ARTICLE

Clinical and Microbiological Profile of Cryptococcosis in a Tertiary Care Hospital in South India

V. Anil Kumar[1], Rachna Babu[1], Anil Venkatachalam[2]

[1]Department of Microbiology and [2]Neurology, Amrita Institute of Medical Sciences
Ponekara, Kochi, Kerala, India.

Corresponding author: Dr. V. Anil Kumar Email: vanilkumar@aims.amrita.edu

Published: 17 November 2014
Ibnosina J Med BS 2014;6(6):306-312
Received: 21 June 2014
Accepted: 07 July 2014
This article is available from: http://www.ijmbs.org

Abstract

Objectives: We aimed to 1) estimate the prevalence of cryptococcal infections among clinically suspected cases of chronic meningitis in a tertiary care center, 2) evaluate the clinical, radiological, microbiological features and 3) assess outcome of these patients. **Materials and Methods:** Medical records of 756 patients investigated for meningitis and any case which was positive for cryptococcus by culture or antigen detection, from January 2006 to December 2012 were abstracted using a standardized abstraction form. The patients' demographic data, underlying diseases, clinical manifestations, radiological findings, laboratory data, and disease outcomes were retrospectively analyzed. Blood and cerebrospinal fluid specimens were processed by standard microbiological procedures. **Results:** The proportion of probable cryptococcal meningitis was only 2.4% with male preponderance who were predominantly non-HIV patients. No risk factors could be documented in 16.6% (3/18) cases. India ink was found to be positive in 76.9%, CSF culture in 86.6% (13/15), blood culture in 56.25%, and latex agglutination in 100%. Half the cases were confirmed cryptococcal meningitis while 16.6% had only cryptococcal septicemia. No case of pulmonary and cutaneous cryptococcosis were seen. **Conclusion:** We underline the need to rule out cryptococcal infection in non-HIV patients and patients with previous history of tuberculosis, as we have documented a higher mortality in this group.

Key words: *Cryptococcus neoformans*, mortality, cryptococcal antigen detection, tuberculosis.

Introduction

Cryptococcosis is caused by encapsulated yeast *Cryptococcus neoformans*. It is most commonly seen in immunocompromised host and the species involved is *Cryptococcus neoformans* var. *neoformans* (*C. neoformans*). Reports of cryptococcosis in immunocompetent host are

on the rise and are more likely caused by *Cryptococcus neoformans* var. *gatti* (*C. gattii*) (1-4). Clinically and radiologically systemic cryptococcosis can masquerade as tuberculosis. Patients with past history of tuberculosis, cryptococcal meningitis is not considered as the first diagnosis and they get treated as tuberculous meningitis. Even conditions like brain tumor, cerebral stroke and enteric fever can mimic as cryptococcal meningitis (5). Untreated cryptococcal meningitis is always fatal and needs to be differentiated from pyogenic and aseptic meningitis (6). Among all fungi causing meningitis *Cryptococcus neoformans* remains the most common and cryptococcal antigen detection is a highly sensitive, specific and rapid test, and should be used for initial screening in these patients. A retrospective study from Pune showed that only 16.7% of the patients clinically diagnosed with cryptococcal meningitis were culture positive (7). Similarly a studies from Delhi and Lucknow found that among with chronic meningitis only 10% and 16.6% were positive for cryptococcus respectively (5,8). Our study objective was to estimate the prevalence of cryptococcal infections among clinically suspected cases of chronic meningitis in a tertiary care center and to evaluate the clinical, radiological, microbiological features and outcome of these patients. Prevalence of cryptococcosis has been reported as 9 to 27% by previous Indian studies (7). Cryptococcal meningitis is a leading cause of infectious morbidity and mortality in AIDS patients (7). However cryptococcal infections can also occur in immunodeficient hosts such as diabetes, solid cancer, haematological malignancy, solid organ transplant, sarcoidosis, autoimmune hemolytic anemia and on long term steroid therapy (9-11).

Material and Methods

Settings

A retrospective study in a 1300 bedded tertiary care referral hospital, from January 2006 to December 2012. Due approval was obtained from the institutional ethics committee. The medical records of 756 cases investigated for meningitis and any case which was positive for cryptococcus by culture or antigen detection were analyzed retrospectively. Of these only cases that were positive for cryptococcal infection were included in the study. Patient's clinical and epidemiological data, laboratory records were analyzed.

