

Prevalence of Cryptococcal meningitis among Immunocompetent and immunocompromised Individuals in Bellary, South India – a Prospective Study

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ABSTRACT

Background and objectives: Cryptococcal meningitis is now the leading cause of community acquired meningitis. It is generally thought to be associated with AIDS individuals. However, other predisposing factors like leukemia, lymphoma, Diabetes mellitus, connective tissue disorders and organ transplantation also contribute to the infection. Cryptococcal meningitis also occurs in apparently immunocompetent individuals. Opportunistic infections are the major life threatening complications of the acquired immunodeficiency syndrome (AIDS). Early diagnosis can help the clinician to treat cryptococcal meningitis and thus help in reducing the rate of mortality. The present study was taken up to study the occurrence of cryptococcal meningitis among the immunocompromised and immunocompetent individuals and also to analyse the predisposing factors in relation to its occurrence.

Methods: A total of 242 CSF samples of clinically suspected cryptococcal meningitis were screened for *Cryptococcus neoformans* irrespective of their immune status. Identification was

based on direct microscopy, culture and biochemical reactions by conventional methods. CD4 cell count was performed by flow cytometry in all culture positive patients. The overall prevalence of cryptococcal meningitis was 8.3% and the prevalence of cryptococcal meningitis among immunocompromised patients was found to be 16.6%. 60% of the patients were in the age group of 21-40 years. Infection with HIV (100%) was the most common predisposing factor, followed by diabetes mellitus (40%), chronic smoking (20%) and prolonged steroid therapy (5%). *Cryptococcus neoformans* var. *neoformans* was the etiologic agent in all the culture positives in our study. Cryptococcal meningitis was AIDS defining illness in 50% of the patients. The mean CD4 Count was 59.55. 65% of the patients had a CD4 count of <100. High prevalence of cryptococcal CNS infections in HIV infected patients underscores the importance of precise and early microbiological diagnosis. A high index of clinical suspicion and mycological surveillance is required to help in an early diagnosis and appropriate therapy.

Key Words: Cryptococcal meningitis, AIDS, Immunocompromised, *Cryptococcus neoformans*, CNS infections

INTRODUCTION

Cryptococcus neoformans is the second most common lethal fungal opportunist after *Candida albicans*, causing symptomatic cryptococcosis in 6–13% patients with AIDS [1,2]. *C. neoformans*, the causative organism, is ubiquitous in distribution. The vast majority of the isolates responsible for infections are *C. neoformans* var. *neoformans* serotype A [3]. The organism gains access to the host usually via respiratory route and is generally contained there by an intact cell mediated immune system. However, in presence of immunodeficiency, *C. neoformans* disseminates widely especially to the central nervous system (CNS) [4]. It has a special propensity for invading the CNS. Meningitis is the commonest CNS manifestation of cryptococcal infection. [4] It presents as a sub-acute or chronic illness without overt meningeal disease or encephalopathy [5]. Diagnosis is established by CSF analysis demonstrating organism on India ink preparation or culture. Cryptococcal meningitis is an important fatal infection of the CNS where in, the signs and symptoms are indistinguishable from other sub acute and chronic CNS infections. Early diagnosis

can help the clinician to treat cryptococcal meningitis and thus help in reducing the mortality rate. It is generally thought to be associated with AIDS individuals.

The perceived importance of *C. neoformans* as a human pathogen, has led to the species being subjected to intense study during the last decade. The present study was taken up to study the occurrence of cryptococcal meningitis in immunocompromised and immunocompetent individuals in Bellary region of South India. Patient's demographic data, predisposing factors for cryptococcal meningitis, risk factors for acquisition of HIV infection, AIDS defining conditions, other concurrent infections, presenting clinical symptomatology, physical findings, laboratory parameters, cerebrospinal fluid (CSF) examination findings were analyzed in relation to its occurrence.

