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Original Article

Spectrum of Opportunistic Fungal Infections in HIV-Infected Patients and Their Correlation with CD4+ Counts in Western India

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Introduction: The decreased level of immunity in Human Immunodeficiency Virus (HIV) infected patients increases their vulnerability to various opportunistic fungal infections. Oral candidiasis has been found to be the most common fungal infection among HIV infected patients. The present study was conducted to evaluate the spectrum of various opportunistic fungal infections and their correlation with CD4+ counts. **Methods:** A total of 163 clinically suspected cases of fungal infections with HIV seropositive status were studied. **Results:** The most common infections observed were oropharyngeal candidiasis (39.26%) followed by cryptococcal meningitis (6.74%). The study showed opportunistic fungal infections in 46.6% of HIV infected patients with CD4+ counts ≤200 cell/µl. **Conclusion:** Early diagnosis and prompt antifungal treatment is necessary to decrease the morbidity and mortality associated with the infections to increase the survival of HIV infected patients. *J Med Microbiol Infec Dis, 2014, 2 (1): 19-22.*

Keywords: Opportunistic Infections, HIV infection, Candida albicans, Cryptococcus neoformans.

INTRODUCTION

The emergence and pandemic spread of Acquired Immunodeficiency Syndrome (AIDS) is a great challenge to public health at present time. After the first reported case in early 1980's, Human Immunodeficiency Virus (HIV) infection has emerged as a leading cause of death infecting approximately 33 million people worldwide. In India, about 2.1 million people are living with HIV infection [1]. The major causes of morbidity and mortality in HIV infected patients are the opportunistic infections. This could be attributed to the decreased level of immunity in such patients due to destruction of CD4+ cells. Thus, these patients become vulnerable to various opportunistic infections, particularly those caused by fungi [2].

Various studies have been carried out in India to analyse the spectrum of opportunistic infections. Among the fungal infections, oral candidiasis has been reported as the most common opportunistic infection in many studies [3-8]. Although *Candida albicans* have been found to be the most commonly isolated organism, some studies have shown that non-albicans *Candida* species including *C. tropicalis*, *C. krusei*, and *C. galbrata* are more prevalent than *C. albicans* [9-12].

The single best predictor of AIDS onset is the percentage or absolute number of CD4+ T cells [13]. There is a direct correlation between the number of CD4+ cells and the incidence of opportunistic infections. Opportunistic fungal infections are mainly caused by non or 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 [14, 15].

HIV infection leads to destruction of CD4+ cells. In the absence of treatment, viral load increases leading to further decrease in these cells, making such patients vulnerable to opportunistic infections and eventually leading to death of these patients [16]. With better awareness about the immune status of the HIV infected patients and background knowledge of the prevalent opportunistic fungal infections in a particular region, an early diagnosis and prompt treatment may decrease the mortality rate. Therefore the present study was aimed to determine the spectrum of various opportunistic fungal infections in the Western India and their correlation with CD4+ counts.

MATERIALS AND METHODS

The present study was conducted in the Department of Microbiology, Government Medical College, Aurangabad, Maharashtra, India during January 2007 to July 2008. Ethical clearance was taken from ethical committee of the college for sample collection. One hundred sixty three clinically suspected cases of fungal infections with HIV seropositive status were included in the study. HIV status of the patients was confirmed by guidelines provided by

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National AIDS Control Organization (NACO, Strategy III) [17]. These patients were investigated to find out the spectrum of opportunistic fungal infections. From each selected patient, samples were obtained based on signs and symptoms. The samples taken were oral swabs (n=132x2), cerebrospinal fluid (n=23), and induced sputum (n=8). Three ml of blood was also collected from each patient in an ethylenediamine-tetraacetic acid (EDTA) vacutainer tube for evaluation of CD4+ counts by Flow Cytometery (n=163).