Cultures

Blood and cerebrospinal fluid (CSF) samples were processed by standard microbiological (mycological and bacteriological) techniques for microscopy and culture (12). Fungal cultures were incubated at 30°C while bacterial cultures at 37°C. Cultures were declared negative only after 21 days incubation. *Cryptococcus neoformans* grew as mucoid colonies which were identified by the ID 32C/mini API system (BioMerieux, Inc., St. Louis, Mo). Susceptibility testing for amphotericin B, itraconazole, fluconazole, voriconazole and 5-flucytosine was done using ATB FUNGUS 3/mini API system (BioMerieux, Inc., St. Louis, Mo.)

CSF microscopy and antigen detection

All CSF samples were subjected to India ink examination and Latex agglutination (LA) for cryptococcal polysaccharide antigen using Pastorex CRYPTO Plus (Bio-Rad, France) which uses pronase to eliminate rheumatoid factor, which can produce false positives. CSF was also analyzed for protein, glucose and cell count. Diagnosis of cryptococcosis was made when at least one or more of these tests were positive with compatible clinical features. When more than one site was culture positive diagnosis of disseminated cryptococcosis was made (4).

Results

The clinical, microbiological and radiological characteristics are summarized table 1. Among the investigated 756 cases, 18 (2.4%) cases only were positive for probable cryptococcal meningitis. Of these 18 patients who were reviewed 14 (77.7%) were males and 12 (66.6%) were not HIV positive.

Mycological data

Lesions suggestive of cryptococcoma were seen in the magnetic resonance images of five cases (Figures 1-5). Among the non-HIV patients, 60% either had malignancy or were on steroids for an autoimmune disorder. No risk factors could be documented in 3/18 case (16.6%). India ink exemplified in figure 6 was positive in 10/13(76.9%) of the CSF cultures in 13/15 (86.6%) of the blood cultures. The corresponding LA positivity's in 9/16 (56.3%) of the blood cultures, all 13 results of the CSF cultures. Half the cases had only cryptococcal meningitis while 3/18 cases (16.6%) had cryptococcal sepsis. Disseminated cryptococcosis evident by the culture being positive in both blood and CSF was documented in one third of cases i.e. 6 out of 18 cases (33.3%).

Clinical aspects

The main clinical manifestations were headache (72.2%), fever (55.5%), vomiting (44.4%), altered sensorium

Table 1. Summary of 18 cases of probable cryptococcal meningitis (2006-2016)

