC, Lesourd M, Kamphuis H, et al. Rat monoclonal antibodies against Aspergillus galactomannan. Infect Immun. 1992;60(6):2237-45.
[5] American Society for Microbiology; Clinical Microbiology Reviews, 1988, 1(4),349-364; 0893-[5]8512/88/04349-16 Copyright © 1988.
[6] Giri TK, Pande I, Mishra NM, Kailash S, Uppal SS, Kumar A. Spectrum of clinical and laboratory characteristics of HIV infection in northern India. J Commun Dis. 1995;27(3):131-41.
[7] Cartledge JD, Midgley J, Gazzard BG. Non albicans oral candidosis in HIV-positive patients. J Antimicrob Chemother. 1999;43(3):419-22.
[8] Abbas J, Bodey GP, Hanna HA, Mardani M, Girgawy E, Abi-Said D, et al. *Candida* krusei fungemia: An escalating serious infection in immunocompromised patients. Arch Intern Med. 2000;160(17):2659-64.
[9] Banerjee U, Dutta K, Majumdar T, Gupta K. Cryptococcosis in India: The awakening of a giant. Med Mycol. 2001;39(1):51-67.

[10] Marques SA, Robles AM, Tortorano AM, Tuculet MA, Negroni R, Mendes RP. Mycoses associated with AIDS in the Third World. Med Mycol. 2000;38(Suppl 1):269-79.
[11] Dictar MO, Maiolo E, Alexander B, Jacob N, Veron MT. Mycoses in the transplanted patient. Med Mycol. 2000;38:251-58.
[12] Patterson TF. New agents for treatment of invasive aspergillosis. Clin Infect Dis. 2002;35(4):367-69.
[13] Gladdy RA, Richardson SE, Davies HD, Superina RA. *Candida* infection in pediatric liver transplant recipients. Liver Transpl Surg. 1999;5(1):16-24.
[14] Larone DH. Medically important fungi: A guide to identification. 5th ed. Washington, DC: ASM Press. 2011;122-24.
[15] Ahmaad S, Khan Z, Mustafa AS, Khan ZU. Semi-nested PCR for diagnosis of candidemia: Comparison with culture, antigen detection and biochemical methods for species identification. J Clin Microbiol. 2002;40(7):2483-89.
[16] Kumarasamy N, Solomon S, Jayaker Paul SA, Venilla R, Amalraj RE. Spectrum of opportunistic infections among AIDS patients in Tamil Nadu, India. Int J STD AIDS. 1995;6(6):447-49.
[17] Surana A, Vajpayee M, Wig N, Seth P. Spectrum of opportunistic infections among HIV infected North Indian patients. Poster Exhibition: The XIV International AIDS Conference: Abstract no. ThPeB7188.
[18] Chakravarty J, Mehta H, Parekh A, Attilli SV, Agrawal NR, Singh SP, et al. Study on clinico-epidemiological profile of HIV patients in Eastern India. J Assoc Physicians India. 2006;54:854-57. PMID: 17249252.
[19] Morace G, Pagano L, Sanguinetti M, Posteraro B, Mele L, Equitani F, et al. PCR-restriction enzyme analysis for detection of *Candida* DNA in blood from febrile patients with hematological malignancies. J Clin Microbiol. 1999;37 (6):1871-75.
[20] Misra SN, Sengupta D, Satpathy SK. AIDS in India: Recent trends in opportunistic infections. Southeast Asian J Trop Med Public Health. 1998;29(2):373-76.
[21] Odds FC, Kerridge D. Morphogenesis in *Candida albicans*. Crit Rev Microbiol. 1985;12(1):45-93.
[22] Iyer RS, Banker DD. Cryptococcal meningitis in AIDS. Indian J Med Sci. 2002;56(12):593-97.
[23] Chakraborty N, Mukherjee A, Santra S, Sarkar RN, Banerjee D, Guha SK, et al. Current trends of opportunistic infections among HIV-seropositive patients from Eastern India. Jpn J Infect Dis. 2008;61(1):49-53.
[24] Sharma SK, Kadhiravan T, Banga A, Goyal T, Bhatia I, Saha PK. Spectrum of clinical disease in a series of 135 hospitalised HIV infected patients from north India. BMC Infect Dis. 2004;4:52. Available on https://doi.org/10.1186/1471-2334-4-52.
[25] Mulla SA, Patel MG, Waghela G, Motala N, Desai V, Shrivastava RK. A study of opportunistic infections in HIV-seropositive patients. Indian J Community Med. 2007;32(3):208-09.
[26] Mbanya D, Assah F, Ndembi N, Kaptue L. Monitoring antiretroviral therapy in HIV/AIDS patients in resource-limited settings: CD4 counts or total lymphocyte counts? Int J Infect Dis. 2007;11(2):157-60.

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