Original Articles

Cryptococcal Meningitis in a Tertiary Care Hospital

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[Received: 10, April 2008. Accepted: 15, December 2008]

Abstract

Seven cases of cryptococcus meningitis in a tertiary care hospital from 2004-2007 were reviewed. 85.7% of the patients had headache as their predominant clinical feature. The spectrum of CT/MR findings ranged from no abnormality, basal ganglion lesion, to intracerebral and intraventricular granulomas. Findings of cerebrospinal fluid (CSF) cytology and biochemistry analysis were inconclusive. Patients were diagnosed by India ink (100%), CSF cryptococcal antigen detection (100%), and CSF culture in 6 (85.7%). With the exception of two patients, co-morbidities associated were HIV, diabetes mellitus, and idiopathic CD4 + lymphocytopenia. Six patients were successfully treated with amphotericin B and discharged. A high index of clinical suspicion and laboratory diagnosis achieved early can reduce the overall morbidity and mortality among patients with cryptococcosis.

Key words : cryptococcal meningitis

Introduction

Cryptococcosis is a systemic granulomatous infectious disease caused by an encapsulated yeast-like fungus, *Cryptococcus neoformans*. Two varieties and five serotypes of *C. neoformans* were recognized, but the varieties have now been accorded a species status: var. *neoformans*, now *C. neoformans* (serotypes A, D and AD; based on capsular polysaccharide antigens), and var. *gattii*, now *C. gattii* (serotypes B and C). *Cryptococcus neoformans* is found worldwide in association with soil contaminated with bird, particularly pigeon, excreta and usually causes infection in immunosuppressed individuals. *Cryptococcus gattii*, on the other hand, found primarily in tropical and subtropical regions, has been associated with several species of eucalyptus trees and causes infection predominantly in apparently immunocompetent individuals¹⁾.

Cryptococcosis occurs as a sporadic infection. The more frequent underlying conditions, in order of

decreasing association, are human immunodeficiency virus (HIV) infection, extensive treatment with corticosteroids, organ transplantations, chronic leukemias, lymphomas, and sarcoidosis²⁾. Cryptococcosis is increasingly being reported from patients with no apparent immune suppression³⁾. A retrospective study was undertaken to analyze the clinical and laboratory characteristics of cryptococcal meningitis patients admitted in a tertiary care hospital.

Material and Methods

During a four year time period, CSF specimens from 191 clinically diagnosed chronic meningitis patients were received. CSF was processed in the laboratory as follows: wet mount, India ink and Gram's stain of centrifuged deposits of CSF were examined. CSF specimens were cultured on Sabouraud dextrose agar without cyclohexamide for 4 weeks at 37°C. Concanavine – glycine thymol blue (CGB) agar was used to discriminate *C. gattii* from *C. neoformans* isolates. The laboratory diagnosis was made on the basis of a positive CSF India ink staining or a positive culture for the organism or a positive cryptococcal antigen latex agglutination test (CALAS; Meridian Diagnostics). Cryptococcus was identified on the basis of colony morphology,

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India ink stain, urease production, nitrate assimilation, sugar assimilation and fermentation tests. A detailed clinical history was recorded for all the cases diagnosed as cryptococcal meningitis.

Results

In this retrospective study, a total of seven patients were confirmed to have cryptococcal meningitis by one or more tests during 2004-2007. The mean age of the patients was 43.4 years. With the exception of two who were immunocompetent, the remaining patients had the following underlying diseases: HIV 2 (28.5%), non-insulin dependent diabetes mellitus (NIDDM) 2 (28.5%), and idiopathic CD4 + lymphocytopenia (14.3%) in one case. The clinical profile of all the cases is shown in Table 1. The CD4 cell counts in cases no. 6 and 7 were 98/cu mm³ and 153/cu mm³, respectively. The other opportunistic infections associated were tuberculosis, oropharyngeal candidiasis and toxoplasmosis. Duration of symptoms ranged from 10 days to one year; 85.7% of the patients had headache as their predominant clinical feature. One patient had disseminated infection with the involvement of skin as evidenced by skin biopsy. Examination was unremarkable except papilloedema 6 (85.7%), Kernig's sign in 5 (71.4%) patients, hemiparesis in 2 (28.6%) and altered sensorium in 3 (42.8%).