N	Age / Sex / Year	Predisposing factors	Radiological signs	Culture: Blood/CSF	Isolate	Symptoms & Signs	Treatment/ Outcome
1	53/F/2006	Wegener's granulomatosis	Normal study.	Pos / Pos	CN	Headache, vomiting	AMB/ Died
2	77/F/2006	NHL, Nodal PTB, IHD	Microangiopathy with diffuse white matter confluent infarcts.	Neg / Neg	CN	Seizures, brain stem stroke	AMB, fluconazole / Died
3	38/F/2007	HIV, Leucopenia	Normal study.	Pos / Pos	CN	Fever, headache, vomiting, weight loss	AMB, fluconazole / Died
4	52/M/2007	Pyogenic eningoencephalitis, lymphoma	ND	ND / Pos	CN	Fever, headache, seizures, altered sensorium.	AMB, fluconazole-/ Died
5	50/M/2008	Old PTB meningitis	Obstructive hydrocephalus, acute infarct of right lentiform nucleus and midbrain.	Pos / Pos	CN	Headache, vomiting	AMB, fluconazole / Alive
6	53/M/2008	HIV, Old PTB	Normal pressure hydrocephalus / extra ventricular obstructive hydrocephalus.	Neg / Neg	CN	Altered sensorium, Loss of weight/ appetite, unsteadiness of gait	AMB, fluconazole / Alive
7	37/M/2008	HIV, TB Lymphadenitis	Infarct of the right internal capsule, right frontal & periventricular region.	Neg / Pos	CN	Loss of weight, papilloedema, Right internal capsule infarct	AMB, fluconazole / Alive
8	48/M/2009	Obstructive hydrocephalus, CLD, Hyponatremia	Diffuse cerebral edema..	Pos / Pos	CN	Fever, headache, Vomiting, unsteadiness of gait, loss of appetite, weight loss	AMB / Died
9	47/M/2009	Sarcoidosis	Normal study	Neg / Pos	CN	fever, headache, altered sensorium	AMB / Alive
10	52/M/2009	Chaurg strauss syndrome, old PTB, ABPA	Diffuse brain edema with absent intra cranial flow voids.	Neg / Pos	CN	Headache ,vomiting	AMB / Died
11	39/M/2009	HIV, PTB left empyema thoracis, Left Pneumothorax	ND	Pos / ND	CN	Loss of weight	Fluconazole / Died
12	36/M/2010	HIV	Hyper intensities in sub cortical white matter of bilateral fronto parietal region, left lentiform nucleus and right cerebellar white matter.	Pos / Pos	CN	Fever ,Headache	AMB, fluconazole / Died
13	51/M/2010	Hemolytic anemia	Normal study	ND / Pos	CN	Fever, headache, vomiting	AMB, fluconazole / Alive
14	63/M/2010	Nil	ND	Pos / ND	CN	Altered sensorium	AMB / Died
15	25/F/2011	Nil	Disseminated encephalomyelitis. Diffuse cerebral edema.	Pos / Pos	CG	Fever, headache, vomiting	AMB/ Died
16	42/M/2011	HIV	Multiple ICSOL with suspicious areas of peripheral enhancement.	Pos / ND	CN	Fever, headache, multiple ICSOL	AMB, fluconazole / Alive
17	40/M/2012	CLD	A 8x8 mm hypo density noted in left head of caudate nucleus.	Neg / Pos	CN	Fever, headache, vomiting	5-flucytosine, AMB / Died
18	52/M/2012	Nil	Normal study	Neg / Pos	CN	Fever, headache, altered sensorium	Lip. AMB, fluconazole / Alive

Abbreviations; NHL, Non-Hodgkins Lymphoma; IHD,Ischemic Heart Disease; CLD, Chronic Liver Disease; ICSOL,Intracranial Space Occupying Lesion; AMB, Amphotericin –B; ND,not done; ABPA,Allergic Bronchopulmonary Aspergillosis; PTB,Pulmonary tuberculosis; Lip.AMB,Liposomal Amphotericin B; HIV,Human Immunodeficiency Virus; CN,Cryptococcus neoformans; CG, Cryptococcus gattii.

Table 2. Cerebrospinal fluid findings in the patients of cryptococcal meningitis	
Variables	Results
White Blood Cell Count[a]	65.3 ± 106.3 cells/μL
Glucose [a]	32.8 ± 19.2 mg/dL
Protein [a]	172.4 ± 213.0 mg/dL
Antigen positive	13/13 [c] (100%)
CSF culture	13/15 [c] (86.6%)
India ink	10/13 [c] (76.9%)
a: Mean ± Standard deviation; b: Positive cases/total patients	

