

Prevalence of cryptococcal meningitis among people living with human immunodeficiency virus/acquired immunodeficiency syndrome in a Tertiary Care Hospital, Southern Odisha, India

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Abstract

Objective: Cryptococcal meningitis (CM) caused by encapsulated opportunistic yeast *Cryptococcus neoformans* is an important contributor to morbidity and mortality in people living with human immunodeficiency virus/acquired immunodeficiency syndrome (PLHAs). Early diagnosis of such patients is the key to their therapeutic success. A retrospective study was conducted to evaluate the clinical features, laboratory findings, and prevalence of CM among hospitalized PLHAs in a tertiary care setting. **Materials and Methods:** A total of 112 clinically diagnosed CM patients were subjected to cerebrospinal fluid analysis and tests for human immunodeficiency virus antibodies by the standard laboratory operating procedures. **Results:** Out of 112, 16 showed a definite diagnosis of *C. neoformans* with the prevalence of 14.3%. Males in the age group of 21-40 years were most commonly affected than females. The clinical manifestations observed were fever and headache (100%), followed by altered sensorium (93.7%), neck stiffness (75%), and vomiting (62.5%). Overall, Cluster of differentiation 4 (CD4) T-lymphocytes count was <100 cells/ μ l except 1 case in which the CD4 T-lymphocytes count was 137 cells/ μ l. No concomitant cryptococcal and tubercular meningitis case was detected. All 16 patients responded initially to induction therapy of IV amphotericin B 1 mg/kg and fluconazole 800 mg daily for 2 weeks. Subsequently, 4 (25%) patients were lost for follow-up and 2 (12.5%) patients expired during their hospital stay. **Conclusion:** As the clinical and radiological pictures of CM are often non-pointing, routine mycological evaluation is necessary for early definite diagnosis and subsequent initiation of appropriate therapy as the majority of patients respond well to treatment if started early.

Key words: CD4 T-lymphocytes, cryptococcal meningitis, *Cryptococcus neoformans* people living with human immunodeficiency virus/acquired immunodeficiency syndrome

INTRODUCTION

Cryptococcal meningitis (CM) caused by *Cryptococcus neoformans* is an opportunistic fungal infection in human immunodeficiency virus (HIV)-seropositive patients.^[1]

This encapsulated yeast is found in soil contaminated with bird droppings particularly from pigeons and chickens, usually inhaled through lungs and remain dormant for many years. Reactivation, which occurs primarily among immunosuppressed individual such as people living with human immunodeficiency virus/acquired immunodeficiency syndrome (PLHAs), leads to infection and most of which is meningitis.^[2] CM is a significant cause of morbidity and mortality among PLHAs world-wide.^[3-6] *Cryptococcus* infect an estimated 1 million people and results in approximately 625,000 deaths annually.^[7] It is the most common central nervous system (CNS) fungal pathogen in PLHAs.^[8-10]

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The clinical signs and symptoms of CM are indistinguishable from those of many other causes of meningitis.^[11] This infection is fatal without treatment. Therefore, rapid recognition, diagnosis, and treatment are required to decrease the mortality. Recent data indicate that the incidence of *C. neoformans* is high among PLHAs in developing countries like India.^[12,13] A retrospective study was carried out in a Tertiary Care Hospital, Southern Odisha, India to evaluate the clinical features, laboratory findings, and prevalence of CM among PLHAs.

MATERIALS AND METHODS

The study was conducted in the Department of Microbiology, a Tertiary Care Hospital of South Odisha, India from January 2010 to June 2012. A total of 112 HIV-seropositive patients, clinically diagnosed as CM were included. A retrospective cross-sectional study of the medical records of these patients and their clinical data were evaluated.