Cryptococcus was detected by India ink and cryptococcal antigen in all the 7 cases but CSF culture was positive in 6 (85.7%). CSF culture on Sabouraud's dextrose agar yielded growth of *C. neoformans* which was nonfermentative, hydrolyzed starch, assimilated inositol, and produced urease. CSF analysis revealed lymphocytic pleocytosis in all the patients. Mean duration of admission was 45.3 days. Neuroimaging of the brain was carried out in the 6 patients, with the result for one patient being normal. The abnormalities found ranged from basal ganglion lesion to intracerebral granuloma. All the patients who survived received conventional amphotericin B for an average duration of 40.5 days (range 20 days to 72 days). One patient received 5-flucytocine concurrently and 6 were discharged on oral fluconazole at 100 to 800 mg/day for a mean duration of 6-8 weeks. One patient with uncontrolled diabetes expired due to cardiac arrest before amphotericin B therapy could be initiated.

Patient 1. A 42-year-old man was admitted with complaints of headache, fever, vomiting, altered sensorium and weakness of left half of the body with nodular cutaneous lesions. Examination of the CSF revealed capsulated yeasts on India ink and culture yielded *C.*

neoformans. Cryptococcal antigen test by latex agglutination kit (CALAS, Meridian Bioscience) was positive (titre 1:4) and skin biopsy showed multiple capsulated yeast cells. Serology for anti-HIV-1 and HIV-2 was negative. Absolute CD4 count was 295 cells/ μ l on two occasions with normal IgM, IgG and IgA. The patient was diagnosed as a case of idiopathic CD4 + lymphocytopenia. CT head of the patient showed multiple intracranial and intraventricular granulomas. Conventional amphotericin B at a dosage of 30 mg/day was started. There was regression of cranial and cutaneous lesions and CSF culture became sterile after six weeks of therapy. The patient was discharged with fluconazole after 63 days of amphotericin B (cumulative dose 1.89 g).

Patient 2. A 32-year-old man was admitted because of fever, headache and vomiting for 10 days. Examination of the CSF revealed a positive India ink result. Fungal culture grew *C. neoformans*. HIV serology was negative and the patient was immunocompetent. Cryptococcal antigen test by latex agglutination kit was positive with titre of 1:4. Conventional amphotericin B was started at a dose of 0.7-mg/kg/day. Fungal culture became sterile after 8 weeks of therapy. The patient improved after a cumulative dose of 2.5 gm of amphotericin B over 72 days.

Patient 3. A 45-year-old man developed neck pain, and headache associated with vomiting without fever. He also developed difficulty in walking. His neurological examination showed sluggish deep tendon reflexes and decreased plantar reflexes bilaterally. Examination of the CSF revealed a positive India ink result with negative cultures. Cryptococcal antigen test by latex agglutination kit was positive with titre of 1:4. HIV serology was negative and the patient was immunocompetent. He was managed with conventional amphotericin B 30 mg/day and mannitol to relieve elevated intracranial pressure, and was also managed with physiotherapy during his hospital stay. He improved with a total dose of amphotericin B of 870 mg over 29 days followed by fluconazole for 4 weeks.