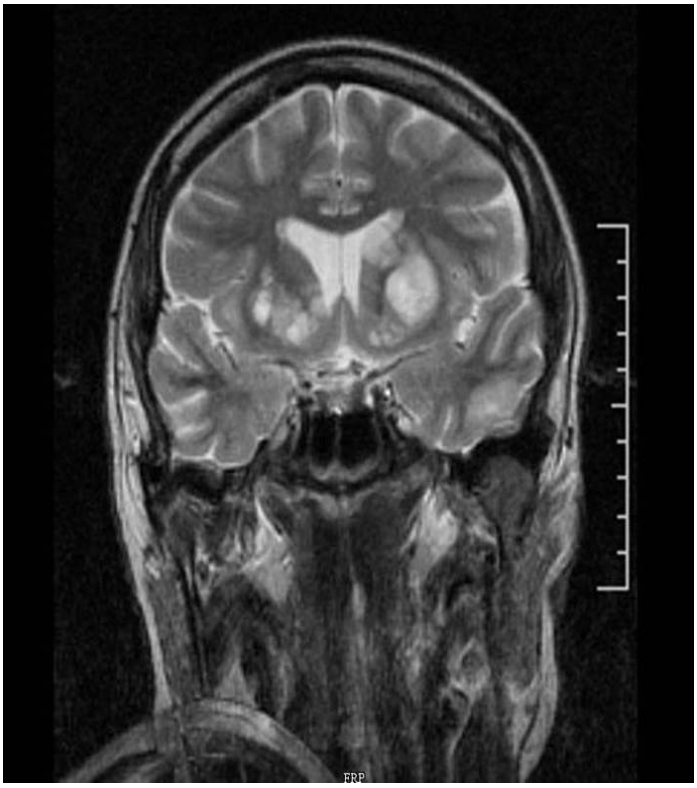

Figure 1. T2 coronal image of case No.5, showing multiple basal ganglia hyperintensities (left > right) and left temporal lobe hyperintensity with perilesional oedema and compression of the ipsilateral lateral ventricle.

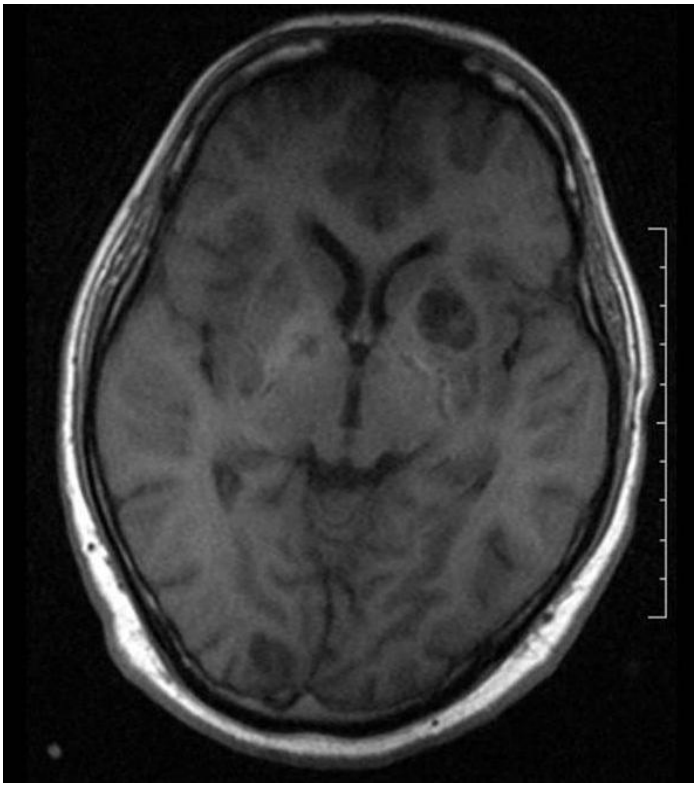

Figure 2. T1 axial image of case No.7, showing a large hypointense lesion around the left basal ganglia with mass effect (cryptococcoma) . Multiple small iso to hypointense lesions over the right basal ganglia, right occipital , left frontal and left parietal regions.

(27.7%) and seizures (11.1%). The CSF findings are summarized in table 2. Interestingly, one case presented as a neuropsychiatric disorder and was initially treated with antipsychotic drugs. *C. gattii*, which was confirmed by sequencing, was isolated in one case showing pseudohyphal forms in the CSF. All isolates were sensitive to amphotericin B, flucytosine and fluconazole. Previous history of tuberculosis was documented in 6/18 cases (33.3%) of which half of them died. The total mortality was 61.1% (i.e. 11/18) and was particularly higher in non-HIV group (72.7%; i.e. 8/11). Among the non-HIV patients who died, lymphoma, autoimmune disorder and chronic liver disease were documented six cases (two each) while the remaining two had no underlying disorder. All the patients received intravenous amphotericin B and fluconazole as per standard recommended dose.