MATERIALS AND METHODS

This study was carried out in the Department of Microbiology, Vijayanagar Institute of Medical Sciences, Bellary, India from December 2007 – December 2008. The catchment area of patients to this

hospital is predominantly from Bellary district and the neighbouring districts. A total of 242 CSF samples were processed during the study period. Clinically suspected cryptococcal meningitis cases, irrespective of the immune status were included in the study. Meningitis cases due to other etiology were excluded from the study.

All CSF samples were collected before instituting therapy. The samples were considered as potential biohazard and handled with care using universal precautions. Haemogram and CD4 cell count was performed in all patients who were involved in the study. The samples were subjected for cell count and cell type, proteins, sugar, direct microscopy by India ink preparation and Gram's stain and culture for speciation and biotyping.

The CSF samples were inoculated on duplicate sets of Sabouraud's dextrose agar slopes devoid of cycloheximide for culture. They were incubated at 25°C and 37°C, separately over a period of four weeks. Cultures yielding smooth, cream-buff coloured, moist, mucoid colonies at 37°C were taken as positive. The suspected colonies on SDA were confirmed by doing Gram's stain and India ink preparation. Following biochemical tests were performed for speciation. A. Urease test: Urease test was performed using Christensen's urea agar with phenol red indicator [6]. B. Inositol assimilation test: It was performed by using Yeast nitrogen base with bromocresol purple indicator [7]. C. Test for phenol-oxidase: It was performed using Staib's bird seed agar [8].

Biotyping was done using Canavanine Glycine Bromothymol blue (CGB) agar. CGB agar was prepared according to Kwon Chung et al.[9] CD4 cell count was performed by flow cytometry using BD FACScout machine.

Cryptococcal meningitis was diagnosed by clinical features of meningitis/meningoencephalitis along with positive CSF India ink preparation and/ or the isolation of *C. neoformans* in the CSF culture. Patients presenting with clinical features of meningitis/meningoencephalitis of less than four weeks duration were defined as having acute or subacute presentation. The following criteria were used to define common concomitant infections: Pulmonary tuberculosis (PTB): Clinical features suggestive of TB with radiological features compatible with TB on chest radiograph or computed tomographic (CT) scan and/or demonstration of acid-fast bacilli (AFB) in sputum smears or growth of *Mycobacterium tuberculosis* in sputum culture. *Pneumocystis jiroveci* pneumonia (PCP): Bilateral, diffuse interstitial infiltrates on chest radiograph or high-resolution CT, with hypoxemia (PaO₂ <12 kPa) and sputum smears/cultures negative for aerobic bacteria and AFB and/or demonstration of *Pneumocystis jiroveci* in induced sputum.

Descriptive statistical analysis was carried out in the present study. Results on continuous measurements are presented as mean ± SD (min-max) and results on categorical measurements presented as per cent. Significance was assessed at 5% level of significance. Prevalence of Cryptococcal meningitis (culture positivity) was estimated and its prevalence was correlated according to the age, gender, marital status, partner status, CSF analysis and CD4 count analysis by using the single proportion test. Diagnostic statistics viz: sensitivity, specificity, positive predictive value, negative predictive value and accuracy were computed for evaluating the India ink against the culture positivity. The statistical software namely SPSS 15.0, Stata 8.0, MedCalc 9.0.1 and Systat 11.0 were used for the analysis of the data and Microsoft word and Excel were used to generate graphs and tables.

RESULTS

The study population consisted of 242 patients, of which 168 were males and 74 females. Of the 242 patients, 122 patients were immunocompromised and 120 of them were immunocompetent. The demographic profile of the study population is shown in [Table/Fig 1]. Out of 242 patients, *C. neoformans* var. *neoformans* was isolated in 20 patients. Twenty patients diagnosed to have cryptococcal meningitis were analyzed. All of them had compromised immune status. Cryptococcal meningitis was not prevalent in immunocompetent patients. Immunocompromised state is significantly associated with culture positivity [Table/Fig 2]. The overall prevalence of cryptococcal meningitis in the study population was 8.3% and that in the immunocompromised group was 16.6%. The mean age of presentation was 28.6±6 years. Most of the patients (95%) were in the age group of 21-40 years [Table/Fig 4]. None of the patients were above the age of 40 years. Among the culture positives, 55% of the patients were males. Male: female ratio was 1.2:1.

