Original Article

Opportunistic Fungal Infection in HIV Positive Patients Attending a Tertiary care Hospital in Eastern Nepal

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Abstract

Background: HIV infection continues to be a major health problem with more than millions of AIDS related death annually. The risk of opportunistic infections increases with the depletion of CD4+ count in HIV positive patients which are responsible for the high mortality and morbidity. The spectrum of opportunistic infection (OIs) varies from one region to another. This study was carried out to see the occurrence of opportunistic fungal infection among the HIV positive patients in Eastern Nepal.

Method: This was a hospital based descriptive study carried out in Microbiology laboratory, BPKIHS, Dharan, Nepal over a period of one year (15th May 2013 to 14th May 2014). Total 60 HIV positive patients with CD4+ count ≤200 cells/mm^3 and suspected of having fungal infections were included. Samples were collected after taking an informed written consent from the patient. Isolation and identification was done as per standard Microbiological procedure.

Result: Opportunistic fungal infection was identified in 51.66% patients. The most common fungi isolated were *Candida* species, *Cryptococcus neoformans, Aspergillus* species and Dermatophytes respectively being 33.3% (n= 20), 10% (n= 6), 3.3% (n= 2) and 8.3% (n= 5). *Candida* species comprised 60%, *Cryptococcus neoformans* 20%, *Aspergillus* species 5.7% and dermatophytes 14.3% of total fungal isolates.

Conclusion: The common fungus isolated were *Candida* species, *Cryptococcus neoformans, Aspergillus* species and Dermatophytes in HIV positive patients in this hospital of Eastern Nepal.

Key words: Fungus, HIV, Opportunistic Infection.

Introduction

Human immunodeficiency virus (HIV) is the most significant emerging infectious pathogen of the 20th century.[1] HIV positive patients are vulnerable to a wide range of clinical consequences from asymptomatic carriage to life threatening Opportunistic infections (OIs) and malignancies.[2] In decreasing order of frequency, frequent OIs and malignancies that occur in Asia are: *Mycobacterium tuberculosis, Cryptococcus neoformans, Candida* species, *Herpes simplex virus, Cryptosporidium parvum, Pneumocystis jiroveci, Toxoplasma gondii*, non-Hodgkin's lymphoma and Kaposi sarcoma.[3]

Major causes of mortality and morbidity in HIV infected people are OIs. Therefore, identification of the specific pathogen(s) is important for management of such cases. The spectrum of OIs in the HIV-infected patients varies from one region to another.[3,4] The most common mycoses seen in HIV/AIDS patients are Candidiasis, Cryptococcosis, Histoplasmosis, Aspergillosis and Dermatophytosis.[5] Other fungal infections that are also seen in HIV positive patients are coccidioidomycosis, blastomycosis, *Pneumocystis jiroveci*, Sporotrichosis, Penicillosis.[1]

This study aims to establish the occurrence of Opportunistic fungal infections among the HIV positive patients with lower CD4+ count (≤200 cells/mm^3) in Eastern Nepal. Along with establishing the magnitude of problem, this study will also help in the management, both in the treatment as well as in the prophylaxis against these infections.

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Methodology

This was a hospital based descriptive study carried out in the department of Microbiology, BPKIHS, Dharan, Nepal over a period of one year (15th May 2013 to 14th May 2014). Ethical clearance was taken from the institutional review board and informed written consents were also taken.

Study subject:

Total 60 HIV positive cases with CD4+ count ≤200 cells/mm^3 participated in the study. Samples like sputum (n= 32), oral swab (n= 28), bronchoalveolar lavage (BAL) (n= 1), cerebrospinal fluid (CSF) (n= 8), skin scrapping (n= 3), nail clipping (n= 3) and stool sample (n= 1) were taken for identification of fungal infection.

Microbiological procedure:[6]

1. Gram's staining was done for the sputum, BAL, oral swab, CSF. Potassium Hydroxide mount was done for the nail and skin scrapping.
2. India ink preparation was done for the cerebrospinal fluid sample to demonstrate the capsule production by Cryptococcus.
3. Giemsa stain with prior sulphation was done for the sputum and BAL to demonstrate the cyst and trophozoite of *Pneumocystis jerovecii*.
4. Samples like sputum, oral swab, BAL and cerebrospinal fluid were cultured on Blood agar, MacConkey agar, Sabouraud's Dextrose Agar (SDA) and also on SDA containing chloramphenicol, cyclohexamide and gentamicin. Skin and nail samples were inoculated on SDA and SDA with antibiotics.