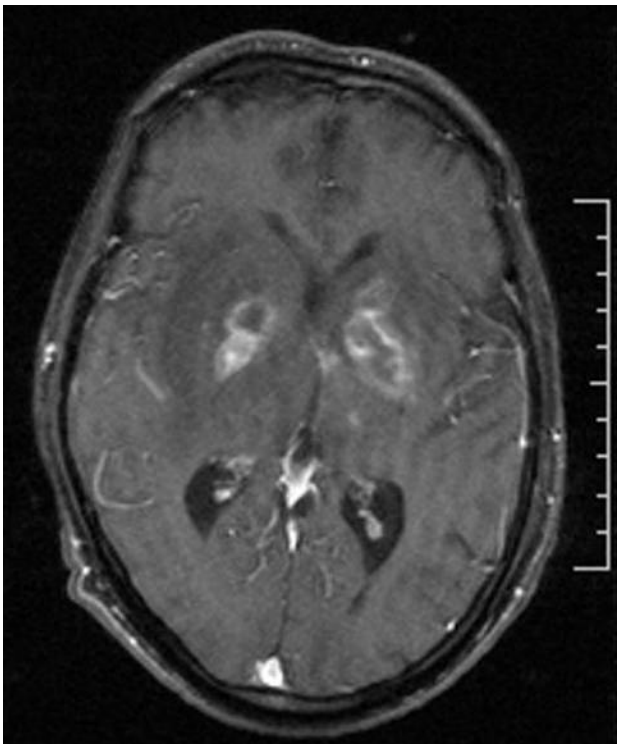

Figure 3. T1 axial contrast images of case No.12, showing bilateral subcortical and basal ganglia lesions with contrast enhancement (cryptococcomas).

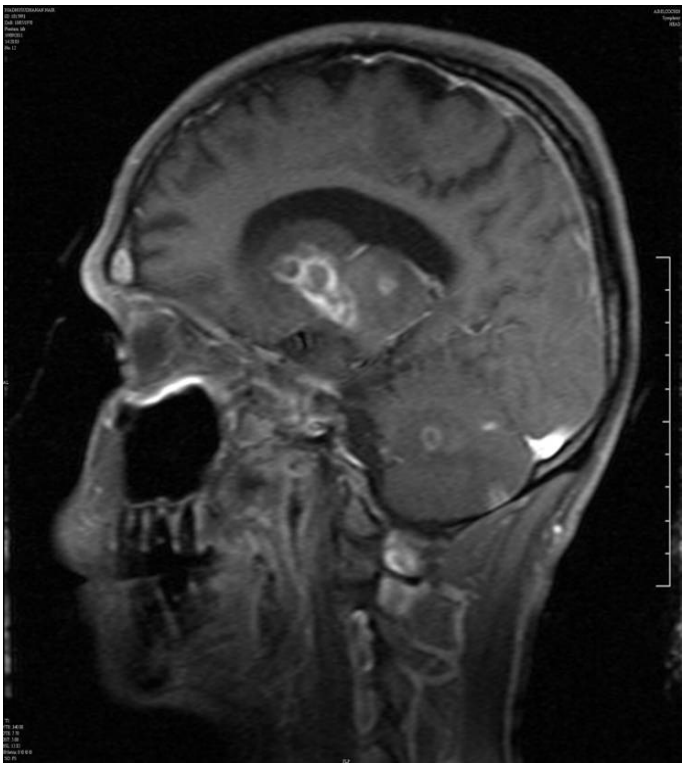

Figure 4. T1 sagittal post contrast scan of case No.16, showing multiple ring enhancing lesions over the subcortical region and cerebellum

