

# Central Nervous System Cryptococcosis among a Cohort of HIV Infected Patients from a University Hospital of North India

CHAITANYA NIGAM, RUPAM GAHLOT, VIKAS KUMAR, JAYA CHAKRAVARTY, RAGINI TILAK

## ABSTRACT

**Background:** *Cryptococcus neoformans* is a ubiquitous encapsulated yeast that causes significant infections which range from asymptomatic pulmonary colonization to the life threatening meningoencephalitis, especially in immunocompromised individuals. Cryptococcal meningitis is one of the AIDS-defining illnesses. Recent data have indicated that, the incidence of the cryptococcal infection is high in developing countries like India. We conducted this study to find out the incidence of cryptococcosis in this area.

**Material and Methods:** The Cerebrospinal Fluid (CSF) specimens were collected from known HIV positive cases that had a clinical diagnosis of meningitis and they were processed by standard

microbiological procedures. The cryptococcal isolates were identified by microscopy, their cultural characteristics, sugar assimilation and by the hydrolysis of urea.

**Results:** The incidence of cryptococcal meningitis was 12.9%. All the strains were susceptible to amphotericin B, fluconazole, itraconazole and voriconazole.

**Conclusion:** The cryptococcal infection should be suspected in all cases of meningitis, especially among HIV infected persons. An early diagnosis and treatment may alter the prognosis of these patients and hence, an examination of the CSF for cryptococcosis should be considered in all the HIV infected persons who have the symptoms of meningitis.

**Key Words:** Cryptococcal meningitis, HIV, Latex agglutination, India ink

## INTRODUCTION

Cryptococcal meningitis is an opportunistic infection which occurs in immunocompromised patients. It is caused by *Cryptococcus neoformans*, an encapsulated yeast, which represents the most common cause of the fungal infections of the central nervous system among these patients [1-3]. The incidence of cryptococcal meningitis has increased in the recent years, both in the HIV-positive and negative patients [4]. Cryptococcal meningitis may be the presenting manifestation of AIDS. The most common sites of occurrence of this infection are the central nervous system and the lungs [5-7]. The *C. neoformans* infection occurs after the inhalation of this organism through the respiratory tract. This organism disseminates hematogenously and it has a propensity to localize in the central nervous system, causing meningitis/meningoencephalitis [8,9]. Although the incidence of cryptococcal meningitis has declined in the HIV patients who undergo anti-retroviral therapy, cryptococcal disease remains a leading cause of mortality in the developing world [10]. The clinical signs and symptoms of *C. neoformans* meningitis are indistinguishable from those of many other causes of meningitis, especially tubercular meningitis. The early cases with no symptoms which are referable to the Central Nervous System (CNS) may have a positive culture of the CSF, with no other abnormality in the fluid [11]. An early diagnosis of the cryptococcal infection is therefore necessary for its appropriate management. The present work is a prospective study of suspected cases of cryptococcal meningitis among HIV infected patients during a period of 1 year.

## MATERIAL AND METHODS

All the CSF samples from HIV positive, clinically suspected meningitis patients, which were received for microbiological analysis, constituted the study material during March 2009 to February 2010. The CSF was collected after taking an informed written consent from the patients. The details of the demographic profile of the patients (viz. name, age, sex, address, date of admission and date of discharge/death) were recorded. After the preliminary microscopic examination (India ink staining and gram staining) and antigen detection (Remel Crypto-LA test kit) [Table/Fig-1], all the samples of CSF were processed for routine microbiological culture on Sabouraud's dextrose agar, which was incubated at 25°C in a BOD incubator and processed according to a predetermined protocol which was based on the presence of any visible growth. The fungal cultures were followed for 4 weeks. The colonies of *C. neoformans* were identified, based on their yeast like colony morphology, the presence of spherical yeast cells without hyphae/pseudohyphae on microscopy, a negative germ tube test, a positive urease test [Table/Fig-2] and the assimilation of sugars viz. inositol, lactose, cellobiose, melibiose and trehalose. The culture isolates were sent to SGPGI, Lucknow for biotyping. The anti-fungal susceptibility was determined by the disk diffusion method [12] [Table/Fig-3].

## RESULTS

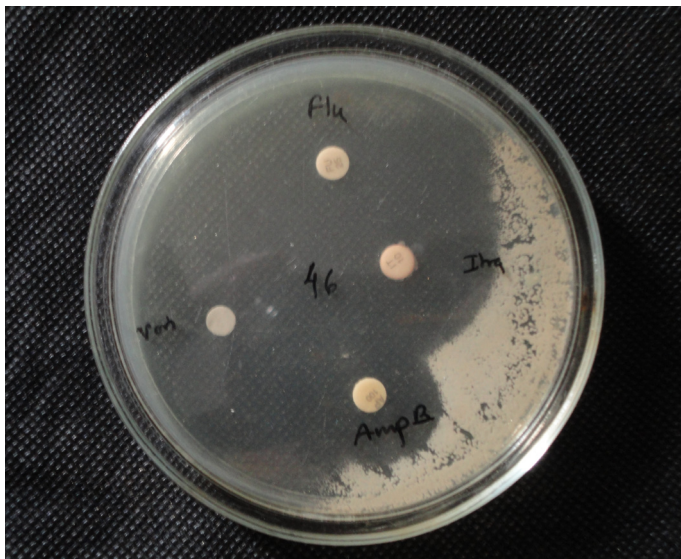
A total of 132 consecutive, non-repetitive CSF samples from HIV infected patients with a clinical diagnosis of chronic meningitis