Infection with HIV (100%) was the most common predisposing factor. followed by chronic smoking (50%), diabetes mellitus (40%) and prolonged steroid therapy (5%) [Table/Fig 3]. Cryptococcal meningitis was the initial presenting illness of HIV seropositive status in 50% of the patients. Two (10%) patients had associated pulmonary tuberculosis, two (10%) had oral candidiasis, two (10%) patients had bronchopneumonia (*Pneumocystis jiroveci*) infection and one (5%) patient had herpes zoster infection. An identifiable risk factor for HIV infection was present in 11 (55%) patients. Eleven (55%) patients were from the high risk groups including truck drivers and intravenous (IV) drug abusers. The commonest mode of transmission was multiple heterosexual unprotected sexual contacts, seen in nine patients. One patient each had history of unsafe blood transfusion and intravenous drug abuse.

Headache was the most common presenting symptom. It was present in 85% of the patients. In all the patients who had headache it was of severe intensity and was situated bi frontally. Vomiting was the second most common presenting symptom, and was seen in 80% of the patients. Low grade fever was present in 60 % of the patients. Other presenting symptoms included altered sensorium (40%), seizures (20%) and motor deficits (10%). Most of the patients (75%) had symptoms of less than four weeks duration (acute – subacute); 25% of patients presented with chronic meningitis. The CD4 cell count in our study ranged from 10 to 156 cells/μl. Median CD4 cell count of the whole group was 59.55 cells/μl. Twenty per cent of the patients had CD4 count of less than 50 cells/dl and 65% of the patients had a CD4 count of less than 100 cells/dl.

Culture was taken as gold standard against which India ink preparation was compared. The sensitivity, specificity, positive predictive value, negative predictive value and accuracy of India ink preparation were 90%, 100%, 100%, 99.1% and 99.2% respectively. [Table/Fig 4] CSF India ink was positive in 90 % of the patients. CSF cryptococcal culture was positive in all the 20 patients. All the positive cultures yielded *C. neoformans* var. *neoformans*. Since Cryptococcal meningitis was prevalent only in immunocompromised patients in our study, comparison could not be done between the two groups.

DISCUSSION

The prevalence of cryptococcal meningitis varies world wide, ranging between 6-13% in patients with AIDS [1,2,10]. In the present study, cryptococcal meningitis was seen predominantly in immunocompromised individuals. It was not prevalent in immunocom-

Variables	Total (%)	No.of males (%)	No. of females (%)
Age group			
< 15	02 (2.7)	0 (0)	02 (2.7)
15-20	20 (27.0)	13 (7.7)	07 (9.5)
21-25	35 (47.3)	23 (13.7)	12 (16.2)
26-30	85 (114.9)	57 (33.9)	28 (37.8)
31-35	39 (52.7)	30 (17.9)	09 (12.2)
36-40	47 (63.5)	33 (19.6)	14 (18.9)
41-45	10 (13.5)	09 (5.4)	01 (1.4)
46-50	04 (5.4)	03 (1.8)	01 (1.4)
Marital status			
Married	236 (97.5)	164 (97.6)	72 (97.3)
Unmarried	06 (2.5)	04 (2.4)	02 (2.7)
Partner status			
Positive	57 (33.9)	25 (33.8)	82 (33.9)
Negative	90 (53.6)	34 (45.9)	124 (51.2)
Not known	21 (12.5)	15 (20.3)	36 (14.9)
Immune status			
Immunocompromised	122 (50.4)	91 (54.2)	31 (41.9)
Immunocompetent	120 (49.6)	77 (45.8)	43 (58.1)

[Table/Fig-1]: Demographic Profile Of Study Population:

Immune status	Number of cases	Culture positivity	%	P value
Immunocompetent	122	0	0.0	-
Immunocompromised	120	20	100.0	<0.001**
Total	242	20	8.3	-

