

# Cryptococcal Meningitis in an Apparently Immunocompetent Individual: A Case Report

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## ABSTRACT

*Cryptococcus neoformans*, a saprophyte, is commonly found in soil contaminated with pigeon droppings but has also been isolated from the wood of several tree species in South America and India, including the flowers and bark of eucalyptus trees. Inhalation of small, encapsulated yeasts may lead to an initial pulmonary infection. However, this is usually short-lived and frequently silent, especially in immunocompetent individuals. Immunocompromised patients are usually tested for *Cryptococcus* as a causative agent when presenting with features of meningitis, but when dealing with immunocompetent patients, it is seldom considered. This report presented a case of a 39-year-old male who was apparently immunocompetent, yet developed cryptococcal meningitis. He had no prior history of any chronic illness, malignancy, immunosuppressive medication, or steroid use. Routine blood investigations were within normal limits. Autoimmune workup and viral markers were negative. He had raised Intracranial Pressure (ICP), Cerebrospinal Fluid (CSF) leukocytosis, and encapsulated yeast forms consistent with cryptococcal meningitis initially demonstrated by CSF cytology examination with Papanicolaou stain and May-Grunwald-Giemsa, and further confirmed by special stains. This case highlights the role of initial cytological workup in detecting cryptococcal infection through morphological and special stain studies in an immunocompetent host.

**Keywords:** *Cryptococcus*, Invasive fungal infections, Meningitis, Mycoses

## CASE REPORT

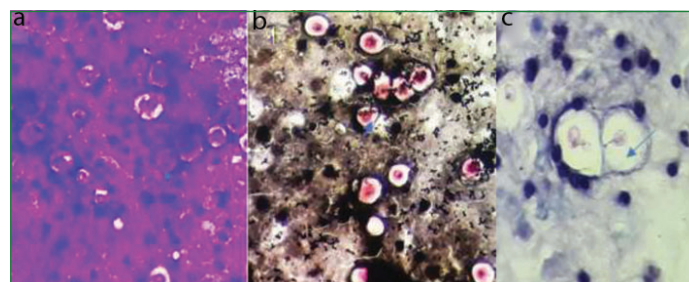
A 39-year-old male presented to the hospital with complaints of severe headache, vomiting, and high-grade fever lasting for three days. He had a history of diplopia for two months. He works as a driver and has no significant past medical history. There was no history of weight loss, malignancy, treatment with steroids, or use of other immunosuppressive drugs. He had no recent infectious contacts.

On examination, the patient was febrile (39.2° C), conscious, and oriented. Neurological examination revealed neck stiffness, bilateral papilloedema, and lateral rectus palsy. The plantar reflex was normal bilaterally. Examination of other systems revealed no significant abnormalities. Routine investigations, including complete blood count (CBC), renal function tests, and liver function tests, were within normal limits. Autoimmune work-up, such as antinuclear antibody (ANA), anti-neutrophilic cytoplasmic antibodies (ANCA), rheumatoid factor (RF), and C-reactive protein, were done and all were negative. Tests for syphilis and tuberculosis were also negative. Human immunodeficiency virus (HIV), hepatitis B surface antigen (HBsAg), and hepatitis C virus (HCV) tests were non-reactive. Dengue non-structural protein (NS1) test was negative.

Magnetic resonance imaging (MRI) of the brain showed focal leptomeningeal enhancement in the right parasagittal parietal cortex with associated gyral swelling. Computed tomography (CT) of the brain showed no significant intracranial pathology. CT angiogram of the brain was normal.

A lumbar puncture was performed, which showed raised opening pressure (29.5 cm H<sub>2</sub>O), but the CSF was clear. CSF examination revealed a total of 201 white blood cells/mm<sup>3</sup>, of which 69% were lymphocytes and 31% were polymorphs. The sugar level was <20 mg/dL, the protein level was 117 mg/dL, and Adenosine Deaminase (ADA) was negative. The Venereal Disease Research Laboratory test (VDRL) was non-reactive. Blood and

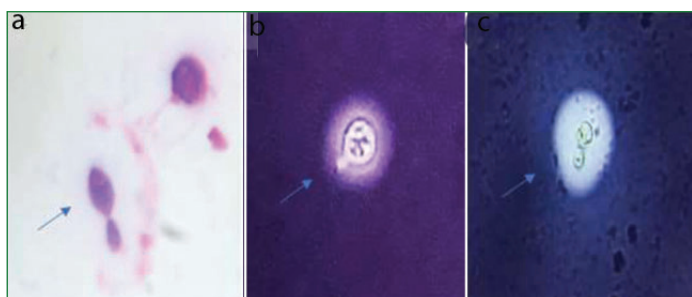
CSF cultures were sterile. Gram stain of the initial CSF sample was negative. CSF cytology examination with Papanicolaou stain and May-Grunwald-Giemsa revealed encapsulated refractile round to oval pale eosinophilic structures with a few budding forms, resembling fungal organisms (*Cryptococcus*). Mucicarmine stain showed encapsulated yeast forms with intense red colour [Table/Fig-1].



**[Table/Fig-1]:** CSF Cytology: (a) May-Grunwald-Giemsa stain(40X); and (b) Mucicarmine stain (40X) showing encapsulated yeast form with intense red colour; (c) Papanicolaou stain(40X) showing encapsulated refractile round to oval pale eosinophilic structures with few budding forms morphology resembling fungal organism-*Cryptococcus*.

Since the initial gram stain was negative, despite the cytology revealing fungal organisms and the patient not showing improvement with ongoing therapy, a false negative gram stain was suspected. A repeat CSF sample was taken, and the gram stain showed round budding yeast cells. India ink preparation and Nigrosine staining of the CSF sample revealed yeast cells with a surrounding halo morphology resembling *Cryptococcus*. The Sabouraud's Dextrose Agar (SDA) culture was negative. The Cryptococcal Antigen (CrAg) test was also negative [Table/Fig-2]. The patient was then further evaluated for any immunodeficiency state; however, no such clues were found.