Blood agar and MacConkey agar was incubated at 37^0C overnight (24 hour) and then re-incubated for 48 hour before it was sterile. SDA and SDA with antibiotics were incubated at 5^0C and 37^0C and were examined to look for the growth.

In blood agar, white non-haemolytic colonies and in SDA tubes, cream coloured pasty colonies were suggestive of yeast species. MacConkey agar showed no growth. In SDA tubes showing cottony, woolly colonies were suggestive of molds.

The growth which resembled yeast cells were further identified by gram stain, germ tube test for *Candida*, Urea hydrolysis test for *Cryptococcus*, slide culture using cornmeal agar for detection of chlamydospore for *Candida*, growth on CHROMagar, thermo tolerance test for *Candida*. Growth which resembled molds was identified by Lacto-phenol cotton blue mount. SDA and SDA with antibiotics were incubated for two months before it was considered sterile.

Result

This study was conducted over a period of one year from 15 May 2013 to 14 May 2014 in the Department of Microbiology, BPKIHS. A total of 60 individuals were included in this study where two-third of the participants were male (Figure 1). The mean age of the participants was 35.4 (SD±9.5). Majority of the studied individuals were in the age range of 31-40 years.

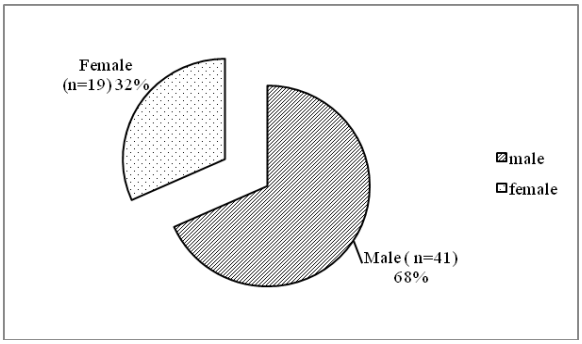

Figure 1: Distribution of studied patients according to gender (n= 60)

The most common mode of HIV transmission in the studied population was sexual contact (66.6%), followed by intravenous drug abuse (10%) while seven patients did not know about the mode of acquisition of HIV infection. The common clinical presentation observed were fever (67%), weight loss (60%), cough (50%) and oral lesion (42%).

Opportunistic fungal infection was identified in 31(51.66%) patients. Fungus was isolated from

11 sputum sample, 12 oral swab samples, 6 Cerebrospinal fluid sample (Figure 2). The mean CD4+ count of the patients with fungal infection in our study was 116.1cells/µl.

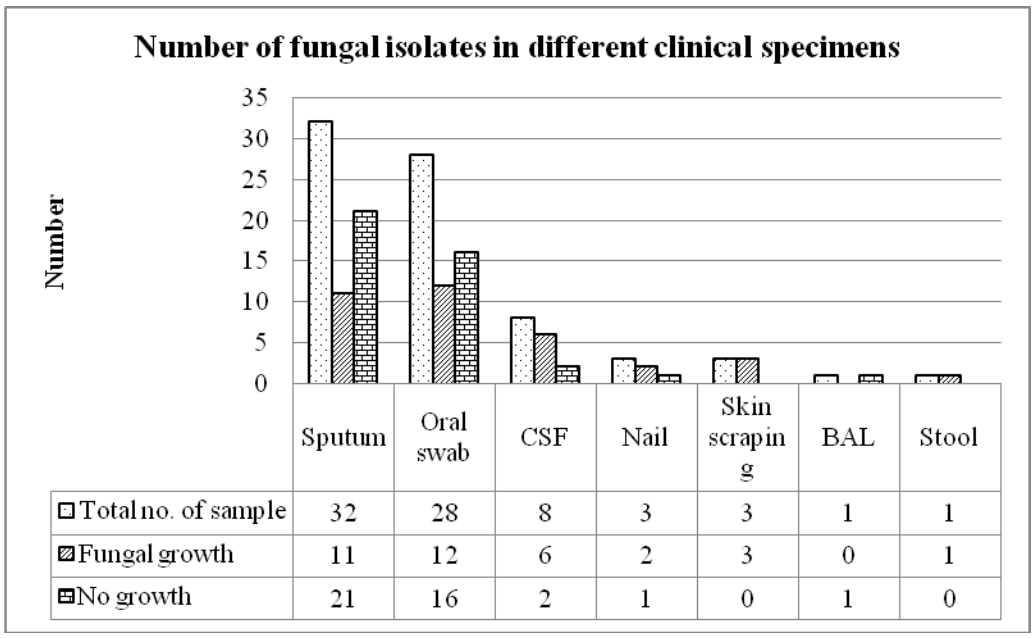

Figure 2: Frequency of opportunistic fungus isolated in different specimen

The different fungi isolated were: *Candida* species isolated from 20 (33.3%) patients comprising 60% of total fungal isolates, among the *Candida* species, the most common species was *Candida albicans*. Similarly, *Aspergillus* species was isolated from 2 patients; *Cryptococcus neoformans* from 6 patients and dermatophytes from 5 patients (Figure 3).

