

Cryptococcus Infections: Microbiological Insights from A Tertiary Care Centre in Eastern U.P

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Introduction: Cryptococcosis is a clinically significant systemic mycosis caused by encapsulated yeast of the genus *Cryptococcus*, predominantly *Cryptococcus neoformans* and other species of *Cryptococcus*. These fungi are ubiquitously present in the environment and are acquired primarily through inhalation of infectious propagules, initially affecting the lungs with a strong propensity for hematogenous dissemination to the central nervous system. Cryptococcal meningitis remains one of the leading causes of fungal-related morbidity and mortality, particularly among immunocompromised individuals such as patients with HIV/AIDS, malignancies, organ transplants, or those receiving long-term immunosuppressive therapy. In recent decades, the global burden of cryptococcosis has increased due to the expanding population of immunocompromised hosts, highlighting its importance as an opportunistic pathogen. Early diagnosis and appropriate management are critical for improving patient outcomes. 1,3

Objectives: To determine the prevalence of cryptococcal infection and associated risk factors in patients attending a tertiary care hospital in Eastern Uttar Pradesh.

Materials and Methods: A prospective cross-sectional study was conducted in the Department of Microbiology, Moti Lal Nehru Medical College Prayagraj from January to December 2024. A total of 500 clinical specimens including CSF, blood, and BAL fluid were collected from suspected cases of cryptococcal infection. Samples were processed using India ink preparation, culture on Sabouraud Dextrose Agar, KOH preparation, gram stain and urease test. Relevant clinical data, including HIV status and comorbidities, were recorded.

Result: Out of 500 samples, 37 (7.6%) were positive for *Cryptococcus* species. Majority were from CSF (71%). *Cryptococcus neoformans* was the predominant species (86.8%), followed by other species of *Cryptococcus* (13.2%). The mean age of affected patients was 36 years, with male predominance (M: F = 2.3:1). HIV infection was the most common underlying risk factor (65.7%), followed by uncontrolled diabetes mellitus (15.7%). Clinically, headache, fever, and altered sensorium were predominant features.

Conclusion: *Cryptococcus* remains an important cause of meningitis in immunocompromised patients in Eastern Uttar Pradesh. Early diagnosis using culture is essential for better outcomes. Strengthen routine screening for cryptococcal infection in high-risk patients is recommended.

Keywords: Sabouraud Dextrose Agar, *Cryptococcus neoformans*, BAL (Bronchoalveolar lavage) fluid & Cerebrospinal fluid (CSF).

INTRODUCTION

Cryptococcosis is a life-threatening opportunistic systemic mycosis caused predominantly by *Cryptococcus neoformans* and, less commonly, by other species of *Cryptococcus*. Globally, cryptococcal disease remains a significant public health problem, particularly among immunocompromised individuals. Recent estimates from 2010 onwards indicate that

approximately 200000-280000 cases of cryptococcal meningitis occur globally each year, with a substantial proportion of associated mortality occurring in low- and middle-income countries.

In India, cryptococcal meningitis is one of the most common invasive fungal infections among people living with HIV/AIDS, especially in individuals with CD4 counts below 100 cells/ μ L. Despite the availability of antifungal therapy, the disease continues to contribute significantly to morbidity and mortality. Several hospital-based studies from different regions of the country have reported a rising burden of disease, between 2014 and 2022, it is estimated that approximately 11500 to 18000 cases of cryptococcal meningitis occur annually, largely attributed to late presentation, limited diagnostic facilities, and overlapping clinical features with other forms of chronic meningitis. In Uttar Pradesh, particularly in tertiary care centres catering to large populations from rural and semi-urban areas, cryptococcal infections remain underdiagnosed, highlighting the need for increased clinical suspicion and improved laboratory diagnostic support.^{3,7}

The infection is acquired through inhalation of desiccated yeast cells or basidiospores from environmental sources, primarily contaminated soil and avian excreta. Primary pulmonary infection may be asymptomatic or mild, followed by possible hematogenous dissemination. The central nervous system is the most frequent site of dissemination, manifesting as cryptococcal meningitis, particularly in individuals with impaired cell-mediated immunity. Clinically, cryptococcal meningitis commonly presents as a subacute or chronic illness characterized by headache, fever, nausea, vomiting, neck stiffness, altered mental status, photophobia, cranial nerve palsies, and raised intracranial pressure. In advanced cases, seizures, visual disturbances, and coma may occur, contributing to high mortality if not promptly treated.

Important risk factors include diabetes mellitus, prolonged corticosteroid therapy, malignancy, solid organ transplantation, and other causes of immunosuppression. Clinically, cryptococcal meningitis often presents with features overlapping those of tuberculous meningitis, frequently leading to misdiagnosis and delays in initiating appropriate antifungal therapy.

