

Meningitis Due to *Cryptococcus gattii* in an Immunocompetent Patient

RAJESH T PATIL¹, JYOTI SANGWAN², DEEPAK JUJAL³, SUMIT LATHWAL⁴

ABSTRACT

The incidence of cryptococcal infection is high in developing countries such as India. *Cryptococcus gattii*, formerly known as *Cryptococcus neoformans var gattii*, is an encapsulated yeast that causes disease in both immunocompetent and immunosuppressed individuals. The organism enters via respiratory tract and causes a spectrum of illness ranging from asymptomatic infection to severe illness, including pneumonia and disseminated infection involving multiple sites, including the central nervous system, eyes and skin. Cryptococcal meningitis is generally considered as rare in immunocompetent patients; therefore, specific treatment is not implemented until the organism is identified or a cryptococcal antigen is detected. We describe the case of a 30-years-old man without prior medical history who presented with meningitis and was treated successfully. This case illustrates the importance of considering infectious causes such as *C.gattii* in the differential diagnosis of meningitis, regardless of the patient's immune status.

Key words: Amphotericin, *Cryptococcus gattii*, Immunocompromised, Meningitis

CASE REPORT

A 30-years-old male, driver by occupation, was admitted to neurology department with chief complaints of high-grade fever, intermittent, moderately severe headache lasting for 30 days associated with multiple episodes of vomiting. He had no history of seizures, ear discharge or earache, nor any focal neurological deficit, head trauma, weight loss, chronic cough, drug abuse including steroids, blood transfusion, or high-risk behavior. He did not have any history of cutaneous or respiratory manifestations neither he had any history of tuberculosis, diabetes, malignancy or any other such chronic illness.

On examination, the patient was febrile (39°C) and conscious, alert, oriented to time, place and person. Higher motor functions were intact and speech was normal. Signs of meningeal irritation (nuchal rigidity and Kernig's sign) and bilateral papilloedema was present. Examination of other systems revealed no obvious abnormality.

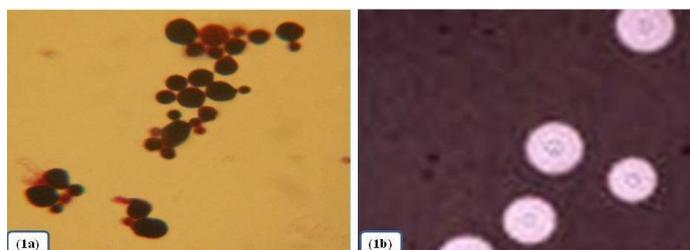
Laboratory investigations revealed Haemoglobin of 11mg/dl, raised total leukocyte count (13,000/mm³) with 78% lymphocytes. Serum electrolytes, renal function tests, and liver function tests were within normal limits. A Cerebrospinal Fluid (CSF) examination revealed 210 cells/mm³, predominantly lymphocytes, with protein 92mg/dl and glucose 22 mg/dl (corresponding blood glucose was 136 mg/dl). A Computerized Tomography (CT) scan of the head and a Chest X-ray were both normal. The CSF specimen was received in laboratory for microbiological investigations such as staining, culture and sensitivity. Macroscopically, the CSF was clear and without coagulum. On microscopy, Gram stain showed round budding yeast cells varying in size [Table/Fig-1a]. There were no acid fast bacilli on Ziehl-Neelsen (ZN) stain. India ink preparation showed characteristic round budding yeast cells varying in size with distinct halos [Table/Fig-1b]; a bacterial culture was sterile. The CSF Cryptococcal Latex Agglutination Test (CALAS, Meridian Diagnostics, Cincinnati, Ohio) for Cryptococcal Antigen (CRAG) was positive, with a titer of 1:1024. Serum was also tested for CRAG and was positive. A presumptive diagnosis of cryptococcal meningitis was given to the clinicians and patient was promptly put on Amphotericin B and Flucytosine. Subsequently, culture on Sabouraud's dextrose agar yielded smooth colonies of yeast after five days of incubation at 37°C, with no growth at 30°C. With a battery of tests and biochemical reactions performed [Table/Fig-2],

[Table/Fig-3a and b] the isolate was characterized as *C.gattii*. Serotyping of the organism was not possible at this point.

The anti-fungal susceptibility testing of the isolate was performed by using microbroth dilution technique and results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines [1]. The Minimum Inhibitory Concentration (MIC) values for fluconazole, Amphotericin B (Amp B), and voriconazole were $\leq 1 \mu\text{g/ml}$, $0.50 \mu\text{g/ml}$, and $\leq 0.12 \mu\text{g/ml}$ respectively. Also E-Test (AB Biodisk, Sweden) was used for Amphotericin B, which showed MIC of $0.38 \mu\text{g/ml}$.

The patient was tested for HIV antibodies and found to be non-reactive for HIV1 and HIV2. His immunoglobulin levels (IgG, IgA, IgM) were normal and CD4 cell counts were 696 cells/mm³, thus ruling out any immune deficiency.