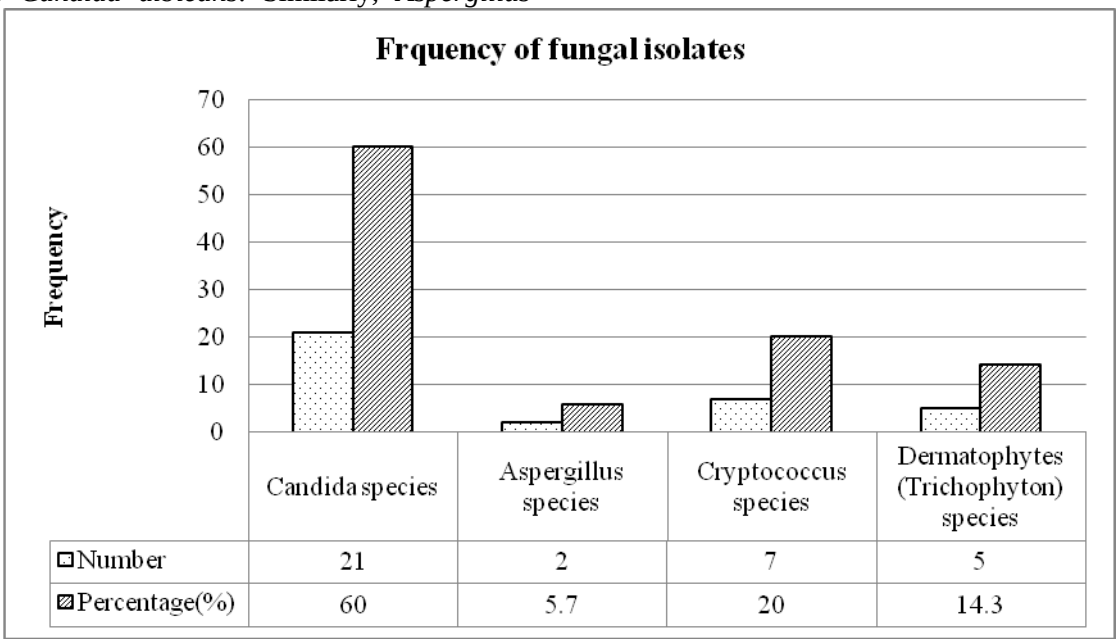

Figure 3: Percentage frequency of different fungal isolates

Table 1: Types of fungal isolates in different clinical samples with percentage frequency and mean CD4+ count

Type of sample	Type of infection	Frequency (%)	Mean CD4+ count cells/µl
	1. *Candida* species	8 (22.8)	62.8
Sputum	2. *Aspergillus* species	2 (5.7)	60.8
	3. *Cryptococcus* species	1 (2.8)	10
Oral swab	1. *Candida* species	12 (34.2)	132.6
Cerebrospinal fluid	1. *Cryptococcus neoformans*	6 (17.1)	111.5
Nail	1. *Trichophyton* species	2 (5.7)	148.5
Skin scrapping	1. *Trichophyton* species	3 (8.5)	86
Stool	1. *Candida* species	1 (2.8)	140
	Total	35 (100)	

Out of 31 patients with fungal infection, 4 patients had mixed fungal infection as shown in table 2.

Table 2: Showing mixed fungal infection

Mixed fungal infection	Number
Disseminated cryptococcal infection (sputum + CSF)	1
Candidiasis (sputum) + Cryptococcal (CSF)	1
Candidiasis (sputum) + *Trichophyton* species	1
Candidiasis (oral swab + stool)	1
Total	4

Among 31 patients with fungal infection, 24 patients were ART naïve while seven patients were on ART. No significant variation was found in fungal infection among ART naïve patients and those on ART (p= 0.068).