**[Table/Fig-1]:** Crypto LA test: well 4 -showing positive agglutination whereas wells 1,2,3,5,6 showing negative test results



**[Table/Fig-2]:** Urease test: pink-positive and yellow-negative



**[Table/Fig-3]:** Antifungal susceptibility test, strain is susceptible to all antifungals used, showing zone of clearing around discs

were screened during a period of 1 year. Fourteen (10.6%) were found to be positive by the India ink examination, 20 (15.1%) were found to be positive by antigen detection and 17 (12.9%) of them were found to be culture positive for *Cryptococcus*, which were further identified by one or more confirmatory tests [Table/Fig-4]. The ages of the patients who were positive for cryptococcal meningitis ranged from 26 to 55 years with a mean age of 40.35

years; the male: female ratio was 16:1. In our study, the incidence of cryptococcal meningitis was 12.9%, among which 94.11% (16/17) were *C.neoformans* var. *neoformans* and 5.88% (1/17) were *C.neoformans* var. *gattii*. All the strains were susceptible to Amphotericin B, fluconazole, Itraconazole and Voriconazole. The mortality rate was 15% and the average hospital stay was 21 days.

### DISCUSSION

The incidence of cryptococcal meningitis has increased after the pandemic of AIDS and it varies from place to place [13]. The incidence rate in our study was 12.9%, which was comparable to the findings of other north Indian studies [14] but it differed from the reports from south India [13,15,16] and other parts of the world [17,18]. The age range of the patients in our study population was very diverse (19-60 years) and there was a male predominance. The mean age of the patients who were positive for cryptococcal meningitis was 38.86 (40.35years), with a range of 19-60 (26-55 years). Most of the cases belonged to the age group of 30-60years [13,17,15] [Table/Fig-5]. There is a difference in the incidence and the prevalence of cryptococcal meningitis in males and females in different countries and in different regions in the same country. In our study, the ratio of its incidence among males and females was 16:1 and the reason may have been different exposures as has been explained in other studies rather than different susceptibilities [15], which was comparable to the findings of other studies [12,19,20]. The sensitivity of the Crypto-LA test was more than 100%, which detected 20 (15%) cases, but three of these cases did not show any growth on culture. Only 14 (10.6%) cases were India ink positive and the sensitivity was 82.35%, which was similar to the findings of other studies [13,17] and all of them were culture positive too [Table/Fig-4]. Among all the culture positive cases, 16 (94.11%) were *Cryptococcus neoformans* var. *neoformans* and only 1 (5.88%) was *Cryptococcus neoformans* var. *gattii*. The isolation of *C. gattii* reflects the changing pattern of cryptococcosis

	India ink	Culture	Crypto LA
Positive	14	17	20
Negative	118	115	112
Total	132	132	132

**[Table/Fig-4]:** Comparison of different tests for diagnosis of Cryptococcal meningitis

Age	Sex	Culture		Total
		Positive	Negative	
<30years	Male	2	25	27
	Female	0	5	5
30-60 years	Male	13	68	81
	Female	2	13	15
>60 years	Male	0	3	3
	Female	0	1	1
Total		17	115	132

**[Table/Fig-5]:** Distribution of study population

Crypto LA			
Culture	Negative	Positive	Total
Negative	112	3	115
Positive	0	17	17
Total	112	20	132

**[Table/Fig-6]:** Comparison of Culture and Crypto LA



[21, 22]. The average hospital stay was about 3 weeks (21 days) as they could be susceptible to various nosocomial infections during this period and this much abstinence from work was really cumbersome for the poor people. The case fatality rate in this study was 17.6%, which differed from the findings of the studies which were conducted by P Mwaba *et al.*, and Shaikh Mohammed *et al.*, which showed 100% and 32% fatality rates respectively [23,20]. All the culture positive strains were tested for their anti-fungal susceptibilities. All the strains were equally susceptible to all the four anti-fungal drugs (Amphotericin B, Fluconazole, Itraconazole and Voriconazole) which were used to do their in vitro antifungal susceptibility testing by the disk diffusion method [Table/Fig-6].

## CONCLUSION

The CSF examination (gram staining, India ink staining) employs simple and easy techniques and this can be performed in any clinical microbiology laboratory. The incorporation of antigen detection tests provides additional benefit due to their high sensitivities. Repeated attempts to demonstrate yeast cells in microscopy and culture on lumbar puncture specimens both pre and post treatment are an important requirement in the diagnosis and the detection of their resistance. We believe that awareness among clinicians and microbiologists will go a long way in arriving at a definite clinical and aetiological diagnosis of cryptococcosis, which would have otherwise been missed or the patients would have undergone treatment for tuberculosis.

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