The cerebrospinal fluid (CSF) samples of 112 cases were processed for fungal culture after preliminary screening by microscopic examination, comprising of wet mount, Gram's staining and negative staining with 10% Nigrosin. All the samples were inoculated on two sets of Sabouraud's dextrose agar (SDA) without cycloheximide, one incubated at 37°C, another at 24°C, in special biological oxygen demand incubator. The colony morphology was noted. *C. neoformans* was identified base on yeast like mucoid colony on SDA and urease test. For a definite diagnosis, colony from SDA was sub-cultured on Niger seed agar,

which was incubated at 37°C and observed for appearance of brownish to blackish colored colonies due to melanin pigment production by *C. neoformans*.^[14]

Written consent was sought before HIV testing was carried out. All the serum samples were tested for HIV antibodies by three rapid tests protocol as per the guidelines laid down by World Health Organization (testing strategy III) and Government of India.^[15] The CD4 T-lymphocytes enumeration was performed by using the BD FACSTM Calibur system (Becton Dickinson, Fluorescent antibody cell sorter, Singapore). All the tests were performed in accordance with the Medical College Institutional Ethical Committee guidelines.

The values of mean, and *P* value were calculated by using the GraphPad statistical software. Statistical significance was defined when *P* < 0.05.

RESULTS

From 112 processed CSF samples, 16 yielded growth of *C. neoformans*, showing prevalence of 14.3% [Table 1]. Out of all laboratory confirmed CM cases, 12 (75%) were male and 4 (25%) were female. Most of the CM positive patients were in the age group of 20-40 years (81.3%) ranging from 22 years to 53 years of age with a mean of 35.1 years [Table 2]. Out of 16 patients, 3 (18.7%) were aware of their HIV status, but subsequently did not come for antiretroviral therapy (ART) counseling and follow-up, 10 (62.5%) were diagnosed only after hospital admission and three (18.7%) were on ART.

The clinical presentations were almost same in most of the patients showing features of meningitis. Most common signs and symptoms being fever and headache (100%), followed by altered sensorium (93.7%), neck stiffness (75%), vomiting (62.5%), and wasting (56.2%) [Figure 1]. The CSF cell counts, glucose, and protein concentrations were non-specific.

Preliminary microscopic examination by negative staining with 10% Nigrosin could identify 13 (81.3%) cases of

Table 1: Year wise distribution of CM in PLHAs

Year	Total clinically suspected CM cases (%)	Total laboratory confirmed CM cases (%)	<i>P</i> value
2010	37	05	<i>P</i> =1.000 (not significant)
2011	48	07	
2012*	27	04	
Total	112 (100)	16 (14.3)	

CM: Cryptococcal meningitis,*Data up to June 2012, *P* ≤ 0.05 (statistically significant), PLHAs: People living with HIV/AIDS

Table 2: Age and sex wise distribution of clinically suspected and laboratory confirmed CM in PLHAs

Age group in years	Clinically suspected CM cases (n=112)			Laboratory confirmed CM cases (n=16)		
	Male	Female	Total (%)	Male	Female	Total (%)
0-10	02	01	03 (2.7)	0	0	0
11-20	03	02	05 (4.5)	0	0	0
21-30	25	11	36 (32.1)	04	01	05 (31.3)
31-40	27	14	41 (36.6)	05	03	08 (50)
41-50	13	06	19 (17)	02	0	02 (12.5)
51-60	04	02	06 (5.3)	01	0	01 (6.2)
≥61	02	0	02 (1.8)	0	0	0
Total	77 (68.8)	35 (31.2)	112 (100)	12 (75)	04 (25)	16 (100)

CM: Cryptococcal meningitis, PLHAs: People living with HIV/AIDS

C. neoformans, whereas both microscopy and fungal culture identified all 16 positive cases. The CD4 T-lymphocytes count of all these cases except 1 case were <100 cells/ μ l [Figure 2].

All laboratories confirmed CM patients were given induction therapy with amphotericin B and fluconazole daily for 14 days during their hospitalization. After clinical improvement, patients were discharged with the advice of taking tablet fluconazole 400 mg daily for 8 weeks followed by fluconazole 200 mg daily for 12 months. Unfortunately, 2 patients were expired during their hospital stay. The mean duration of hospital stay was 10 days (range from 7 days to 16 days).