Early laboratory diagnosis is essential for the prompt initiation of antifungal treatment and improved patient outcomes. Conventional diagnostic methods such as direct microscopy, culture, and India ink preparation continue to play a vital role, particularly in resource-limited settings. The present study was undertaken to assess the prevalence, microbiological characteristics and associated risk factors of cryptococcal infections among clinically suspected cases attending a tertiary care centre in Eastern Uttar Pradesh.

MATERIAL AND METHOD

Prospective cross-sectional study was conducted in the Department of Microbiology, Moti Lal Nehru Medical College Prayagraj, over a period of one year from January to December 2024. The study included clinically suspected cases of cryptococcal infection.

Study population and specimens: A total of 500 clinical specimens were collected from patients with clinical suspicion of cryptococcosis. The specimens included cerebrospinal fluid (CSF), blood, and bronchoalveolar lavage (BAL) fluid, collected following standard aseptic precautions. Detailed demographic and clinical information such as patients aged between 18 - 70 years were included in the study. Among these, 349 (69.8%) were males and 151 (30.2%) were females, demonstrating a clear male predominance with a male-to-female ratio of 2.3:1, headache, fever, nausea, vomiting, neck stiffness, altered mental status were the presenting symptoms. HIV status, and associated comorbidities was recorded for each patient.

All specimens were processed immediately according to standard mycological procedures.

Macroscopic finding of the sample: Cerebrospinal fluid in cryptococcal meningitis is typically clear or mildly turbid, with raised opening pressure, and does not form a clot on standing. **Direct microscopic examination:**

Potassium Hydroxide (KOH) wet mount: KOH mount is a simple, rapid screening test used for the detection of fungal elements by dissolving host cellular material while preserving fungal structures. A wet mount was prepared such as, a drop of specimen was placed on a clean glass slide, mixed with KOH, and examined under low and high power magnification. The presence of round to oval budding yeast cells suggested fungal infection.

India ink preparation: India ink preparation is a negative staining technique used to demonstrate the thick polysaccharide capsule of *Cryptococcus* species. India ink staining was primarily performed on CSF samples. A drop of CSF was mixed

with a drop of India ink on a glass slide, covered with a coverslip, and examined under high power. Cryptococcus appeared as round budding yeast cells surrounded by a clear halo against a dark background, representing the capsule.

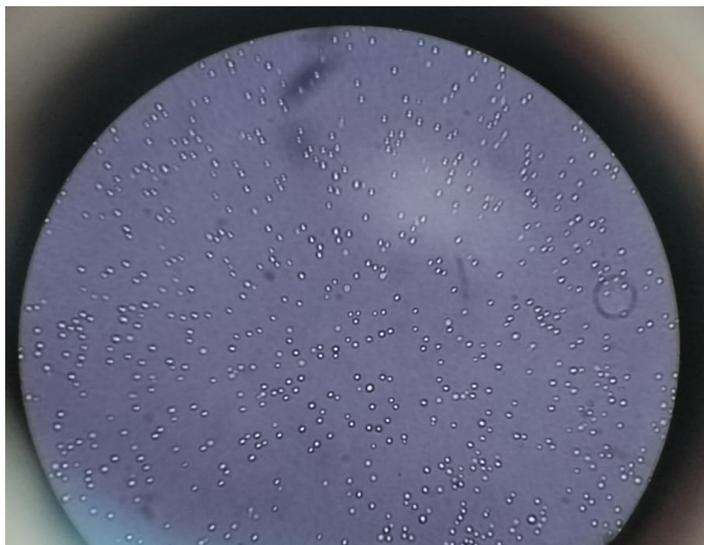


Fig. 1 India Ink stain (40x) shows round to oval budding yeast cells surrounded by a wide, clear, unstained halo, representing the thick polysaccharide capsule of Cryptococcus.

Gram staining: Gram staining is a differential staining method used to classify organisms based on their cell wall properties. Direct Gram stain was performed on all specimens except blood, and examined under oil immersion objectives(100x) visible as Gram-positive, round to oval, budding yeast cells, sometimes with a faintly visible capsule. It provides supportive evidence of yeast cells.

