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Prevalence of various fungal infections among HIV/AIDS patients

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ABSTRACT

Objectives: Human immunodeficiency virus (HIV)-associated opportunistic fungal infections (OFI) are a major cause of mortality and morbidity in HIV-seropositive patients. This prospective study aimed to isolate various fungal pathogens from HIV seropositive patients and to identify and characterize these fungal pathogens at the species level in India.

Material and Methods: Based on clinical signs and symptoms, various clinical specimens (n=323) were collected from (n=200) HIV-seropositive patients in the adult age group of either sex and underwent direct microscopy and fungal culture. Fungal isolates were identified and specified according to a standard protocol. Statistical analysis: All data were collected and analyzed using Microsoft Excel.

Results: Out of 323 samples from 200 HIV-seropositive patients with a suspected fungal infection, fungal isolates were found in 89 cases, or 27.56% of cases. The most frequently isolated fungal organism was *Candida* species (75.28%), followed by *Cryptococcus neoformans* (17.97%), *Aspergillus* species (4.48%), *Alterneria* species (1.12%), and *Trichophyton* mentagrophyte (1.12%). Amongst 67 *Candida* species, *Candida albicans* had the highest isolation rate (88.05%), followed by *Candida tropicalis* (5.97%), *Candida parasilosis* (2.98%), and *Candida auris* (2.98%).

Conclusion: Oropharyngeal candidiasis followed by cryptococcal meningitis was the most common OFI among other fungal infections. This study would help clinicians in the proper diagnosis and early treatment of these infections to prevent their devastating effects in developing countries like India.

Keywords: *Aspergillus*, *Candida*, Cryptococcus, Opportunistic infections

INTRODUCTION

Acquired immunodeficiency syndrome (AIDS) is an immune system disorder that affects humans, leading to a loss of CD4 cells below 200 cells/mm^3 and a decrease in immunity.[1,2]

In 2016, one million people died from AIDS-related diseases globally.[3] According to the United Nations Programme on HIV/AIDS (UNAIDS), an estimated 38 million people were living with human immunodeficiency virus (HIV) infection worldwide in 2019. It has been observed that opportunistic infections, particularly fungal infections, are the leading cause of morbidity and mortality in HIV-infected patients due to the progressive decline in CD4+ T cells.[4]

With an estimated 2.6 million HIV-infected individuals, India has the third-largest population of HIV-positive patients in the world.[2] Opportunistic infections in people with late-stage HIV infection are primarily caused by fungi.[3,4] A rapid decline in peripheral blood CD4+T cells is a key mechanism associated with the occurrence and progression of opportunistic fungal infections (OFI) in HIV seropositive patients.[5-7] Opportunistic respiratory infections are major causes of morbidity and mortality in HIV patients, and pulmonary involvement is the first manifestation in approximately 65% of cases.[8]

The spectrum of fungal infections ranges from asymptomatic mucosal candidiasis to disseminated infections, fungal pneumonia, and meningitis.[6] Fungal infections by *Candida albicans, Cryptococcus neoformans, and Aspergillus fumigatus* are the most prevalent mycoses in immunocompromised individuals.[5] Systemic infections include *Pneumocystis jirovecii* (pneumocystosis), *Cryptococcus neoformans* (cryptococcosis), *Histoplasma capsulatum* (histoplasmosis), and *Talaromyces*, previously known as *Penicillium marneffei* (talaromycosis).[1] The purpose of the present study was to characterize the various fungal pathogens found in HIV-positive patients and their susceptibility to different antifungal drugs.

