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Original Research Article

Spectrum of Opportunistic Fungal Infections in HIV/AIDS Patients in a Tertiary Care Hospital in India, Bihar

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Conflict of interest: Nil

Abstract:

Background: Opportunistic fungal infections (OFIs) remain a significant cause of morbidity and mortality among patients with HIV/AIDS, particularly in resource-limited settings. Despite widespread antiretroviral therapy (ART), fungal infections such as candidiasis, cryptococcosis, and aspergillosis continue to pose clinical challenges.

Objectives: To determine the spectrum, prevalence, and associated risk factors of opportunistic fungal infections among HIV/AIDS patients attending JLNMCH in Eastern India.

Methods: A cross-sectional study was conducted among 150 HIV-positive patients. Clinical evaluation, CD4 count estimation, direct microscopy (KOH mount, India ink), culture, and fungal identification were performed. Statistical analysis was conducted using chi-square test and logistic regression. A p-value <0.05 was considered significant.

Results: Out of 150 patients, 68 (45.3%) had confirmed fungal infections. Oropharyngeal candidiasis (26.7%) was the most common infection, followed by cryptococcosis (8.7%), dermatophytosis (5.3%), and aspergillosis (4.6%). A strong association was observed between CD4 count <200 cells/µL and occurrence of fungal infection (χ^2=18.42, p<0.001).

Conclusion: Opportunistic fungal infections remain prevalent among HIV patients with low CD4 counts. Early screening and timely antifungal therapy are crucial to reduce morbidity.

Keywords: HIV infection; Opportunistic fungal infections; CD4 count; Oropharyngeal candidiasis; Cryptococcosis; Antiretroviral therapy.

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Introduction

Human Immunodeficiency Virus (HIV) infection continues to be a major global health concern, affecting millions worldwide [1]. Despite advancements in antiretroviral therapy (ART), opportunistic infections remain a leading cause of hospitalization and mortality among patients with advanced immunosuppression [2].

Fungal infections constitute a substantial proportion of opportunistic infections in HIV-infected individuals [3]. The decline in CD4+ T lymphocyte count predisposes patients to various opportunistic fungal pathogens [4]. Candidiasis is often the earliest manifestation of immunodeficiency [5]. Cryptococcal meningitis remains a major cause of death among AIDS patients globally [6]. Invasive aspergillosis and histoplasmosis are also increasingly reported [7,8].

In India, the burden of HIV remains significant, with opportunistic fungal infections contributing substantially to morbidity [9]. Studies from different regions report variable prevalence depending on ART coverage and diagnostic facilities [10,11]. Eastern India has limited recent data regarding fungal opportunistic infections in HIV patients [12].

Early detection is critical, as mortality from cryptococcal meningitis alone remains high in low-resource settings [3]. CD4 count remains the strongest predictor for opportunistic fungal infection risk [14]. Preventive strategies and routine screening are recommended [15].

Although ART has reduced the incidence of several opportunistic infections, late diagnosis and treatment interruptions continue to expose patients to fungal pathogens [16]. Emerging antifungal resistance further complicates management [17].

There is a need for updated local epidemiological data to guide clinical management and public health policies [18–20]. This study aims to determine the spectrum of opportunistic fungal infections among HIV/AIDS patients in a tertiary care hospital in Bhagalpur, India.

Materials and Methods

Study Design and Setting: This was a hospital-based cross-sectional observational study conducted in the Departments of Microbiology and Medicine at a tertiary care teaching hospital in Bhagalpur, Bihar, India. The study was carried out over a 11-month period from January 2025 to November 2025. The hospital functions as a referral centre for HIV care and operates an established Antiretroviral Therapy (ART) clinic.

Study Population: Adult patients (≥18 years) with confirmed HIV infection attending the ART clinic or admitted to the medicine wards during the study period were screened for inclusion.