DISCUSSION

The asexual yeast *C. neoformans* has been classified as four serotypes based on the capsular polysaccharide,

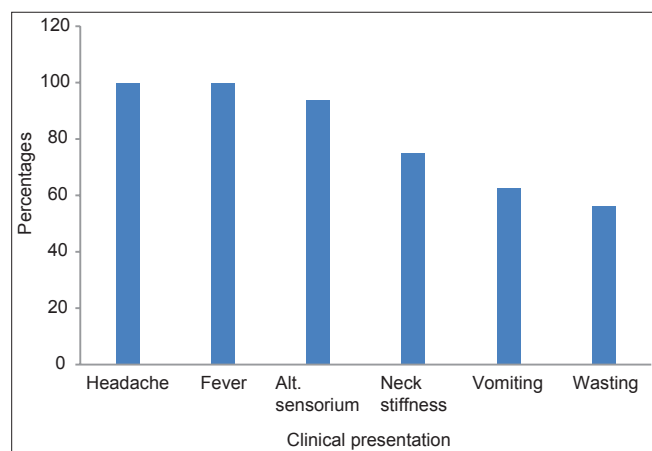


Figure 1: Clinical presentations of laboratory confirmed cases of cryptococcal meningitis in people living with human immunodeficiency virus/acquired immunodeficiency syndrome

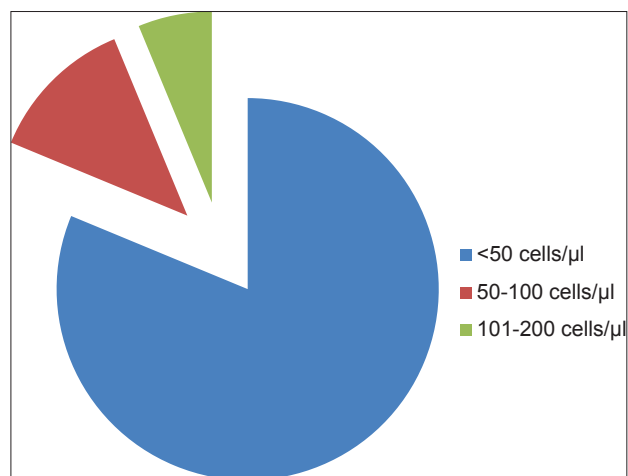


Figure 2: CD4 T-lymphocytes count of laboratory confirmed cryptococcal meningitis cases

glucuronoxylomannan. Capsular types A through D correspond to the variants *C. neoformans var. grubii* (A), *C. neoformans var. gattii* (B and C), and *C. neoformans var. neoformans* (D). Recently, *Cryptococcus gattii* has been classified as a separate species as it has shown to be genetically distinct from *C. neoformans*.^[16] It is a well-recognized opportunistic infection among cell-mediated immunodeficient patients, such as HIV infection, organ transplantation, and rheumatologic conditions requiring immunosuppressive agents. In HIV patients, it is classified as an acquired immunodeficiency syndrome (AIDS)-defining condition.^[17] Characteristics of *Cryptococcus* that permit its survival within the host include a polysaccharide capsule, and phenol oxidase enzyme uses catecholamine as a substrate to produce melanin, which accumulates in the cell wall. It is the use of catecholamine that may produce a predilection for involvement of the CNS.^[18]