With a diagnosis of Cryptococcal meningitis, the patient was referred to a higher centre and treated with liposomal amphotericin B (3-4 mg/kg/day) and flucytosine (100 mg/kg/day) for two weeks.



**[Table/Fig-2]:** CSF Microbiology: (a) Gram stain (100X) showing round budding yeast cells; (b) Nigrosin stain (100X); and (c) India Ink stain (40X) showing yeast cells with surrounding halo morphology resembling *Cryptococcus*.

The patient showed symptomatic improvement. A therapeutic CSF tap was also performed to lower the ICP. On subsequent follow-up after two weeks, the patient improved considerably, with resolution of symptoms.

## DISCUSSION

Cryptococcosis was identified in 1894 when the pathologist Otto Busse and physician Abraham Buschke jointly identified *Cryptococcus species* as the cause of a chronic granuloma of the tibial bone in a 31-year-old woman [1]. There are more than 50 species of *Cryptococcus*, of which *Cryptococcus neoformans* and *Cryptococcus gattii* cause the majority of infections, and some consider them the only pathogens in humans.

The vast majority of cases globally are caused by *Cryptococcus neoformans* [2]. *Cryptococcus* grows readily in soil contaminated with avian excreta, particularly that of pigeons, and is transmitted to humans via inhalation of the contaminated aerosol. In accordance with previous studies of cryptococcal meningitis, this patient also presented with severe headache, vomiting, high-grade fever, and diplopia [2,3].

CSF examination showed pleocytosis with predominantly lymphocytes, decreased CSF sugar, and elevated CSF protein, favouring tuberculous meningitis. Similar findings have been reported earlier in some cases [4]. Further work-up showed negative Cartridge-Based Nucleic Acid Amplification Testing (CBNAAT) and ADA. Papanicolaou stain and May-Grunwald-Giemsa of the CSF sample received in the cytopathology lab showed encapsulated refractile round to oval pale eosinophilic structures with a few budding forms, morphologically resembling *Cryptococcus*. Mucicarmine staining in the CSF sample showed encapsulated budding yeast forms with an intense red colour. India ink microscopy has historically been a quick, low-resource method to detect *Cryptococcus* in the CSF [5]. The stain fills the background field, but it is not taken up by the thick *Cryptococcus* capsule, forming a halo of light by which it can be visualised using a light microscope. India ink and Nigrosine staining of the CSF sample of the patient revealed yeast cells with surrounding halo morphology resembling *Cryptococcus*. Gram stain showed round budding yeast cells.

CSF culture is considered the gold standard for the diagnosis of cryptococcal meningitis. However, the diagnosis can take days and up to one to two weeks for definitive results. Thus, other diagnostic methods such as India Ink and detection of Cryptococcal Antigen (CrAg) by Lateral Flow Assay (LFA) or Latex agglutination have been used to expedite diagnosis and treatment [6]. In the present case, the CSF culture was negative at 48 hours, and the patient was referred immediately with a positive India ink test. In accordance with previous studies, culture-negative cryptococcosis can be seen in cases with a low fungal burden [7,8]. Culture-negative cryptococcosis may be a significant contributor to undiagnosed meningitis [8]. The

patient's CrAg test was negative. If cryptococcosis is suspected, serological screening for CrAg should be performed using a test that can detect not only *Cryptococcus neoformans* but also a broader range of specificity and sensitivity for *Cryptococcus gattii* and interspecies hybrids [9]. Serum CrAg in non-HIV patients should not be used due to its low sensitivity in detecting varied Cryptococcal species [10].

MRI of the brain showed focal leptomeningeal enhancement in the right parasagittal parietal cortex with associated gyral swelling, while CT of the brain showed no significant intracranial pathology. This is in accordance with a similar case report showing that MRI is more sensitive in detecting cryptococcal CNS infections, such as leptomeningeal enhancement and Virchow-Robin space dilatation [10].

Meningitis in immunocompetent hosts is usually attributed to *Cryptococcus gattii* worldwide [9]. In the present case, the patient was immunocompetent, and the culture negativity may be due to the fact that most cultures usually test for *Cryptococcus neoformans* and miss out on *Cryptococcus gattii*. The diagnosis of cryptococcal meningitis was suggested based on the detection of *Cryptococcus* in the CSF mainly by special stain studies such as India ink, nigrosine, and mucicarmine, and the lack of evidence for alternate diagnoses. Without liposomal amphotericin B, the patient's neurological status deteriorated and developed infarcts, despite antibiotic coverage. This further strengthened the diagnosis.

The management of cryptococcal meningitis is done with three basic principles: 1) the use of antifungal regimens for the clearance of the fungus; 2) early detection and treatment of raised ICP and Immune Reconstitution Inflammatory Syndrome (IRIS); and 3) the use of liposomal amphotericin B for kidney protection.

## CONCLUSION(S)

Cryptococcal meningitis caused by *Cryptococcus neoformans* is a relatively infrequent condition in apparently immunocompetent individuals. When obvious signs of immunosuppression are absent, it is important to explore less commonly considered factors that could contribute to minor immunosuppression. These factors may encompass conditions such as diabetes, alcoholism, and chronic liver or kidney disease, among others. Nonetheless, cryptococcal meningitis can be associated with mortality in individuals without a known underlying immunocompromised state, and diagnosis is often delayed because the symptoms tend to develop gradually. In conclusion, the present case highlighted the role of initial cytological work-up in the detection of cryptococcal infection by morphological and special stain studies, even in an immunocompetent host.

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