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MATERIAL AND METHODS

Study population and design

This cross-sectional observational study was carried out from November 01, 2019, to March 31, 2021, after obtaining approval and ethical clearance from the Institutional Ethics Committee (No. TP (MD/MS) 34/2019/IEC/ABVIMS/RMLH 698/19). According to various studies, the isolation rate of fungal infections in HIV-positive patients was 41.1%. Based on this figure, a minimum sample size of 166 samples was determined for analysis, allowing a 7.5% margin of error and a 5% significance level. The calculation was performed using the formula,

$$N \geq (p\ (1\text{-}p)\ /(ME/Z_{\alpha})^2$$

Where Z_{α} = Value of Z at two-sided alpha error of 5%

ME = Margin of error

P = Isolation rate

Two hundred HIV seropositive patients in the adult age group (>18 years) of either sex, with suspected fungal infection, were enrolled in the study. The CD4 cell count was conducted at the Integrated counseling and testing center using the flow cytometry method. A total of 323 different clinical specimens were received from these 200 HIV seropositive patients who exhibited relevant clinical signs and symptoms while attending the antiretroviral therapy (ART) clinic or being admitted to the department of medicine.

Isolation and identification of fungal isolates

Oral and throat swabs were obtained by swabbing the oropharyngeal mucosa. cerebrospinal fluid (CSF), sputum, urine, and blood samples were collected according to the protocol and placed directly into sterile containers. Strict confidentiality was maintained throughout the study. A standard procedure was followed to process the samples.

Microscopy

Depending on the type of specimen and the suspected fungal infection, all samples were examined directly under a microscope using Gram stains, potassium hydroxide preparations/Wet preparations, and India ink preparations.

Fungal culture

Fungal cultures were grown on sabouraud dextrose agar (SDA) (with and without antibiotics) and blood agar as appropriate. Chloramphenicol (50 mg/L), cycloheximide (500 mg/L) (Hi-media), and gentamicin (40 mg/L) were added to prepare SDA with antibiotics. Samples were streaked in duplicate on slants, incubated at 25°C and 37°C, and examined for growth after 48 hours, then weekly for up to 6 weeks until being discarded as negative. Specimens inoculated onto blood agar were incubated for 24–48 hours.[5] Cream-colored, smooth pasty colonies grown on SDA slant or blood agar were Gram-stained and processed further for the identification of yeast. Molds were identified based on morphological appearances and microscopic features under lactophenol cotton blue stain and Riddles Slide culture in accordance with standard procedures.[9] Yeast isolates were identified and classified based on germ tube formation, Calcofluor White Staining, morphology on corn meal agar with Tween 80 (Hi-Media), Hi-Crome *Candida* agar (Hi-Media), and automated VITEK 2 compact system (BioMerieux) according to standard protocol.[9,10]

RESULTS

Among the 200 HIV-positive individuals, 144 (72%) were predominantly male. Most patients, 105 (52.50%), were between the ages of 30 and 45 years, with 59 (29.50%) under 30 years [Table 1].

Among the 200 HIV-seropositive patients, weight loss (50.56%) was the most prevalent clinical symptom, followed by oral ulceration (46.06%), fever (37.07%), and cough (29.21%) [Figure 1].

A total of 323 specimens were received from 200 HIV seropositive patients with suspected fungal infections. Among these, 89 specimens (27.56%) tested positive for various fungal infections.

The CD4 levels of the patients in this study ranged from 5 to 927 cells/mm^3. The mean CD4 cell count among the 200 patients with suspected fungal infection was 221.20 cells/mm^3. Specifically, 18% showed a count of less than 50, 35.5% had counts between 50 and 199, 36.5% had counts between 200 and 500, and 10% had a CD4 cell count above 500 cells/mm^3. In the 89 confirmed fungal-positive patients, the mean CD4 cell count was 140.8, with 24.71% having a count of less than 50, 48.31% between 50 and 199, 23.59% between 200 and 500, and only 3.37% of having a count above 500 cells/mm^3.