Patient 4. A 48-year-old female with diabetes mellitus type II was admitted with a one and a half month history of headache, fever and vomiting. The patient had uncontrolled DM and CSF examination revealed capsulated yeasts on India ink, culture yielded *C. neoformans*. Cryptococcal antigen test by latex agglutination kit was positive with titre of 1:64. Treatment with amphotericin B (0.3 mg/kg/day) and flucytocine (100

Table 1. Clinico-pathological profile of seven cases of cryptococcal meningitis

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7
Age/Sex	42/M	32/M	45/M	48/F	52/M	40/M	45/M
C/F	Headache Fever Vomiting Hemiparesis	Fever Headache vomiting	Headache Vomiting Neck stiffness	Headache Fever Vomiting	Fever Headache Vomiting Altered sensorium	Fever Headache Altered sensorium	Right Hemiparesis Right Facial palsy
Duration	2 months	10 days	1 year	1.5 months	1.5 months	1 month	2 months
HIV status	Negative	Negative	Negative	Negative	Negative	Positive	Positive
Underlying illness	CD4 Lymphocytopenia	–	–	NIDDM	NIDDM	AIDS	AIDS
Outcome	Discharged	Discharged	Discharged	Discharged	Expired	Discharged	Discharged
CSF findings							
Protein mg/dl	26	96	46	46	20	160	139
Sugar mg/dl	63	32	40	80	6.7	34	43
Total cells	10	20	60	400	180	80	50
Predominant Cell type	Lymphocyte	Lymphocyte	Lymphocyte	Neutrophils	Lymphocyte	Lymphocyte	Lymphocyte
India Ink	Positive	Positive	Positive	Positive	Positive	Positive	Positive
Culture	<i>C. neoformans</i>	<i>C. neoformans</i>	Negative	<i>C. neoformans</i>	<i>C. neoformans</i>	<i>C. neoformans</i>	<i>C. neoformans</i>
Latex Agglutination	Positive	Positive	Positive	Positive	Positive	Positive	Positive
Fundus	Papilloedema	Papilloedema	Papilloedema	Papilloedema	–	Papilloedema	Papilloedema
CT/MRI findings	Multiple intraventricular and intracerebral granuloma	NAD	Hyperintense regions in cerebrum	Contrast enhancing lesion (frontal)	Not done	B/L Lesions in basal ganglia	Left basal ganglia lesions
Miscellaneous	Cutaneous lesions	–	–	–	–	–	–
Antifungal Dose	Amphotericin B 30 mg/day	Amphotericin B 35 mg/Day	Amphotericin B 30 mg/day	Amphotericin B (0.3 mg/kg/day); Flucytosine 100 mg/kg/day	Amphotericin B (0.2 mg/kg/Day)	Amphotericin B 18 mg/day	Amphotericin B (0.3 mg/kg/day)
Antifungal duration	63 days	72 days	29 days	30 days	48 hrs	20 days	29 days
Hospital stay	79 days	75 days	31 days	34 days	48 hrs	22 days	31 days

mg/kg/day) was started. The patient recovered with 540 mg of conventional amphotericin B and flucytocine followed by fluconazole for 6 weeks.

Patient 5. A 52-year-old male with diabetes mellitus type II was admitted with altered sensorium, fever and vomiting. His neurological examination showed positive Kernig's sign. CSF examination revealed capsulated yeasts on India ink and culture yielded *C. neoformans*. Cryptococcal antigen test by latex agglutination kit was positive with titre of 1:8. The patient was put on amphotericin B (0.2 mg/kg/day), mannitol and human insulin. His condition deteriorated in the next 48 hrs and he had sudden cardiopulmonary arrest. Despite all resuscitative measures, he could not be revived. No autopsy was performed.

Patient 6. A 40 year old seropositive for HIV-I with a history of chronic alcoholism and opium addiction was admitted with complaints of altered sensorium and fever for one month. The patient was a known case of pulmonary tuberculosis with irregular treatment. MRI brain showed symmetrical lesions involving bilateral caudate and putamen and lesions in right globus pallidus and thalamus. On examination, there was sign of meningeal irritation. CSF examination revealed capsulated yeasts on India ink and culture yielded *C. neoformans*, however, culture and PCR were negative for tuberculosis. Cryptococcal antigen test by latex agglutination kit was positive with titre of 1:128. The patient was started on amphotericin B (0.3 mg/kg/day), antitubercular drugs and highly active antiretroviral therapy (HAART) [stavudine (D4T), lamivudine (3TC), and efavirenz (EFV)]. He received 522 mg of amphotericin B for 20 days, followed by 200 mg of fluconazole twice daily. The patient expired after 4 months of taking HAART.