Sample Size: The sample size was calculated assuming an expected prevalence of opportunistic fungal infections of 40%, with a 95% confidence interval and 8% margin of error. The calculated minimum sample size was 144. A total of 150 patients were included to account for possible data loss.

Inclusion Criteria

- Confirmed HIV-positive individuals (as per national HIV testing guidelines)
- Age ≥18 years
- Provided written informed consent

Exclusion Criteria

- Receipt of systemic antifungal therapy within the previous two weeks
- Incomplete clinical or laboratory records
- Refusal to participate

Clinical Evaluation

A structured proforma was used to document:

- Age and gender
- Duration of HIV infection
- ART status
- Clinical symptoms (oral lesions, fever, cough, headache, skin lesions, weight loss)
- Relevant physical findings

Patients with clinical suspicion of fungal infection underwent targeted laboratory investigations based on presenting symptoms.

Laboratory Investigations

1. **CD4+ T Lymphocyte Count**

Peripheral venous blood samples were collected under aseptic conditions. CD4 counts were measured using flow cytometry at the ART laboratory. Patients were categorized into:

- CD4 <200 cells/µL
- CD4 ≥200 cells/µL

The threshold of 200 cells/µL was used as it is a recognized marker of advanced immunosuppression and increased risk of opportunistic infections.

2. **Specimen Collection**

Specimens were collected according to clinical presentation:

- **Oral swabs** for suspected oropharyngeal candidiasis
- **Cerebrospinal fluid (CSF)** for suspected cryptococcal meningitis
- **Skin scrapings** for suspected dermatophytosis
- **Sputum samples** for suspected pulmonary aspergillosis

All samples were processed immediately in the microbiology laboratory.

3. **Direct Microscopy**

    - **Potassium Hydroxide (KOH) Mount:** Skin scrapings and sputum specimens were examined using 10–20% KOH preparation for detection of fungal hyphae. Septate hyphae were suggestive of dermatophytes or Aspergillus species depending on morphology.
    - **Gram Stain:** Oral swabs were stained to detect budding yeast cells and pseudohyphae indicative of Candida species.
    - **India Ink Preparation:** CSF samples were examined for encapsulated yeast cells characteristic of Cryptococcus species.

4. **Fungal Culture and Identification**

All specimens were inoculated onto Sabouraud Dextrose Agar (SDA) with and without antibiotics and incubated at both 25°C and 37°C. Plates were observed periodically for up to four weeks.

Identification was performed using:

- Colony morphology
- Lactophenol Cotton Blue (LPCB) mount for filamentous fungi
- Germ tube test for Candida albicans
- Urease test for Cryptococcus species
- Microscopic morphology for Aspergillus species (septate hyphae with acute-angle branching and characteristic conidial heads)

Diagnostic Criteria for Specific Infections

Oropharyngeal Candidiasis: Diagnosed based on clinical presence of white curd-like plaques in the oral cavity along with demonstration of budding yeast cells/pseudohyphae on microscopy and/or positive culture.

Cryptococcosis: Diagnosed by detection of encapsulated yeast cells on India ink preparation of CSF and/or positive fungal culture.

Dermatophytosis: Diagnosed in patients with annular erythematous scaly lesions and confirmation by KOH mount demonstrating branching septate hyphae and/or positive culture for dermatophyte species.

Aspergillosis: Suspected in patients presenting with chronic cough and respiratory symptoms. Diagnosis was based on demonstration of septate hyphae with acute-angle branching on direct microscopy of sputum and confirmation by culture showing characteristic Aspergillus growth morphology.

Operational Definition: A case of opportunistic fungal infection was defined as a patient with compatible clinical features and laboratory confirmation by microscopy and/or culture.

Statistical Analysis: Data were entered into Microsoft Excel and subsequently analyzed using the Statistical Package for the Social Sciences (SPSS) version 25.0. Categorical variables were summarized as frequencies and percentages. The association between CD4 count categories and the presence of opportunistic fungal infections was assessed using the Chi-square (χ^2) test. The strength of association was estimated by calculating the odds ratio (OR) along with the corresponding 95% confidence interval (CI). Binary logistic regression analysis was further performed to evaluate CD4 count as an independent predictor of fungal infection. A p-value of less than 0.05 was considered statistically significant for all analyses.