[Table/Fig-2]: Immune Status Of The Patients And Culture Positivity

Predisposing factors	Number of patients (n=20)
HIV infn	20 (100%)
Chronic smoking	10 (50%)
Diabetes mellites	08 (40%)
Prolonged steroid therapy	01 (5%)

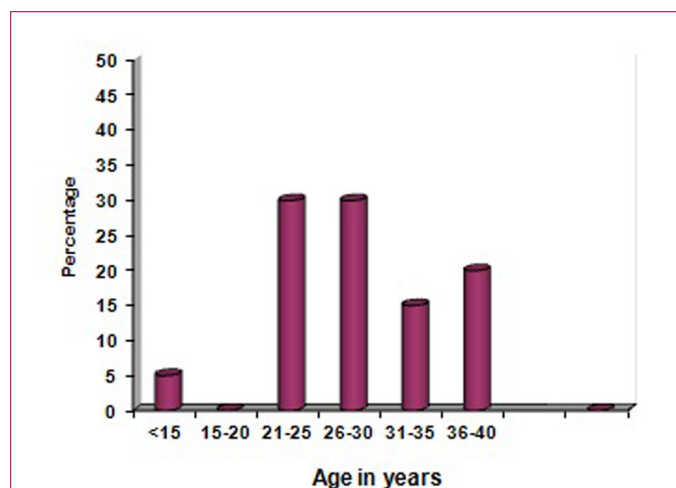
[Table/Fig-3]: Predisposing Factors In Culture Positive Patients

India Ink and culture positivity	Number (n=242)	%
India Ink		
Positive	18	7.4
Negative	224	92.6
Culture		
Positive	20	8.3
Negative	222	91.7

Comparison of India Ink positivity vs Culture positivity

True positive	18	-
False positive	0	-
False Negative	2	-
True negative	222	-
Sensitivity	-	90.0
Specificity	-	100.0
PPV	-	100.0
NPV	-	99.1
Accuracy	-	99.2
Significance	<0.001**	

[Table/Fig-4]: Comparison Of India Ink Positivity Vs Culture Positivity



[Table/Fig-5]: Age Distribution Of Culture Positive Patients

petent patients. Cryptococcal meningitis is generally considered rare in immunocompetent patients and the diagnosis is often delayed because of non-specific symptoms. The overall prevalence of cryptococcal meningitis in the present study was 8.3%, which was similar to the findings of the studies which were done elsewhere in India and abroad [11,12]. Cryptococcal meningitis may affect persons of any age. However, the average age ranges from 30-40 yrs. In the present study, the patients had a mean age of 28.6+6 years, with 95% of the patients being in the age group of 21-40 years, the

most productive age group of the country. This was in consonance with the technical report providing national level statistics published by National AIDS Control Organization (NACO) in the year 2006.

Cryptococcal meningitis is more frequently reported in men than women and rarely from children [13]. This may reflect a difference in exposure rather than a difference in susceptibility. In the present study, the number of males was more as compared to the number of females (1.2:1). This is in accordance with the findings of other studies, which have demonstrated a higher prevalence of cryptococcal meningitis in men [14]. In the present study, while the males belonged to a wider age spectrum, the females were of a considerably younger population, and most of them had acquired infection from their spouses, thus reflecting the male dominance in the Indian society. This emphasizes an increased need for awareness and counseling of both the spouses. Cryptococcal meningitis is generally thought to be associated with AIDS individuals. However, other predisposing factors like leukemia, lymphoma, diabetes mellitus, connective tissue disorders and organ transplantation also contribute to the infection. In the present study, cryptococcal meningitis was prevalent only in immunocompromised patients; infection with HIV being predominant followed by chronic smoking, diabetes mellitus and prolonged steroid treatment. In the present study, among the 20 culture positives, all the 20 patients were found to be HIV seropositive; of which 50% of the patients were found to be HIV seropositive during prior admission for pulmonary tuberculosis, oral candidiasis and herpes simplex infection. Cryptococcal meningitis was the first manifestation of AIDS in 50% of the patients. In other words, cryptococcal meningitis was the AIDS defining illness in 50% of the patients. This finding is almost similar to the study which was done by Chuck et al., [5] wherein cryptococcal meningitis was the AIDS defining illness in 45.28% of the patients. In this study, two patients had associated pulmonary tuberculosis, one patient had oral candidiasis, two patients had bronchopneumonia and one patient had herpes zoster infection. This is in consonance with two large studies on HIV-infected patients which were reported from the Indian subcontinent [15,16].