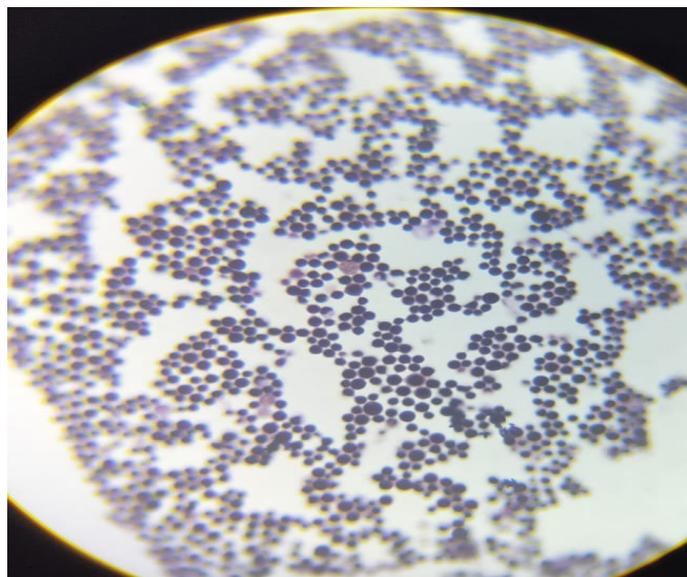


Fig. 2 Gram Staining shows Gram-positive, round to oval, budding yeast cells, clear halo(100x) suggestive of Cryptococcus.

Culture and isolation: All specimens were cultured on Sabouraud Dextrose Agar (SDA) with chloramphenicol to inhibit bacterial growth. SDA is a selective fungal culture medium with acidic pH, commonly used for isolation of yeasts and moulds, two sets of Sabouraud Dextrose Agar are inoculated and incubated at 25°C and 37°C, separately for 7days, on eighth day colony characteristics on SDA appears smooth, moist, round, convex, glistening, creamy to white in colour, mucoid in consistency due to thick polysaccharide capsule.



Fig. 3 Culture on Sabouraud Dextrose Agar

Urease test: *Cryptococcus* species are characteristically urease positive, urease test serves as an important phenotypic tool in their laboratory identification. The test is commonly performed using Christensen's urea agar, where *Cryptococcus* hydrolyses urea through the enzyme urease, producing ammonia and carbon dioxide. The release of ammonia increases the pH of the medium, resulting in a colour change of the phenol red indicator from yellow to pink. Most clinical isolates of *Cryptococcus neoformans* demonstrate a rapid positive reaction within 24–48 hours at 35–37 °C. Urease positivity helps in differentiating *Cryptococcus* from other yeast-like fungi, particularly *Candida* species, which are typically urease negative. Thus, the urease test remains a simple, rapid, and reliable adjunctive method for the presumptive identification of *Cryptococcus*.

Identification criteria: Identification of *Cryptococcus* species was based on a combination of direct microscopic examination, staining characteristics, culture morphology, and biochemical testing. Direct microscopy of the clinical specimen using KOH mount revealed round to oval yeast cells, and India ink preparation demonstrated characteristic encapsulated budding yeast cells showing a clear halo against a dark background. On Gram staining, the organism appeared as gram-positive, spherical to oval budding yeast cells. Culture on Sabouraud dextrose agar showed smooth, creamy, mucoid colonies, suggestive of capsulated yeast. Further confirmation was obtained by a positive urease test on Christensen's urea agar, indicated by a colour change of the medium to pink, supporting the identification of *Cryptococcus* species.

RESULT

A total of 500 clinical samples processed during the study period, 37 samples (7.6%) were positive for *Cryptococcus* species by one or more microbiological methods. Among the 37 positive cases, the majority were from cerebrospinal fluid (CSF) (71.0%), followed by bronchoalveolar lavage (BAL) fluid and blood samples.

Microscopic examination: KOH wet mount was positive in 37/37 cases (100%), showing round to oval budding yeast cells suggestive of fungal infection.

India ink preparation: Demonstrated encapsulated yeast cells with a clear halo in 35/37 cases (94.6%), predominantly in CSF samples.

Gram staining: Revealed Gram-positive, round to oval budding yeast cells in 32/37 cases (86.5%), serving as a supportive diagnostic finding.