Patient 7. A 45-year-old man seropositive for HIV developed gradually progressing weakness in the right half of the body including his face in the form of mouth deviation. There was no history of fever, headache, vomiting or loss of consciousness. Examination of the CSF revealed a positive India ink result. Fungal culture grew *C. neoformans*. Cryptococcal antigen test by latex agglutination kit was positive with titre of 1:256. His absolute CD4 count was 153 cells/mm³. He was referred to antiretroviral therapy clinic (ART) clinic and counseling and put on lamivudine (150 mg), stavudine (30 mg), nevirapine (200 mg), and conventional amphotericin B (0.3 mg/kg/day). The patient defaulted 2 months after initiation of

HAART.

Discussion

Cryptococcus neoformans is the second most common fungal opportunist after *Candida albicans*, causing symptomatic cryptococcosis in up to 8.5% of HIV-infected individuals⁴⁾. Cryptococcal meningitis is a relatively understudied entity in developing countries like India, although high incidence has been reported from various localized studies in the country⁵⁻⁷⁾. In the present study, a total of 7 (3.6%) patients were found to be positive for cryptococcal meningitis by one or more of the tests. Isolation of the organism from the clinical sample is the gold standard in the diagnosis of cryptococcal meningitis. All the isolates were speciated to *C. neoformans* on CGB media. Importance of the cryptococcal antigen detection in CSF cannot be underestimated as all seven patients were positive by antigen detection. Detection of antigen in cerebrospinal fluid has been reported as a sensitive and specific test⁸⁻⁹⁾. In this study, India ink examinations were also sensitive and specific.

CT brain or MRI brain in cryptococcal meningitis is usually normal but may reveal diffuse atrophy of the brain or ring enhancing lesions¹⁰⁾. In this study, five patients showed various lesions in neuroimaging.

Out of a total of 7 patients, six received conventional amphotericin B (0.3-0.7 mg/kg BW/day) given IV as monotherapy for 6-8 wks followed by oral fluconazole at 100-800 mg/day. Due to economic constraints and poor availability of flucytocine in India, conventional amphotericin B is given as monotherapy. Since dose related nephrotoxicity has been reported, patients were monitored with regular renal function tests. Although liposomal amphotericin B is less nephrotoxic, the cost is prohibitive¹¹⁾. Two HIV positive patients (WHO clinical stage 4) presented with a first episode of cryptococcal meningitis. The patients (Case no. 6 and Case no. 7) were put on [stavudine (D4T), lamivudine (3TC), and efavirenz (EFV)] and [stavudine (D4T), lamivudine (3TC), and nevirapine (NVP)], respectively, on admission¹²⁾. Disseminated cryptococcosis was seen in case no. 1 with cutaneous lesions over the face and neck. The patient was diagnosed as a case of idiopathic CD4 + lymphocytopenia.

Analysis of the CSF data showed a worse outcome in case no. 5 with lower CSF glucose concentration as also seen in other reports¹³⁾. CSF WBC counts of less than 20/mm³ prior to the start of the antifungal therapy has been reported to be associated with a grave prognosis but such a count did not seem to influence the prognosis of our patients. Moreover, cryptococcal

antigen titer in all the patients was $< 1:1024$ which is considered a significant adverse prognostic factor^{14, 15)}. In our center, patients with NIDDM have emerged as an important risk group in addition to HIV infected patients in cryptococcal meningitis. Excellent clinical outcome is mainly due to early diagnosis and vigorous treatment with amphotericin B and fluconazole combined with aggressive management of elevated intracranial pressure.

In conclusion, cryptococcosis continues to be an important cause of chronic meningitis in immunosuppressed as well as immunocompetent patients. A high index of clinical suspicion and laboratory diagnosis achieved early can reduce the overall morbidity and mortality among patients with this condition.

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