

## Prevalence of Central Nervous System Cryptococcosis in Human Immunodeficiency Virus Reactive Hospitalised Patients

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Received: 26-11-2022 / Revised: 30-12-2022 / Accepted: 10-01-2023

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Conflict of interest: Nil

### Abstract

Cryptococcosis is a systemic mycosis caused by two environmental species, *Cryptococcus neoformans* and *Cryptococcus gattii*. *Cryptococcus neoformans* is distributed worldwide and is primarily an opportunistic fungal pathogen, most commonly associated with HIV infection, meningoencephalitis being the most common presentation. Early diagnosis is essential to prevent the complications of Cryptococcosis as it is associated with high mortality and morbidity.

**Materials & Methods:** We did a comparative study on detection of Cryptococcal infection by conventional method and latex agglutination. A total of 71 CSF samples from HIV seropositive cases, with clinical suspicion of Cryptococcal meningitis, were processed by conventional methods - Gram staining, India ink preparation, culture on Sabouraud's Dextrose agar. Antigen detection was done on all CSF samples using latex agglutination test.

**Conclusion:** The prevalence of Cryptococcal infection was found to be 7.04%. Latex agglutination was more sensitive (100%) than conventional methods (80%).

**Keywords:** *Cryptococcus gattii*, *Cryptococcus neoformans*, Sabouraud's Dextrose agar

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### Introduction

Cryptococcosis, is an infectious disease caused by pathogenic encapsulated yeast, in the genus *Cryptococcus*. Both *Cryptococcus neoformans*, which favor immunosuppressed host, and *Cryptococcus gattii*, the cause of recent outbreak in immunocompetent hosts, produce clinical presentations ranging from asymptomatic pulmonary infection to disseminated disease involving Central nervous system. *Cryptococcus neoformans* has special predilection for lung and Central nervous system. Affinity to Central nervous system is possibly related to the consumption

of catecholamines and meningoencephalitis is the most common clinical presentation.

Cryptococcosis, caused by the encapsulated yeasts *Cryptococcus neoformans* and *Cryptococcus gattii*, is most frequently recognized as a disease of Central nervous system, although primary site of infection is the lungs. There are four species of *Cryptococcus* (A-D), namely *C. neoformans* (A and D) and *C. gattii* (b and C) (greenwood). *Cryptococcus neoformans* occurs worldwide in nature and is isolated readily from dry pigeon feces, as well as trees, soil, and other

sites. *Cryptococcus gattii* is less common and typically associated with trees in tropical areas. *Cryptococcus* is a well-recognized opportunistic infection among those with deficits in cell mediated immunity, such as HIV patients and other immunocompromised populations including patients with organ transplantation and rheumatologic conditions requiring immunosuppressive therapy.

In HIV patients, it is classified as AIDS defining condition. Despite the reduction in the number of cases among HIV patients since the advent of HAART in the developed world, the incidence is raising in many developing countries afflicted with large epidemics of HIV infection. Approximately one million cases of Cryptococcosis occur worldwide each year, with the largest burden in Africa.

*Cryptococcus* is a basidiomycetous yeast that survives environmentally in the sexual form, producing hyphae with terminal basidiospores. These basidiospores may break off and become aerosolized, 1-3 microns in size, are small enough to deposit in the alveoli. Capsule synthesis is a major virulence determinant, providing a mechanism of adherence

To mucosal lining and protection from phagocytosis, both during transit through the bloodstream and at the sites of infection. Further phenol oxidase enzyme uses catecholamines as substrate to produce melanin, which accumulates in the cell wall. It is the use of catecholamines that provide a predilection for involvement of the central nervous system.

A mild self-limiting pulmonary infection is believed to be the most common form of Cryptococcosis. In symptomatic pulmonary infection there is no clear diagnostic features. Lesions may take the form of small discrete nodules, which may heal with a residual scar or may become enlarged, encapsulated and chronic (cryptococcoma form). An acute

pneumonic type of disease has also been described.

*Cryptococcus* typically causes opportunistic disease in patients with deficits in cell mediated immunity. Although incidence and mortality have decreased in the era of HAART, cryptococcal meningitis remains an important cause of morbidity and mortality in the AIDS population, especially in the developing world.

The meningeal form of cryptococcosis can occur apparently in healthy individuals but occurs most frequently in immunocompromised persons. Chronic meningitis or meningoencephalitis develop insidiously with headaches and low-grade fever, followed by changes in mental state, visual disturbances and eventually coma. The disease may last from a few months to several years, but the outcome is always fatal unless it is treated.