Table 1: Age-wise distribution of HIV seropositive patients with suspected fungal infections

Age category	Number of patients	Male	Female	Percentage
<30 Years	59	41	18	29.50
31-45 Years	105	79	26	52.50
ears	28	18	10	14.0
>60 Years	8	6	2	4.0
Total	200	144	56	

HIV: Human immunodeficiency virus

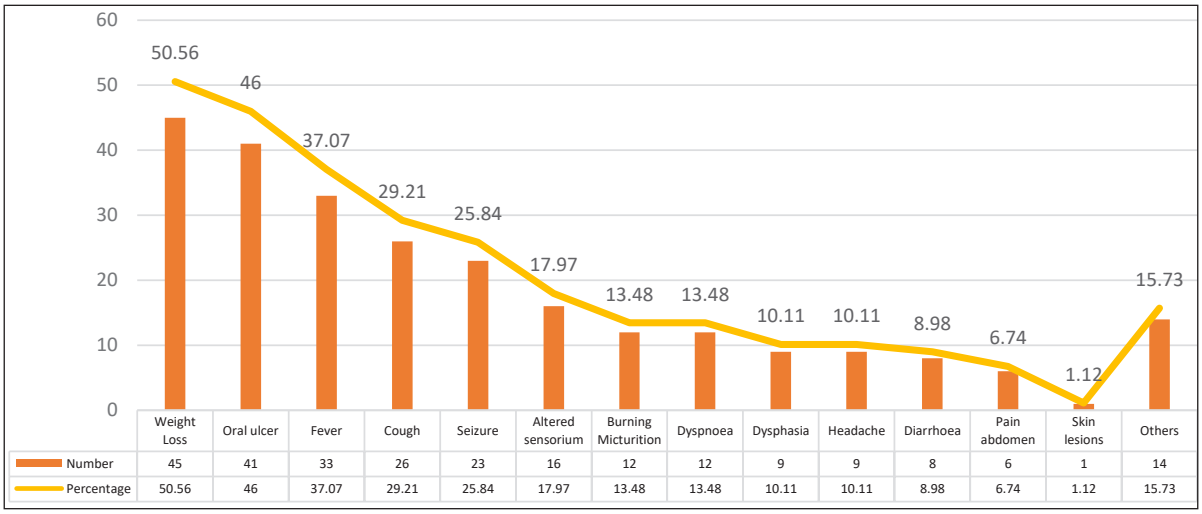

Figure 1: Clinical profile of patients with confirmed fungal infections.

The most common specimens in the study were CSF (62), followed by oral swabs (59), urine (57), sputum (51), and blood (45). Oral swabs (66.10%) and sputum samples (45.10%) had the highest positivity rate for fungal isolates [Table 2].

Candida albicans was the most frequently isolated fungal species in oral swabs (97.43%) and sputum samples (78%). It was also seen that CSF samples (88%) were primarily positive for *Cryptococcus neoformans.*

Candida species were the most frequently isolated fungi, accounting for 75.28%, followed by *Cryptococcus neoformans*, which was identified in 16 patients (17.97%). Among the 67 *Candida* species isolated, *Candida albicans* had the highest isolation rate (88.05%), followed by *Candida tropicalis* (5.97%) [Figure 2].

Table 2: Distribution of fungal isolates from various samples

Sample type	No. of samples	No. of fungal isolates	Isolated fungal species
Oral swab	59	39	*C.albicans*:38 *C.tropicalis*:01
Sputum	51	23	*C.albicans*:18 *C.parasilosis*:01 *Aspergillus niger*:02 *Aspergillus flavus*:01 *Aspergillus fumigatus*:01
CSF	62	15	*Cryptococcus neoformans*:15
Urine	57	7	*Candida* albicans:03 *Candida* tropicalis:02 *Candida* parasilosis:01 *Candida* auris:01
Blood	45	1	*Cryptococcus neoformans*:01
Throat swab	02	1	*Candida* tropicalis:01
Skin scrapping	02	1	*Trichophyton mentagrophyte*:01
Axilla/ Groin swab	01	1	*Candida* auris:01
Nail	01	1	*Alternaria spp*:01
Total	280	89	

CSF: Cerebrospinal fluid

DISCUSSION

In developing countries, opportunistic fungal infections (OFI) in HIV-positive patients are the most important cause of mortality and morbidity.[2-5] While there is extensive literature on the spectrum of OFIs (OFIs) in HIV seropositive patients worldwide, data from India remains inadequate.