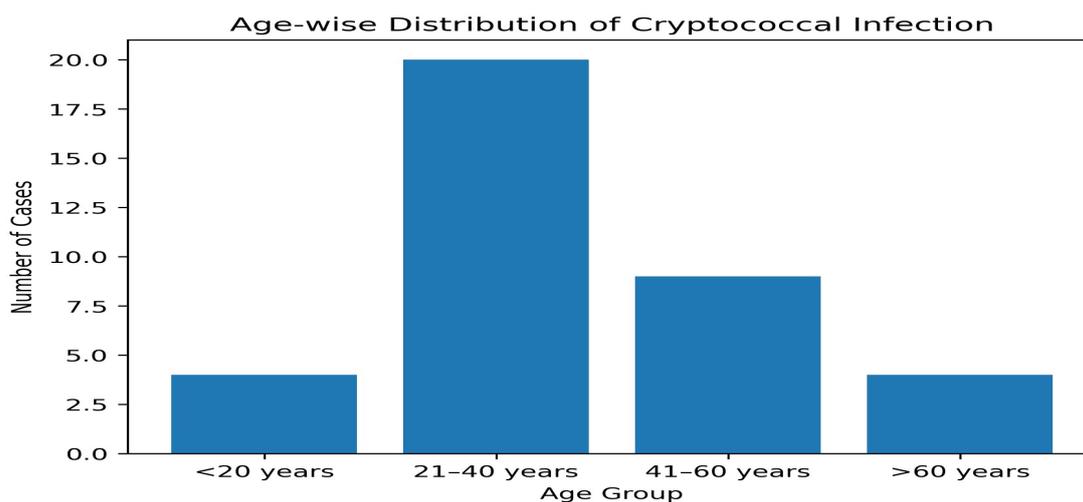
Culture results: All samples positive on direct microscopy were subjected to culture on Sabouraud Dextrose Agar (SDA) yielded growth in 34/37 cases (91.9%), colonies appeared smooth, creamy to mucoid, and white to cream coloured, characteristic of *Cryptococcus* species. On further identification, *Cryptococcus neoformans* was the predominant species, accounting for 86.8% of culture-positive isolates.

Urease test: Urease positivity of *Cryptococcus* helps in differentiating it from other yeasts such as *Candida* species, which are typically urease negative.

Demographic and clinical characteristics: The mean age of patients with cryptococcal infection was 36 years, with a male predominance (Male:Female = 2.3:1).

Age group	No. of cases (%)
<20 years	4 (10.8%)
21–40 years	20 (54.1%)
41–60 years	9 (24.3%)
>60 years	4 (10.8%)

Maximum cases (54.1%) were observed in the 21–40 year age group.

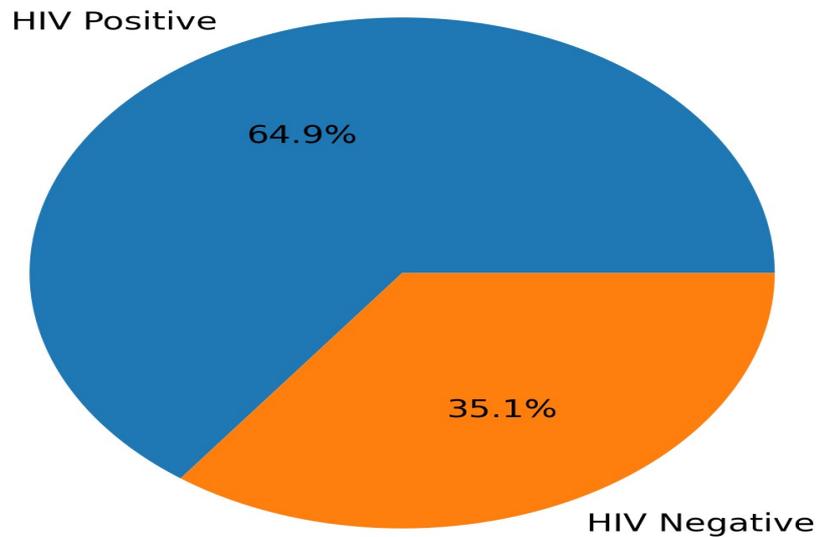


HIV infection (65.7%) was the most common predisposing factor, followed by diabetes mellitus (15.7%).