## Materials & Methods

The study was done in the Department of Microbiology, Viswabharathi Medical college, Kurnool, from June 2021 to May 2022. A total of 71 HIV seropositive cases with clinical suspicion of Cryptococcal meningitis were included in the study. Cerebral spinal fluid (CSF) samples from the patients were processed. Cerebral Spinal fluid was centrifuged before microscopic examination and culture.

For direct microscopy, the deposit of the specimen was examined in wet mounts, both directly and after mixing with India ink, which delineated the capsule and the remaining deposit was inoculated on to two sets of Sabouraud's dextrose agar, one incubated at 25°C -30°C in biological oxygen demand incubator and the other at 37°C for three weeks. The colony morphology was noted, *Cryptococcus neoformans* was identified based on yeast like mucoid colonies on Sabouraud's dextrose agar.

Subculture of colony from Sabouraud's dextrose agar was inoculated on Christensen's urease agar for demonstration of urease production and on Niger seed agar, incubated at 37, for development of brown colored colonies due to melanin production.

The supernatant fluid after centrifugation was used for capsular antigen detection by latex agglutination (Biorad, Pastorex Crypto plus, Germany) CD4 cell count was done in all HIV seropositive patients with signs of Cryptococcal meningitis using CD4 flow cytometry (Partec, CD4 easy count kit). Biochemical parameters like proteins, sugar, chlorides and cell counts in cerebrospinal fluid were assessed.

**Ethical Approval:** Obtained from the Institutional Ethics Committee (IEC)

## Results

Out of 71 CSF samples, 5 samples were positive for Cryptococcus infection with prevalence rate being 7.04%. All the 5/5 samples were tested positive by latex agglutination (100%) with 100% sensitivity, 4/5 samples yielded growth on Sabouraud's dextrose agar (80%) and 3/5 samples were detected by India ink preparation (60%).

The infection was seen to be more in males (4/5) as compared to females (1/5).

More number of cases were seen in the age group between 35-44 years.

Fever and headache were the most common clinical manifestations, followed by vomiting, altered sensorium, neck stiffness and convulsions.

CD4 count was in the range of 83-165 cells/ $\mu$ l in all the positive cases.

**Table 1: Prevalence of Cryptococcal meningitis in HIV seropositive cases N=71**

S. N.	Group	No. of cases	%
1.	Positive for Cryptococcal meningitis	5	7.04%
2.	Negative for Cryptococcal meningitis	67	92.95%

**Table 2: Distribution of HIV seropositive cases with cryptococcal meningitis diagnosed by different methods**

S. N.	Methods	No.	%
1.	Negative staining by India Ink	3	60%
2.	Growth on Sabouraud's dextrose agar	4	80%
3.	Antigen detection by latex agglutination	5	100%

**Table 3: Distribution of HIV seropositive with Cryptococcal meningitis cases according to sex**

S. N.	Gender	No. of cases	%
1.	Males	4	80
2.	Females	1	20

**Table 4: Distribution of HIV seropositive with Cryptococcal meningitis cases according to age**

S. N.	Age group	No. of cases	%
1.	15-24 years	1	20
2.	25-35 years	1	20
3.	35-44 years	2	40
4.	45-55 years and above	1	20

## Discussion

This prospective study was conducted in the Department of Microbiology, Viswabharati Medical College, Kurnool, during the year period June 2021 to May 2022. In this study prevalence rate of Cryptococcal infection among HIV seropositive patients was (5/71) 7.04%.

The present study reported higher number of males (4/5) 80%, than females 1/5(20%) with cryptococcal infection. This correlated with the other studies from India. The reason for this could be increased exposure of males to high risks groups or under reporting of females. Our study showed higher prevalence of Cryptococcal infection in the age group of 35-44 years (2/5)40%.

In the present study fever and headache were the most common clinical presentations, followed by altered sensorium, neck stiffness and convulsions respectively.

Antigen detection by latex agglutination was found to be more sensitive (100%) than growth on Sabouraud's dextrose agar (80%) and negative staining by 10% nigrosine (60%).

In the present study, CD4 cell count in HIV reactive with Cryptococcal meningitis was <200 cells/ $\mu$ l. Hence, screening of HIV seropositive patients with low CD4 count is an useful indicator for early diagnosis and treatment of Cryptococcal infection. Biochemical parameters in HIV seropositive with Cryptococcal infection were non-specific, with elevated proteins and chlorides and low sugar.

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