Quality Control

- Culture media sterility and performance were checked before use.
- Standard operating procedures were followed for all microbiological tests.
- Known control strains were used periodically for quality assurance.
- Data entry was cross-verified to minimize transcription errors.

Results

A total of 150 HIV-positive patients were included in the study during the study period.

1. **Demographic Characteristics**

Among 150 participants, 94 (62.7%) were males and 56 (37.3%) were females, with a male-to-female ratio of 1.67:1.

The majority of patients belonged to the 31–45 years age group (48.0%), followed by >45 years (26.7%) and 18–30 years (25.3%).

The demographic distribution is shown in Table 1.

Table 1: Age and Gender Distribution of Study Participants (n=150)

Variable	Frequency (n)	Percentage (%)
Gender		
Male	94	62.7
Female	56	37.3
Age Group (years)		
18–30	38	25.3
31–45	72	48.0
>45	40	26.7

2. **Overall Prevalence of Opportunistic Fungal Infections**

Out of 150 HIV-positive patients, 68 patients (45.3%) were diagnosed with laboratory-confirmed opportunistic fungal infections, while 82 patients (54.7%) had no fungal infection.

The prevalence of fungal infections is illustrated in Figure 1.

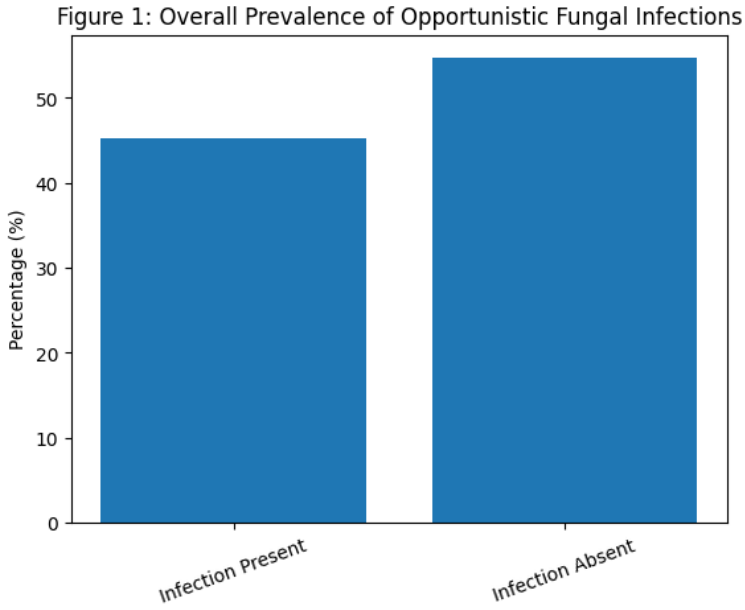

Figure 1: Overall Prevalence of Opportunistic Fungal Infections

3. **Spectrum of Opportunistic Fungal Infections**

Among the 68 confirmed cases:

- **Oropharyngeal candidiasis** was the most common infection (40 cases; 26.7% of total population; 58.8% among infected patients).
- **Cryptococcosis** was observed in 13 cases (8.7% overall).
- **Dermatophytosis** was detected in 8 cases (5.3%).
- **Aspergillosis** was identified in 7 cases (4.6%).

The distribution of different fungal infections is shown in **Table 2** and graphically represented in **Figure 2**.