Typically, CNS opportunistic infection occurs during severe immune deficiency in advanced HIV infection when CD4 T-lymphocytes count is less than 200 cells/ μ l.^[19] *Cryptococcus* meningitis caused by *C. neoformans* is one of the most common opportunistic CNS infections in PLHAs. Before the introduction of ART, 5-10% of patients with AIDS developed CM. Although the incidence has fallen, this disease remains a major concern in sub-Saharan Africa and south and southeast Asia.^[7] In our study, the prevalence observed was 14.3% (16 out of 112). This value is comparable with the reports prepared by Lakshmi *et al.*, they found out 10.86% (39 out of 359) of suspected CM cases showed a definite diagnosis of *C. neoformans*.^[12] Various studies have been conducted in different parts of the world including India, to find the prevalence of CM in HIV-seropositive patients and has been found to vary widely from 2.79% to 55%.^[13,20-22] This discrepancy probably is due to under reporting and misdiagnosis of cases.^[23]

Three successive studies conducted in AIIMS, New Delhi over a period of 12 years (1992-2004) had revealed that parallel to increase in number of HIV cases; HIV cryptococcosis co-infection increased from 20% in 1992-96 to 30% in 1996-2000 and 49% in 2000-04.^[24] In this present study no such increase in prevalence over 2½ years was observed (*P* is 1.000, not significant). Other studies conducted in India did not find any significant increase in the prevalence of CM over the years.^[12,22]

Our study revealed, male in the age group of 21-40 years were most commonly affected than female, which may reflect a difference of exposure and out-door activity rather than a difference in host susceptibility as it was noted earlier.^[24] The present study did not find any confirmed CM cases in the age group of 0-20 years. Though children are

less commonly affected, now there is an increase in the prevalence of CM observed in HIV-infected children.^[24,25]

The clinical manifestations observed in the laboratory confirmed cases were fever and headache (100%), followed by altered sensorium (93.7%), neck stiffness (75%), vomiting (62.5%), and wasting (56.2%). Baradkar *et al.* noted headache, fever and altered sensorium (100%), neck stiffness (90%) and vomiting was present in 52.6% cases, similar to our study.^[22] Our findings are also comparable with a study conducted by Lakshmi *et al.* in India.^[12] Present study differed from Lee *et al.*, they reported fever (72.7%), headache (54.5%), altered mentality (45.5%), dyspnea (36.4%), general weakness (27.3%), dizziness, insomnia, and vomiting (18.2%) cases in South Korea.^[20] Other presentations such as lethargy, coma, papilledema, focal neurological deficits, and cranial neuropathies were observed by different workers,^[14] no such similar complications were noted in our study.

Negative staining with 10% Nigrosin revealed 87.5% confirmed cases of *C. neoformans*, but the combination of negative staining (10% Nigrosin) and fungal culture had detected all 16 cases of *C. neoformans*, in this present study. Microscopic detection with India ink or 10% Nigrosin and fungal culture of CSF are diagnostic for CM.^[16] This present study showed in 93.7% of laboratory confirmed cases CD4 T-lymphocytes count was <100 cells/ μ l and only in 1 case, the CD4 T-lymphocytes count was 137 cells/ μ l. Lakshmi *et al.* reported in all confirmed CM cases the CD4 T-lymphocytes count was <100/ μ l.^[12] In our study, no case of concomitant cryptococcal and tubercular meningitis was detected, similar to study conducted by Thakur *et al.*^[13] In comparison, Lakshmi *et al.* had reported concomitant meningitis up to 33% of AIDS patients in India.^[12]

All 16 patients in our study responded initially to induction therapy of IV amphotericin B 1 mg/kg and fluconazole 800 mg daily for 2 weeks. During their discharge, patient were advised to take oral fluconazole 400 mg daily for 8 weeks followed by fluconazole 200 mg daily for 12 months and frequent follow-up. Unfortunately, 4 (25%) patients were lost for follow-up and 2 (12.5%) patients expired during their hospital stay. No relapse was observed among recovered patients.

CONCLUSION

CM remains a significant cause of morbidity and mortality particularly among PLHAs in resource poor environments. Present study indicates a high prevalence of CM in PLHAs in a tertiary care hospital setting. As the clinical and radiological pictures of CM are often non-pointing,

routine mycological evaluation is necessary for early definite diagnosis and subsequent initiation of appropriate therapy as the majority of patients respond well to treatment if started early.

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