In our study, 200 HIV-positive patients from different age groups were included, with a male predominance of 72%. Most patients (52.50%) were in the age group of 30-45 years, followed by 29.50% who were under 30 years. Similar results in the most sexually active and economically productive age male preponderance of 67% and 73.2% was observed by Kaur *et al.*, (2016) and by Harikrishna *et al.*, (2017) respectively.[5,11] Our findings are consistent with other studies on HIV-seropositive patients in India and Iran.[12,13] Men often migrate away from their homes in search of work, leading to prolonged separations from their wives and visits to brothels, which are significant contributors to HIV infection and the observed male preponderance. Additionally, many women in Indian society are housewives and may not receive treatment due to social stigma and lack of family support.[14,15]

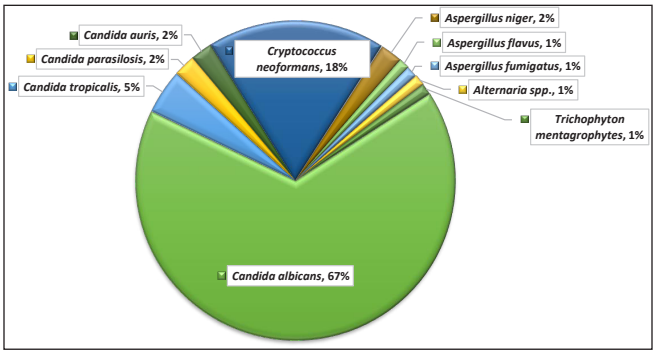

Figure 2: Distribution of fungal species in human immunodeficiency virus (HIV) patients.

Among the 200 HIV-seropositive patients with suspected fungal pathogens, the most prominent clinical features in our study were weight loss (50.56%) followed by oral ulcer (46.06%), fever (37.07%), and cough (29.21%). Similarly, a study from New Delhi, India, reported weight loss (78.2%) and oral ulcers (74.6%) as the prominent clinical features in HIV-positive patients.[5] In a different study from Nepal, Joshi *et al.*, (2004) also identified weight loss (58.8%) as the most common clinical feature, consistent with our findings.[16] However, Chakravorty *et al.*, (2006) reported that the most prevalent clinical feature was fever, observed in 70.6% of patients, followed by gradual weight loss in 53.3% of cases, while 18.0% of patients were asymptomatic.[17]

CSF samples (19.2 %) and oral swabs (18.26%) were the most frequently received samples in our study. The highest positive rates for fungal isolates were observed in oral swabs (66.10%), sputum (45.10%), and CSF (24.19%). In a study from New Delhi (2016), the most common sample was the oropharyngeal swab (100%), followed by induced sputum (32.5%), blood (31.8%), and CSF (26.2%). The highest rate of fungal isolation in that study was found in sputum samples (53.9%), followed by oropharyngeal swabs (49.3%), with only 9.6% of CSF samples testing positive for fungi.[5] Among the 200 individuals suspected of having an opportunistic fungal infection, 89 patients had confirmed fungal infections. In our study, 71% of individuals with confirmed fungal infection had a CD4 count of less than 200 cells/mm^3, indicating a correlation between low CD4 counts and OFIs.[6,18] Most fungal isolates (i.e., *Candida* species) were obtained from oral swabs, which are commonly associated with oropharyngeal candidiasis, the most prevalent opportunistic fungal infection. According to several other studies, oropharyngeal candidiasis is the most common opportunistic fungal infection.[1,18-22]

Although *Candida* species are part of the normal flora in the oral cavity, we categorized them as opportunistic fungal pathogens because patients experienced oral ulcers with burning sensations during eating or swallowing. Oral candidiasis was found to be more prevalent in our study, likely due to patients having irregular access to ART center sessions during the COVID-19 era. Poor oral hygiene and a lack of awareness about opportunistic infections among HIV-seropositive patients may also contribute to the incidence of oral candidiasis. In our study, *Candida albicans* was the most frequently isolated fungal species from oral swabs (97.43%) and sputum samples (78%).

There were two isolates of *Candida auris*, one from urine, for which subsequent surveillance measures were initiated, and another isolated from an axilla/groin swab of the same patient, hence infection control measures for *Candida auris* were reinforced.