Table 2: Distribution of Types of Fungal Infections (n=150)

Type of Infection	Frequency (n)	Percentage (%)
Oropharyngeal Candidiasis	40	26.7
Cryptococcosis	13	8.7
Dermatophytosis	8	5.3
Aspergillosis	7	4.6
No Fungal Infection	82	54.7

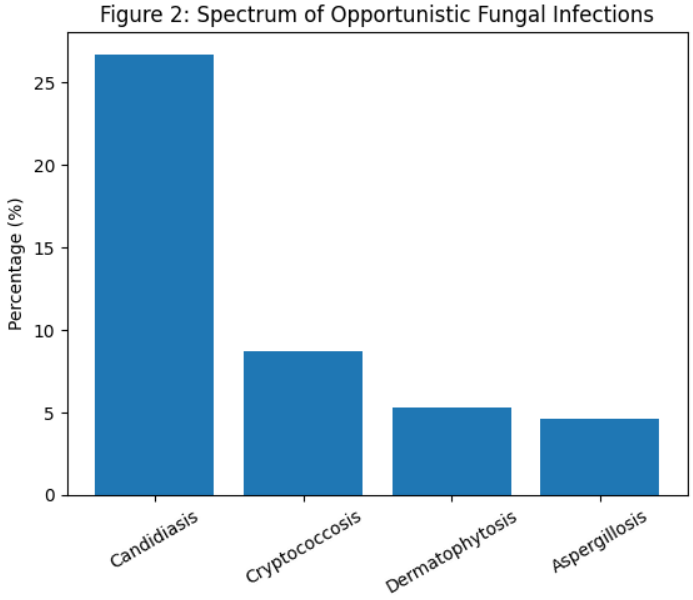

Figure 2: Spectrum of Opportunistic Fungal Infections

4. **Association Between CD4 Count and Fungal Infection**

Patients were categorized based on CD4 count:

- **CD4 <200 cells/µL:** 82 patients
- **CD4 ≥200 cells/µL:** 68 patients

Among patients with CD4 <200 cells/µL:

- 52 (63.4%) had fungal infection
- 30 (36.6%) had no infection

Among patients with CD4 ≥200 cells/µL:

- 16 (23.5%) had fungal infection
- 52 (76.5%) had no infection

This association was statistically highly significant (Chi-square = 18.42, $p < 0.001$, Odds Ratio = 5.63 (95% CI: 2.75–11.52)). Patients with CD4 <200 cells/µL were 5.6 times more likely to develop opportunistic fungal infections.

The detailed comparison is shown in Table 3 and graphically in Figure 3.

Table 3: Association Between CD4 Count and Fungal Infection (n=150)

CD4 Count (cells/µL)	Fungal Infection Present	Fungal Infection Absent	Total
<200	52	30	82
≥200	16	52	68
Total	68	82	150