Two swabs were taken from the oral cavity of each suspected case patient with oropharyngeal candidiasis. Gram stained smear was then prepared from one swab and 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, 42, and 45°C, respectively (strains of *C. dubliniensis* may grow at 42°C, but none of the species grow at 45°C). Tentative identification of *Candida* species was done based on colony morphology. For confirmation of *C. albicans*, germ tube test was performed on grown *Candida* samples by inoculating them in serum and incubating at 37°C for 2-3 h. Also, the slide culture technique and sugar assimilation tests were performed. Slide culture technique was done on corn meal agar to demonstrate chlamydospore formation different-iating various species of *Candida*. Sugar assimilation tests were done by using various disks of sugars including glucose, maltose, sucrose, lactose, cellobiose, galactose, trehalose, raffinose, melibiose, xylose, inositol, and dulcitol [18]. Cerebrospinal fluid (CSF) samples were taken from each suspected patients with cryptococcal meningitis.

The CSF samples were inoculated into two SDA slopes and incubated aerobically at 25°C and 37°C for 2-3 days. A direct wet mount and a gram stained smear were prepared and examined microscopically.

After incubation, the colonies were examined for their morphology. Colonies of *C. neoformans* were moist, white-cream coloured and mucoid and grown equally well at 25°C and 37°C. Gram stained smear was prepared from the colonies and examined for 4-6 µm oval or round yeast cells. An India ink preparation was made to demonstrate the capsule. Christensen's urea agar hydrolysis test was also done. *C. neoformans* were differentiated from *C. albidus*, and *C. laurentii* by sugar assimilation tests using sugar disks of galactose, maltose, sucrose, lactose, cellobiose, xylose, raffinose, melibiose, inositol, and dulcitol. Differentiation of *C. neoformans* from *C. gattii* was done using d-proline assimilation test.

One induced sputum sample was collected from each suspected cases of pneumonia to demonstrate the presence of *Pneumocystis jirovecii* cysts and trophozoites. Smears were prepared and stained by Giemsa and toluidine blue O and examined for the presence of *P. jirovecii* trophozoites and cysts, respectively. Cysts were 4-7 µm in diameter, non-budding and had round, ovoid, or collapsed crescent forms. The trophozoites were pleomorphic tiny bodies, 2-5 µm in size and exist in clusters with basophilic cytoplasm and reddish-purple nuclei in eosinophilic mass. A smear for Ziehl-Neelsen stain was also performed to rule out infection with *Mycobacterium tuberculosis*.

RESULTS

A total of 163 non-duplicate samples were obtained from the HIV positive patients. Opportunistic fungal infections caused by *Candida* spp. were found in 76 (46.6%) patients, representing the most common causing agents. The distribution of various opportunistic fungal infections is documented in Table 1. Demographic profile of various opportunistic infections is documented in Table 2.

Table 1. Distribution of various opportunistic fungal infections among HIV-positive Patients

Opportunistic infections (Sample)#	No. of cases^	Total positive~	Positivity (%)	Prevalence (%)*
Oropharyngeal candidiasis (Oral swab)	132	64	48.48%	39.26% (64/163)
Cryptococcal meningitis (CSF)	23	11	47.82%	6.74% (11/163)
Pneumocystis pneumonia (Induced sputum)	08	01	12.50%	0.61% (1/163)

*= 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

Table 2. Demographic profile of various opportunistic fungal infections

Infections	Age group (Years)					
	0-15	16-30	31-45	46-60	61-75	Total
Oropharyngeal candidiasis						
Male	01 (2.4%)	12 (29.2%)	23 (56%)	03 (7.3%)	02 (4.8%)	41
Female	00	09 (39.1%)	12 (52.1%)	01 (4.3%)	01 (4.3%)	23
Cyrptococcal meningitis						
Male	00	02 (28.5%)	05 (71.4%)	00	00	07
Female	00	01 (25%)	03 (75%)	00	00	04
Pneumocystis pneumonia						
Male	00	00	01 (100%)	00	00	01
Female	00	00	00	00	00	00

Among *Candida* species, *C. albicans* was identified as the most prevalent causative agent (Table 3). Cryptococcal meningitis, observed in 47.82% of suspected cases, was caused only by *C. neoformans*. The means of CD4+ counts associated with various opportunistic fungal infections found in this study is reflected in Table 4. The grading of

CD4+ count range was performed according to World Health organization (WHO) and Centers for Disease Control and Prevention (CDC), Atlanta guidelines [19].