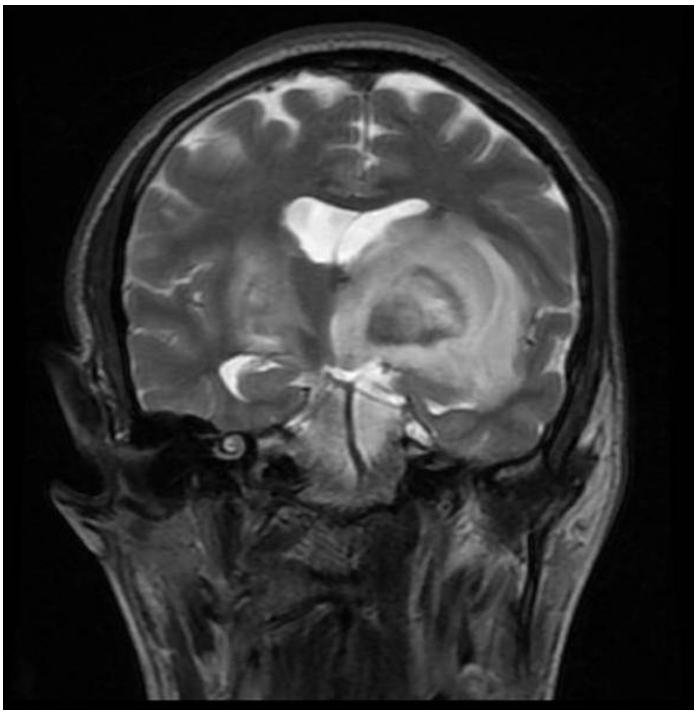

Figure 5. T2 coronal section of case No.17, showing large hyperintense lesion over the left basal ganglia with mass effect and perilesional oedema (cryptococcoma).

Discussion

Cryptococcal infections without any risk factors have been reported in almost 40% of patients in one series (5). In Non HIV infected patients and immunocompetent patients cryptococcosis is rarely suspected and the treatment is initiated only when the patient doesn't improve with antibacterial or antitubercular drugs. Respiratory tract is the route of entry in humans with propensity to localize predominantly in basal ganglia and cortical grey matter of central nervous system causing meningoencephalitis. Certain selective nutritional factors such as simple sources of nitrogen (asparagine and creatinine) present in spinal fluid can stimulate the growth. Classically cryptococcal meningitis is characterized by the triad of headache, fever and vomiting while altered sensorium have been reported in 13% to 73% patients (5). Laboratory investigations of CSF such as fungal culture, India ink preparation and LA for cryptococcal antigen are invaluable aids to make a correct diagnosis. Overall positivity of culture, microscopy and LAT in CSF were comparable to the reports in the literature (70-90% for microscopy, 80-92% for culture and 95-100% for LA) (4). Latex agglutination test was 100% sensitive and specific. Compared to developed countries the morbidity and mortality (10-30%) due to cryptococcosis in

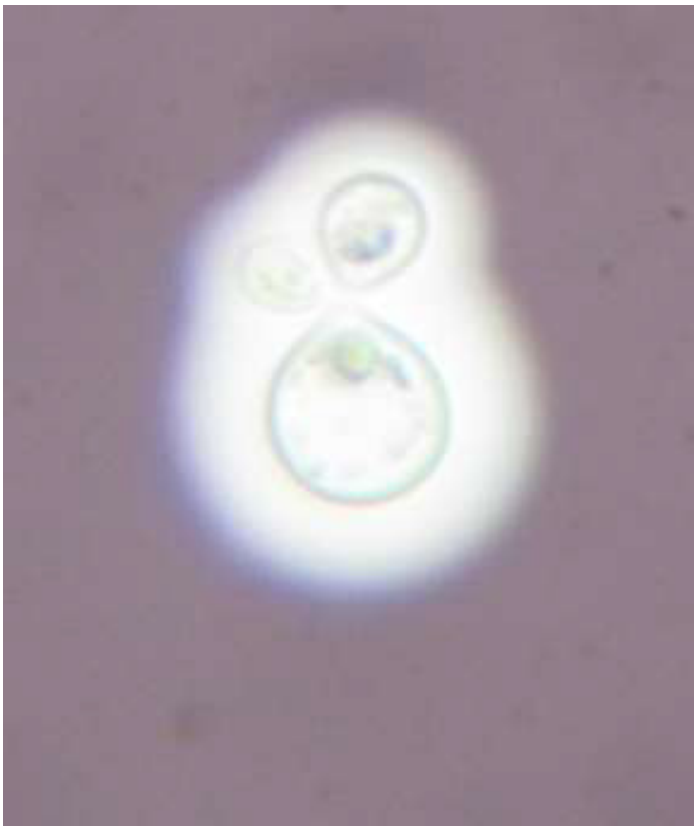

Figure 6. CSF showing capsulated budding yeast cells. (India ink x1000)