Discussion:

Since 1988 when the first case of HIV infection was detected in Nepal, there has been steady rise in HIV infected population.[7] The HIV epidemic in Nepal has evolved from low to concentrate among the high risk group.[8]

The primary target cells of HIV are the CD4+ T-lymphocytes. The progressive loss of these lymphocytes eventually results in loss of immune response to any pathogens and death of the patients at terminal stage of HIV infection.[9] Major cause of morbidity and mortality of such patients are OIs.[10]

Regarding the gender distribution, there was male preponderance (68%) in our study which was consistent with the data published by National centre for AIDS and STD control.[11]

The Data of National centre for AIDS and STD control (NCASC)[8] and other studies conducted in India[12-15] have documented sexual contact as the commonest mode of HIV transmission. Our result was in agreement with the above studies. This shows in spite of various awareness programmes about safe sex and condom promotion by NCASC,[16] it seems the message has not reached to the people.

Initially at higher CD4+ count (> 500 cells/µl), the patients remain asymptomatic. In the advanced stage of HIV infection, more than one symptom are presented by the patient.[12-14,17-20] In the present study; fever, weight loss, cough, oral lesion were the major symptoms presented by the patient and the frequency of these symptoms were 67%, 60%, 50%, 42%, 43% respectively.

The prevalence of fungal infection varies with geographical areas.[1] In the present study, overall prevalence of fungal infection was 51.6% which was similar to the report from south India (53%).[13] Studies conducted in western part of India have documented higher prevalence of opportunistic fungal infection (66%)[1] while other studies done in southern and northern part of India (28-45%)[12,14] and western part of Nepal

(22.2%)[10] have revealed lower prevalence than our study.

Most common opportunistic fungus isolated in our study were *Candida*, *Aspergillus and Cryptococcus neoformans* similar to the study by Parmer *et al*. while study in western Nepal,[10,22] the common fungi isolated were *Candida*, *Pneumocystis* and *Cryptococcus*. Wadhwa A *et al* in Northern India[14] isolated *Candida*, *Cryptococcus*, *Aspergillus*, *Pneumocystis jiroveci* and *Histoplasma capsulatum.*

Although *Pneumocystis jiroveci* is now considered as one of the common opportunistic agents, it was not identified in any of the patients in our study, also the results of studies in different parts of India[15,20,21] do not document *Pneumocystis jirovecii*. Direct examination is the mainstay of diagnosis and BAL is the ideal sample and requires selective staining method like silver methamine staining and immunoflorescence microscopy[22] which we lack in our study. Also *Pneumocystis jirovecii* has decreased in both developed and developing countries due to a combination of chemoprophylaxis with ART.[23]

Candidiasis is mainly found as secondary infection in individuals with some underlying immunocompromised condition and very rarely as the primary disease. The results of study done by Singh A *et al* in South India[24] and Parmer R *et al* in western part of India[1] identified candidiasis in 59% and 55% of cases which were higher than our study where Candidiasis was seen 33.3% (20/60) of cases comprising 60% of total fungal infection. In one study in Vietnam, prevalence of candidiasis was 54%[25] while other studies in India have reported lower prevalence 24.2%, 22.7%.[20,26]

Cryptococcus is considered predominant cause of fatal fungal infection in patients with HIV/AIDS. It may occur at any time during the course of HIV infection but is frequent when CD4+ count falls below 200 cells/µl.[27] In this study, second most common isolated fungus was *Cryptococcus* species found in 6(10%) patients comprising of 20% of total fungal infection. Similar result was observed in study in north India[14] (10%) and Vietnam[25] (9%). However, various studies in different parts of India have reported lower prevalence of *Cryptococcal* infection with 1.51%[20], 4%[15] and 4%.[1]

Although higher prevalence of pulmonary aspergillosis was seen in hospital based study in New Delhi, India[14] (8.3%) compared to our present study where it was identified in 2 (3.3%) patients comprising 5.7% of total fungal infection, our finding was in agreement with the results of study in western[1] and Northern India.[14]

Even if dermatophytes are not considered opportunistic fungus, it was identified in 5 (8.3%) cases and comprised 6.5% of total fungal isolates. Various studies in India have also documented dermatophytical infection in varying frequency amongst the HIV positive patient.[1,28]

Among 31 patients with fungal infection, 24 patients were ART naïve while seven patients were on ART. No significant variation was found in fungal infection among ART naïve patients and those on ART (p= 0.068). This may be due to small sample size of the patients on ART, lack of drug compliance.

Conclusion:

Opportunistic fungal infections like Candidiasis, Cryptococcosis and Aspergillosis are common in HIV positive patients in this Eastern part of Nepal.

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