Treatment was started with combination of Amp B (1 mg/kg/day) and Flucytosine (100 mg/kg/day) as an intravenous infusion along with intravenous fluids and mannitol. A therapeutic CSF tap was also performed to lower intra-cranial pressure. Complete blood counts, serum electrolytes and renal functions were monitored on daily basis for the drug toxicity. Amp B and flucytosine were



[Table/Fig-1a]: Gram staining picture showing gram positive round budding yeast cells (magnification x40)

[Table/Fig-1b]: India ink preparation showing round budding yeast cells with distinct halos (magnification x40)

Test	Result
Growth on Niger seed agar	Mucoid, brownish black colonies after 48 hours of incubation [Table/Fig-3a]
Urease production	Positive within 4 hours
Growth on L-canavanine glycine bromothymol blue (CGB)	Present. CGB media became cobalt-blue in colour after 48 hours of incubation [Table/Fig-3b]

[Table/Fig-2]: Biochemical tests used for identification

continued for 2 weeks; after which a repeat CSF analysis showed no yeast in Gram stain and India ink wet mount. Also CRAG levels have fallen to 1:256. Thereafter, patient was put on oral fluconazole (800 mg/day) for 8 weeks. On subsequent follow up patient improved considerably, with complete resolution of symptoms.



[Table/Fig-3a]: Dark brown colonies on Niger seed agar
[Table/Fig-3b]: L-canavanine glycine bromothymol blue (CGB) media turns cobalt blue after 48 hrs

DISCUSSION

Most cases of the cryptococcal meningitis occurs in patients with conditions that weaken their immune system, such as Acquired Immunodeficiency Syndrome (AIDS). Cryptococcal meningitis has also been sporadically reported in HIV-negative patients caused by organ transplant and chemotherapy related immunosuppression, reticuloendothelial malignancies, corticosteroid therapy and sarcoidosis [2,3]. Occasionally, no obvious underlying cause can be detected [4]. Considering the ubiquitous presence of this organism in environment, the most possible explanation for acquiring meningitis in an otherwise healthy individual could be through inhalational route.

Meningitis in immunocompetent host is usually attributed to *C.gattii* worldwide; although few cases have been caused by *C. var grubii* [3,5]. In our case also, the patient was immunocompetent and developed meningitis due to *C.gattii*. A study of the molecular epidemiology of the human pathogenic fungus *C. neoformans* in India has shown that most Indian isolates are *C. neoformans var. grubii* (serotype A), few are *C. neoformans var. gattii* (serotype B), and very few are *C. neoformans var. neoformans* (serotype D) [6]. Overall, *C. neoformans* showed a higher environmental prevalence than that of *C.gattii* which was not found in the avian habitats. In addition, more than half of the isolates were derived from patients who had no known impairment of their immune systems.

Because the signs and symptoms are similar in both the diseases, cryptococcal meningitis presents late in the course of disease and has a shorter duration of symptoms in AIDS patients. In contrast, in non-AIDS patients, the onset is insidious with a chronic course. Symptoms of meningitis may begin months to years before clinical diagnosis. CT findings may also be normal in 50% of the cases [3]. Our patient had sub-acute presentation with a history of headaches over a duration of 30 days. The CT scan was also normal in our patient.

Current practices of anti-cryptococcal therapy in India for immunocompetent patients generally include Amp B alone or with flucytosine (5-fluorocytosine), and sometimes followed by fluconazole [7]. In immunocompetent patients, initial therapy should be Amphotericin B (0.7-1 mg/kg per day) alone or in combination with flucytosine (100 mg/kg per day in four divided doses). Amphotericin B can be administered alone for six to ten weeks or in conjunction with flucytosine for two weeks, followed by fluconazole for a minimum of ten weeks [8].

Patient in this case was started on combination therapy with Amp B and Flucytosine as soon as the provisional diagnosis was provided on the basis of Gram stain, India ink and CRAG. With early diagnosis, cryptococcal infections, including CNS and disseminated infections, are usually amenable to therapy. In patients with no demonstrable immunosuppression, Amphotericin B therapy, with or without flucytosine, is effective in controlling or terminating infection in 70% - 75% of patients [8]. There had been few cases where lack of timely diagnosis and delayed treatment has resulted in patient death [5]. Therefore, irrespective of the immune status of the patient, the outcome can be severe unless the disease is diagnosed early in the course of illness.

CONCLUSION

Cryptococcosis is a life-threatening infection caused by two main species, *Cryptococcus neoformans* and *Cryptococcus gattii*. Despite appropriate antifungal therapy, mortality from cryptococcal meningitis, the most severe form of cryptococcosis, remains high. Early diagnosis, targeted screening and prompt management have been proposed to reduce meningitis related deaths.

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PARTICULARS OF CONTRIBUTORS:

1. Assistant Professor, Department of Microbiology, Azeezia Institute of Medical Sciences & Research, Meeyannoor, Kollam, Kerala, India.
2. Assistant Professor, Department of Microbiology & Immunology, Veer Chandra Singh Garhwal Government Medical Sciences & Research Institute, Srinagar Garhwal, Uttarakhand, India.
3. Senior Demonstrator, Department of Microbiology & Immunology, Veer Chandra Singh Garhwal Government Medical Sciences & Research Institute, Srinagar Garhwal, Uttarakhand, India.
4. Deputy Assistant Director Health, Indian Armed Forces, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:

Dr. Jyoti Sangwan,
 Assistant professor, Department of Microbiology & Immunology,
 Veer Chandra Singh Garhwal Government Medical Sciences & Research Institute,
 Srinagar Garhwal, Uttarakhand- 246174, India.
 Phone: +919627759902, E-mail: jyolathwal@yahoo.co.in

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