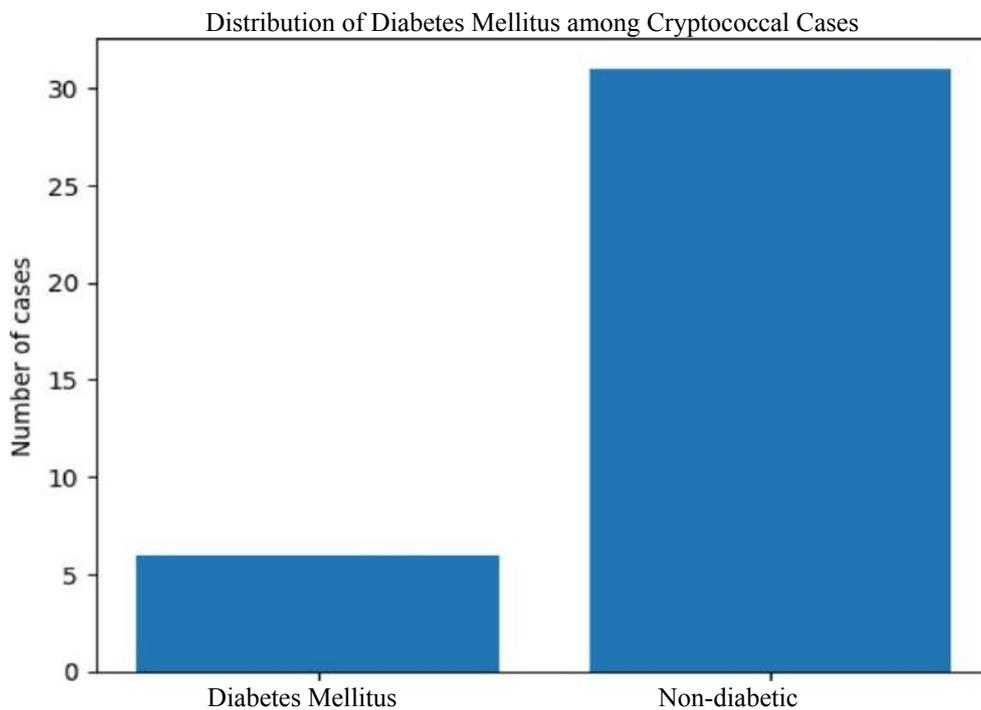
HIV status	No. of cases (%)
HIV positive	24 (65.7%)
HIV negative	13 (34.3%)

Nearly two-thirds of cryptococcal infections occurred in HIV-positive patients*, underscoring the opportunistic nature of the disease.

HIV Status among Cryptococcal Positive Cases



Out of the 37 confirmed cryptococcal cases, 6 patients (15.7%) had diabetes mellitus, while 31 patients (84.3%) were non-diabetic. Diabetes mellitus was the second most common predisposing factor after HIV infection.



Common presenting symptoms included headache, fever, nausea, vomiting, neck stiffness, altered mental status, photophobia, cranial nerve palsies, and raised intracranial pressure, particularly among patients with cryptococcal meningitis.

Diagnostic yield of tests: Direct microscopic examination showed a high diagnostic yield. KOH wet mount was positive in all 37 cases (100%), demonstrating round to oval budding yeast cells. India ink preparation revealed encapsulated yeast cells in 35 cases (94.6%), predominantly in CSF samples. Gram staining demonstrated Gram-positive budding yeast cells in 32 cases (86.5%), serving as a supportive diagnostic method. Fungal culture on Sabouraud Dextrose Agar was positive in 34 out of 37 cases (91.9%), with colonies appearing smooth, creamy to mucoid, and white to cream in colour. *Cryptococcus neoformans* was identified as the predominant species, accounting for *86.8% of culture-positive isolates.

Overall, India ink preparation and culture on SDA were found to be reliable diagnostic methods, with culture remaining the gold standard for confirmation.

DISCUSSION

Cryptococcosis continues to be a significant opportunistic infection, particularly among immunocompromised individuals. In the present study, a positivity rate of approximately 7.6% was observed among clinically suspected cases, with cerebrospinal fluid being the most common sample type. This finding underscores the predominance of cryptococcal meningitis as the major clinical manifestation of the disease.

A clear male predominance and a higher incidence in young to middle-aged adults were noted, HIV infection emerged as the most common predisposing factor, followed by diabetes mellitus, highlighting the role of impaired cell-mediated immunity in disease pathogenesis.

Cryptococcus neoformans was identified as the predominant species, in agreement with most hospital-based studies from India. India ink microscopy, though rapid and inexpensive, has variable sensitivity, especially in cases with low fungal burden. Culture on Sabouraud Dextrose Agar remains the gold standard for definitive diagnosis, despite the longer turnaround time. The continued reliance on conventional diagnostic methods in resource-limited settings emphasizes the need for strengthening basic mycological facilities.

Delayed diagnosis and treatment contribute significantly to the high mortality associated with cryptococcal meningitis. Therefore, heightened clinical suspicion and routine screening of high-risk patients are essential for early detection and improved outcomes.^{2,11}

CONCLUSION

Cryptococcal infection remains an important cause of chronic meningitis in immunocompromised patients in Eastern Uttar Pradesh. Early diagnosis using simple, cost-effective laboratory methods such as India ink preparation and fungal culture is crucial for timely initiation of antifungal therapy. Screening of high-risk groups, including HIV-positive individuals, patients with diabetes, prolonged corticosteroid therapy, malignancy, solid organ transplantation, and other causes of immunosuppression should be emphasized to reduce morbidity and mortality associated with this potentially fatal infection.

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