In our study, among the 62 CSF samples, 15 (24.19%) tested positive for fungal isolates, with the sole fungal species identified being *Cryptococcus neoformans*. However, several other studies have reported significantly lower isolation rates of 9.6%, 6.7%, 2.9%, 4%, and 3.7% for cryptococcal meningitis than the isolation rate observed in our study.[5,18,19,23,24] The higher isolation rate observed in our study may be attributed to the study period coinciding with the COVID-19 era, during which most patients visited the hospital only when their condition worsened, experiencing seizures and altered sensorium that necessitated emergency medical monitoring. Given that cryptococcosis is the most common systemic fungal infection among AIDS patients, its prevalence is directly related to the rapid spread of the disease.[18,25,26]

In sputum samples, *Candida* species predominated at 82.6%, followed by *Aspergillus* species at 17.4%.

Upon further categorization, the percentage of *Candida albicans* was 78%, while non-*albicans Candida* accounted for 4.3%. Among the *Aspergillus* species, *Aspergillus niger*, *Aspergillus fumigatus*, and *Aspergillus flavus* were identified at 8.7 %,4.3%, and 4.3 %, respectively. These findings closely align with a study by Bharathi M. and Rani AU. (2011), which reported that 57% of isolates were *Candida* spp. Including 27% *albicans* and 30% non-*albicans Candida*, followed by 13.5% *Aspergillus* spp. as the major groups.[27] In another study by Chandwani *et al.*, (2016), *Candida albicans* was the most common isolate (31.7%), followed by *Aspergillus niger* (17.7%) and *Aspergillus flavus* (10%).[6] In sputum samples, *Candida* species are typically commensals, while *Aspergillus* species are considered contaminants. However, both can act as opportunistic pathogens in immunocompromised individuals. In our study, the same fungal isolate was found in three consecutive sputum samples, and patients presented with symptoms of fever, cough, and chest pain, prompting us to report these isolates as fungal pathogens.

Candida species (75.28%), *Cryptococcus neoformans* (17.97%), *Aspergillus* species (4.48%), *Alterneria species*

Table 3: Prevalence of various fungal isolates from HIV/AIDS patients

Reference	*Candida spp.*	*Cryptococcus spp.*	*Aspergillus spp.*	Others
Our Study	75.28%	17.97%	4.49%	2.24%
Kaur et al.[5] (2016)	86.5%	3.3%	6.5%	3.7%
Gandham et al.[31] (2013)	71.7%	1.2%	14%	13.1%
Parmar et al.[2] (2012)	83.3%	6.0%	4.7%	6.0%
Bharathi and Usha[27] (2011)	57%	5.2%	13.5%	24.3%
Jahromi and Khaksar[28] (2005)	69.4%	4.2%	13.9%	12.5%

HIV: Human immunodeficiency virus, AIDS: Acquired immunodeficiency syndrome

(1.12%), and *Trichophyton mentagrophyte* (1.12%) were the most frequently isolated fungal species in our study. These results are comparable to a previous study by Parmar *et al.*, (2012), which reported *Candida* species (55%) as the most common fungal isolate, followed by *Cryptococcus* species (4%) and *Aspergillus* species (3%).[2]

Similarly, R. Kaur *et al.*, (2016) from New Delhi found *Candida* species (86.5%) to be the most prevalent fungal isolate, followed by *Aspergillus* species (6.5%), *Cryptococcus* species (3.3%), *Penicillium* species (1.9%). *Alterneria* and *Rhodutorula* species were reported at 0.9% each[5] [Table 3].