Table 3. Distribution of various *Candida* spp.

Species of *Candida*	Total isolates	Percentage
C. albicans	60	93.75%
C. tropicalis	02	03.12%
C. krusei	01	01.50%
C. glabrata	01	01.50%

Table 4. CD4+ counts of various opportunistic fungal infections

Opportunistic infections	MeanCD4+ cells count (cells/µl)
Oropharyngeal candidasis	197
Cryptococcal meningitis	67
Pneumocystis pneumonia	78

DISCUSSION

Though HIV is the causative agent of AIDS, the major cause of morbidity and mortality in these patients is the opportunistic infections as both humoral and cell mediated immunity are decreased. Opportunistic infections range from viral, parasitic and bacterial to fungal. Among these, fungal agents are ubiquitously present becoming the most common cause of life threatening infections in immunocompromised individuals. The opportunistic fungal infections affect immunocompromised individuals when their CD4+ counts falls below 200 cells/mm^3, indicating the value of monitoring the CD4+ counts in these patients.

In our study out of 163 HIV-positive individuals 76 (46.6%) were infected with opportunistic infections. Similar incidences have been reported by Kumaraswamy *et al.* (66%) [20], Surana *et al.* (45.01%) [21], and Chakraborty *et al.* (66.4%) [7]. In our study, the most prevalent *Candida* species obtained from oral thrush lesions was *C. albicans* (93.75%). An increasing trend of non albicans *Candida* infections has also been reported by others [20-22], and this trend has been favoured by the extensive use of fluconazole [23]. Pseudohyphae has long been known as a sign of fungal infection and this was observed in 50 of 65 isolates of *Candida* species. In this study only hyphal forms showed to be invasive, whereas yeast cells were merely commensals [24].

Pneumonia due to *P. jirovecii* is one of the common opportunistic infection in AIDS patients in developed countries, however, its incidence was found to be very low (0.61%) in our study. Similar low incidences of Pneumocystis pneumonia in HIV positive patients from India have been reported by Agarwal *et al.* (1.25%) [25], Chakravarty *et al.* (3.2%) [7] and Surana *et al.* (0.6%) [21]. The main reason for the low incidence of Pneumocystis pneumonia in India is unavailability of high quality diagnostic laboratories and low yield specimens, as bronchoalveolar lavage is not performed in most hospitals [15].

Cryptococcosis is the most common systemic fungal infection among AIDS patients and its incidence is on the rise with the rapid spread of the disease [26]. Our study reports an occurrence of 6.74%; similar occurrences have been reported by Chakraborty *et al.* (4%) [27], Sharma *et al.* (3.7%) [22] and Mulla *et al.* (2.9%) [6].

CNS cryptococcosis is one of the most important risk factors associated with HIV infection contributing to a very high degree of morbidity and mortality among HIV infected patients [14]. Hence, surveillance of mycosis in HIV infected individuals and adopting an appropriate treatment at early stages is necessary [28].

In our study various opportunistic fungal infections showed to be correlated with the absolute CD4+ counts; the CD4+ counts for the patients suffered from orophar-yngeal candidiasis, pneumocystis pneumonia, cryptococcal meningitis was ≤200 cells/µl. This finding is also supported by other published literature [19, 21].

Opportunistic fungal infections, due to severe suppression of the immune system, are prevalent in large number of HIV infected patients. In our 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/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 HIV infected patients more efficiently, which in turn may increase their longevity.

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CONFLICT OF INTEREST

No potential conflicts of interest were disclosed.

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