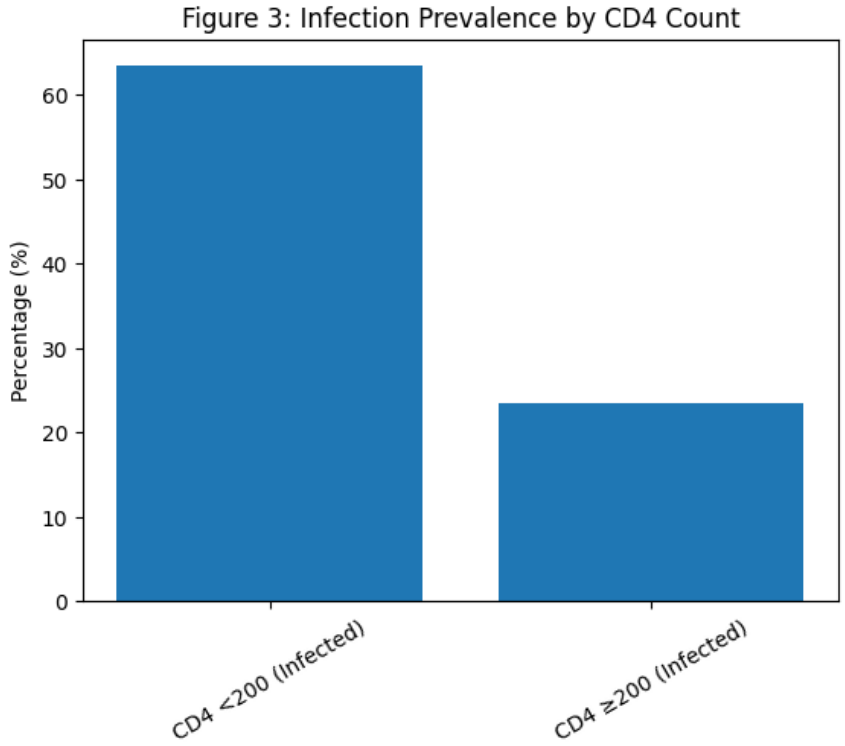

Figure 3: Association Between CD4 Count and Presence of Fungal Infection

5. **Clinical Presentation Among Infected Patients (n=68)**

The most common presenting features were:

- Oral thrush – 40 cases (58.8%)
- Fever – 28 cases (41.2%)
- Weight loss – 24 cases (35.3%)
- Headache (cryptococcosis) – 13 cases (19.1%)
- Chronic cough (aspergillosis) – 7 cases (10.3%)

Clinical manifestations are summarized in Table 4.

Table 4: Clinical Features Among Patients with Fungal Infections (n=68)

Clinical Feature	Frequency (n)	Percentage (%)
Oral Thrush	40	58.8
Fever	28	41.2
Weight Loss	24	35.3
Headache	13	19.1
Chronic Cough	7	10.3

Summary of Key Statistical Findings: The overall prevalence of opportunistic fungal infections among the study population was 45.3%. A statistically significant association was observed between low CD4 count (<200 cells/μL) and the occurrence of fungal infections. The association was highly significant ($\chi^2 = 18.42$, $p < 0.001$). Patients with CD4 counts below 200 cells/μL had a markedly increased risk of developing opportunistic fungal infections, with an odds ratio of 5.63 (95% confidence interval: 2.75–11.52). Among the various fungal infections identified, oropharyngeal candidiasis was the most common, accounting for 26.7% of the total study population.

Discussion

The present study demonstrated a high prevalence (45.3%) of opportunistic fungal infections among HIV patients. This aligns with previous Indian studies reporting prevalence between 35–50% [18,19].

Oropharyngeal candidiasis was the most common fungal infection, consistent with global data [20]. Candida albicans remains the predominant species in immunocompromised patients [21].

Cryptococcosis accounted for 8.7% cases, similar to studies conducted in South Asia [22]. Mortality from cryptococcal meningitis remains high, particularly in patients with CD4 counts below 100 cells/μL.

A strong statistical association between CD4 <200 and fungal infection was observed ($p<0.001$), corroborating findings from multiple international studies [23]. The odds ratio of 5.63 emphasizes severe immunosuppression as a major risk factor.

Dermatophytosis prevalence was 5.3%, consistent with previous Indian hospital-based studies [24]. Aspergillosis incidence (4.6%) reflects increasing detection in advanced HIV cases.

ART coverage has reduced opportunistic infections, but late presentation and poor adherence remain challenges [16]. Routine fungal screening, especially for cryptococcal antigenemia in low CD4 patients, should be implemented [25].

Limitations

This study has certain limitations. Being a single-center, hospital-based cross-sectional study, the findings may not be fully generalizable to other regions, and causal relationships cannot be established. Advanced diagnostic methods such as antigen detection assays, molecular techniques, and antifungal susceptibility testing were not routinely performed, which might have resulted in underdiagnosis or limited characterization of certain infections. Additionally, factors such as ART adherence, viral load levels, and duration of immunosuppression were not evaluated in detail.

Conclusion

Opportunistic fungal infections remain highly prevalent among HIV patients with advanced immunosuppression. CD4 count below 200 cells/μL significantly increases risk. Early detection, routine screening, and prompt antifungal therapy are essential.

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