In another study from Iran by Jahromi SB and Khaksar AA (2005), *Candida* species accounted for 69.4%, followed by *Aspergillus* species (13.9%) and *Cryptococcus* species (4.2%).[28] A study by Pagano *et al.*, (2006) from Italy showed *Aspergillus* species as the most frequently isolated fungal species (57.6%), followed by *Candida* species (32.5%) and *Cryptococcus* species (1.4%).[29] Additionally, a study by Kashyap *et al.*, (2012) reported *Candida* species (18.3%) as the predominant fungal isolate, followed by *Aspergillus* species (6.9%) and *Cryptococcus* species (0.6%).[30] The most isolated fungal species in the study by Gandham *et al.*, (2013) was *Candida* species (71.7%), *Aspergillus* species (14%), *Penicillium* species (1.5%), *Cryptococcus* species (1.2%), and *Rhodutorula* species (0.9%).[31]

The isolation rate of *Cryptococcus* species in our study was 17.97%, which is significantly higher compared to the rates reported in other studies, which were 4%, 3.3%, 4.2%, 1.4%, 0.6%, and 1.2%.[2,5,28-31] [Table 3].

Among the 67 *Candida* species isolated in our study, *Candida albicans* was predominant (88.05%), followed by *Candida tropicalis* (5.97%), *Candida parasilosis* (2.98%), and *Candida auris* (2.98%). Similarly, a study from New Delhi, India, found *Candida albicans* (75.8%) to be the most prevalent among *Candida* isolates, followed by *Candida tropicalis* (9.7%), *Candida krusei* (6.5%), *Candida glabrata* (4.3%), *Candida parapsilosis* (2.7%), and *Candida kefyr* (1.1%).[5] Another study by Gandham *et al.*, (2013) reported similar results, with *Candida albicans* at 51.4%.[31] However, in contrast, Picardi *et al.*, (2012) from the USA found non-albicans *Candida* to be the most prevalent isolate in neutropenic patients.[32] Among *Aspergillus* species, *Aspergillus niger* was the most prominent at 50%, followed by *Aspergillus flavus* and *Aspergillus fumigatus* at 25% each. Another study from India also reported *Aspergillus niger* (50%) as the primary species, followed by *Aspergillus fumigatus* (35.7%) and *Aspergillus flavus* (14.3%).[5] Meanwhile, Gandham *et al.*, (2013) reported *Aspergillus fumigatus* (53.2%), followed by *Aspergillus niger* (25.5%) and *Aspergillus flavus* (14.9%).[31] In our study, only *Cryptococcus neoformans* was isolated among *Cryptococcus* species(100%), which is comparable the findings of Gandham *et al.* (2013).[31] However, Kaur *et al.*, (2016), from New Delhi, India, reported both *Cryptococcus neoformans* (71.4%) and *Cryptococcus gattii* (28.6%) among *Cryptococcus* species (14.9%).[5]

In our study, one isolate of *Alterneria* species (1.12%) was obtained from a nail sample. In contrast, a study from New Delhi reported an isolation rate of 0.9% for *Alternaria alternata* from sputum samples. However, a study by Warthe N *et al.* (2015) found that 5.6% of cancer/HIV patients from central India had *Alternaria alternata* isolated from their blood.[33] However, we did not find any case of *Alternaria* fungemia in HIV-seropositive patients.

CONCLUSION

Oropharyngeal candidiasis followed by cryptococcal meningitis are the most common opportunistic fungal infection in HIV-positive patients, particularly those with low CD4 counts. A detailed comparative study of CD4 cell counts, viral load, and OFIs could establish CD4 count as an indicator of opportunistic mycoses in HIV/AIDS patients. Such research would aid clinicians in t diagnosing and initiating early treatment for these infections, ultimately helping to prevent their severe consequences in developing countries like India.

Authors' contributions: SM, NK: Concept and design; NK, SM, AKC, NC, NKB: Acquisition, analysis, or interpretation

of data; NK, SM, AKC: Drafting of the manuscript; SM, NKB: Critical revision of the manuscript for important intellectual content; NC, AKC: Technical and material support; SM, NKB: Supervision.

Ethical approval: The research/study is approved by the Institutional Ethics Committee at Atal Bihari Vajpayee Institute of Medical Sciences and Dr Ram Manohar Lohia Hospital, number TP (MD/MS) 34/2019/IEC/ABVIMS/RMLH 698/19, dated 22nd October 2019.

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Use of artificial intelligence (AI)-assisted technology for manuscript preparation: The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

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