Triad of headache, fever and vomiting were the predominant symptoms, which were similar to the earlier reports in the literature [17,18]. Headache was the most common presenting symptom. It was present in 85% of the patients. In all the patients who had headache, it was found to be of severe intensity and it was situated bi frontally. Cryptococcal meningitis usually presents in the form of chronic meningitis. In the present study, more than half the patients had acute to subacute presentation. This emphasizes that even with an acute presentation along with the features of meningitis in HIV-infected individuals, the possibility of cryptococcal meningitis must always be considered and patients must be investigated accordingly. The clinical presentation of cryptococcal meningitis in an indolent manner with fever and headache, with absence of signs of meningeal irritation in more than 80% of cases, necessitates a high index of suspicion. The diagnosis of cryptococcal meningitis was relied on CSF culture. Among the 20 culture positives, the India ink preparation showed positive results in 18 patients. In one out of 18 patients, India ink staining showed numerous capsulated budding yeast cells in CSF. In other cases, we had to diligently search all the fields of the smear from the deposit before it could be considered as positive. The CSF India ink preparation was positive in 90% of the patients. The CSF culture was superior to the India ink staining. Considering culture as gold standard, the sensitivity of India ink preparation was 90%. The India ink preparation is a simple procedure and it is used as a screening procedure in cases

of cryptococcal meningitis. The positivity in the present study with respect to India ink preparation was similar to studies done by Imwidthaya et al., [19] Darras-Joly et al., [20] Kovac et al. and Thakur et al [21]. The CSF culture for *C. neoformans* is the diagnostic gold standard test.

A vast majority of the isolates which were responsible for cryptococcal infection were of the *C. neoformans* var. *neoformans* serotype A.3 *Cryptococcus neoformans* var. *neoformans* was found to be the etiologic agent in all the culture positives in our study. It is possible that exposure to *neoformans* variety is more common than exposure to *gattii* variety. It is therefore an epidemiologic problem closely associated with the geographic topography specific to ecological niches of these two varieties. The *neoformans* variety is found in pigeon droppings. The CD4+ T cell count is the best indicator of the immediate state of immunologic competence and also the strongest predictor of the HIV-related complications in these patients. Cryptococcal meningitis was one of the AIDS defining illnesses in patients with the CD4 count less than 100/ μ l. In one study [20], the average CD4 lymphocyte count in patients with AIDS and cryptococcosis was 46 cells/ μ l. Median CD4 cell count in this case series was 59.55 cells/ μ l.

CONCLUSION

In conclusion, the present study demonstrated the prevalence of cryptococcal meningitis only in immunocompromised individuals. It had an acute to sub-acute presentation in a majority of the patients. The frank meningeal signs were less common in the isolated cryptococcal meningitis cases. Infection with HIV continues to be the most important predisposing factor for the development of CNS cryptococcosis followed by chronic smoking, diabetes mellitus and prolonged steroid therapy. The occurrence of meningitis in patients with the HIV infection was most frequently due to opportunistic infections. The biotype which was responsible for cryptococcal meningitis in and around Bellary was *C. neoformans* var. *neoformans*. Cryptococcal meningitis was the AIDS defining illness in 50% of the patients. Cryptococcal infection remains the major opportunistic infection in HIV-infected patients with a CD4 cell count <100 cells/ μ l. A high index of clinical suspicion and mycological surveillance is required to help in an early diagnosis and appropriate therapy.

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