developing countries is very high (50-100%) (6). Previous studies have documented concomitant tuberculosis with cryptococcal meningitis (13). One third of our cases also had a previous history of tuberculosis making the diagnosis of cryptococcal meningitis even more difficult. Similar findings have been reported by Prasad *et al*. who found 60% of patients who died of cryptococcal infections were apparently healthy adults and among them 66.6% had received antitubercular therapy for more than six weeks before being diagnosed with cryptococcal infection (5). In non- HIV infected patients CSF cryptococcal antigen is the test of choice with a positivity of 97% (8). *C*. *gattii* which is predominantly seen in immunocompetent patients was reported in only one patient in our series. In one study from north India 40% of the patients were immunocompetent but none of them had *C*. *gattii* (5). Due to the nonspecific presentation all these cases were initially misdiagnosed tubercular or pyogenic meningitis and are treated with antibiotics which further extended the course of the disease. Initially the patient may respond to antibiotics therefore it does not necessarily exclude the existence of a fungal etiology. Without appropriate treatment Cryptococcal meningitis is invariably fatal with mortality of 83% in one series of patients (8).

In conclusion, untreated cryptococcal meningitis is always fatal and needs to be differentiated from other causes of chronic meningitis such as tuberculous meningitis, especially in countries endemic to mycobacterial infections. Cryptococcal antigen detection is a highly specific and rapid test, and should be used for initial screening in these patients. Our report underlines the need to rule out Cryptococcal infection in non-HIV infected patients such as diabetes, cancer, haematological malignancy, solid organ transplant, sarcoidosis, autoimmune hemolytic anemia and on long term steroid therapy and all patients with previous history of tuberculosis presenting with meningitis , as we have documented a higher mortality in this group.

References

1. Madan M, Ranjitham M, Chandrasekharan S, Sudhakar. Cryptococcal meningitis in immunocompetent individuals. J Assoc Physicians India 1999;47:933-4.
2. Mahapatra LN. Medical mycology in India - an overview. Indian J Med Res 1989;89:351-61.
3. Chakrabarti A, Sharma A, Sood A, Grover R, Sakhuja V, Prabhakar S, et al. Changing scenario of cryptococcosis in a tertiary care hospital in north India. Indian J Med Res 2000;112:56-60.
4. Khanna N, Chandramuki A, Desai A, Ravi V. Cryptococcal infection of the central nervous system: an analysis of predisposing factors, laboratory findings and outcome in patients from south India with special reference to HIV infection. J Med Microbiol 1996;45:376-9.
5. Prasad KN, Agarwal J, Nag VL, Verma AK, Dixit AK, Ayyagari A. Cryptococcal infection in patients with clinically diagnosed meningitis in a tertiary care center. Neurol India 2003;51:364-6
6. Banerjee U, Datta K, Majumdar T, Gupta K. Cryptococcosis in India: Awakening of a giant? Med Mycol 2001;39:51-67
7. Satpute MG, Telang NV, Litake GM, Niphadkar KB, Joshi SG. Prevalence of cryptococcal meningitis at a tertiary care centre in Western India (1996-2005). J Med Microbiol 2006;55:1301-2.
8. Capoor MR, Nair D, Deb M, Gupta B, Aggarwal P. Clinical and mycological profile of cryptococcosis in a tertiary care hospital. Indian J Med Microbiol 2007;25:401-4
9. Dumortier J, Piens MA, Boillot O, Faure JL, Scoazec

JY, Berger F, et al. Late cryptococcal meningitis after liver transplantation. Gastroenterol Clin Biol 1999;23:1251-3.
10. Campisi D, Tumminello A, Bivona A, Listi F. A rare case of cryptococcal meningitis unrelated to AIDS. Recenti Prog Med 2000;91:16-9.
11. Botha RJ, Wessels E. Cryptococcal meningitis in an HIV negative patient with systemic sarcoidosis. J Clin Pathol 1999;52:928-30.
12. Baron EJ, Thomson RB. Specimen Collection, Transport, and Processing: Bacteriology. In: Versalovic J, Carroll KC, Funke G, Jorgensen JH, Landry ML, Warnock DW (eds.) Manual of Clinical Microbiology. 10th Edition. Washington, DC: American Society for Microbiology Press. 2011. p. 228-71.
13. Rawat D1, Capoor MR, Nair D, Deb M, Aggarwal P. Concomitant TB and cryptococcosis in HIV-infected patients. Trop Doctor 2008;38(4):251-2.