

CRYPTOCOCCAL MENINGOENCEPHALITIS DIAGNOSED BY BLOOD CULTURE

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Abstract

Increase in cryptococcal infection has been noticed after acquired immunodeficiency syndrome pandemic. *Cryptococcus neoformans* can be isolated from blood in the process of dissemination to brain. We report a case of cryptococcal fungaemia in a patient whose cerebrospinal fluid was negative for *Cryptococcus neoformans*. Retrospective analysis revealed human immunodeficiency virus seropositivity of the patient. He was treated with amphotericin B and fluconazole. Antiretroviral therapy was started, however, the patient succumbed to the infection.

Key words: *Cryptococcus neoformans*, blood culture, acquired immunodeficiency syndrome

Cryptococcosis is one of the acquired immunodeficiency syndrome (AIDS) defining illnesses. In 45% of AIDS patients, *Cryptococcosis* was the first AIDS defining illness.¹ The infection is most common when the CD4⁺ count falls below 200 cells /cubic mm. Cryptococcal meningoencephalitis is the most commonly encountered manifestation of *Cryptococcosis*. The diagnosis of Cryptococcal meningoencephalitis can be very difficult, given the sub acute onset of symptoms and the nonspecific presentation.² *Cryptococcus neoformans* can be identified by its characteristic round budding yeast cell with capsule. We report a case of *Cryptococcal fungaemia* in a human immunodeficiency virus (HIV) positive patient whose cerebrospinal fluid (CSF) was negative for *Cryptococcus* by India ink preparation and culture.

Case Report

A 55-year-old male complained of headache for one week and fever for five days. Fever was continuous, high grade, associated with chills and rigors. He also had an episode of vomiting. Diagnosis of pyogenic meningitis was made in a private hospital and he was treated with intravenous antibiotics for two days. On the third day, he developed altered sensorium and he was referred to our hospital on February 5th 2005 as a case of partially treated pyogenic meningitis.

On examination, the patient was febrile, disoriented, heart rate - 106/minute, BP-128/72 mm/Hg. Respiratory and cardiovascular systems were normal. Abdomen was soft with no organomegaly. Examination of central nervous system revealed grade II altered sensorium, irritability and pupils

reacting equally. There were no cranial nerve palsies. Tone was increased in all four limbs. Deep tendon reflexes were exaggerated and there was plantar extensor on both sides. Neck stiffness was present.

Haemogram was within normal limits. Blood urea was 65 mg%. Random blood sugar was 103 mg%. Serum electrolytes were within normal limits. Liver function tests were normal. CT scan showed no abnormalities.

CSF was sent to microbiology laboratory on the day of admission. It was turbid and Gram stain showed 7-9 leucocytes per high power field and no bacteria. India ink was negative for any capsulated organism. Culture was sterile after 48h of aerobic incubation.

Blood was sent for culture on the same day in biphasic medium (brain heart infusion agar and broth). It was incubated at 37°C aerobically. On the fifth day of incubation, the bottle was turbid and subculture was done on 5% sheep blood agar and MacConkey agar. After 24h of incubation, minute colonies were noted on blood agar. Gram stain revealed gram-positive round budding yeast cells. Colonies were then subcultured on chocolate agar and Sabouraud dextrose agar. Christensen's urease were inoculated.³

Meanwhile the patient was treated in intensive care unit with ceftriaxone and dexamethasone. Patient improved and was shifted to the ward.

Retrospective analyses of HIV status revealed that he was positive for HIV 1 antibodies by enzyme-linked immunosorbent assay (ELISA) (Microlisa), which was confirmed by Western blot (Transasia).

Colonies on chocolate agar and Sabouraud dextrose agar also showed gram positive round budding yeast cells. Urease was positive after 48h of incubation. Animal pathogenicity test was done according to standard procedure in Swiss albino

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mice.^{3,4} Modified India ink preparation of mouse brain showed capsulated round budding yeast cells.

Repeat CSF sample was positive for Cryptococcal antigen detection by latex agglutination test (CALAS™) but the culture was negative for *Cryptococcus* even after five days of incubation.

The strain was sensitive to amphotericin B, fluconazole, itraconazole and five fluocytosine and moderately sensitive to ketoconazole.

The patient was treated with liposomal amphotericin B for 11 days followed by amphotericin B for three days. Patient tolerated amphotericin B, renal parameters were normal. Antiretroviral therapy was started. Oral fluconazole was given as maintenance therapy. In spite of treatment, patient succumbed to the infection on May 11th 2005.

Discussion

Cryptococcosis has emerged as an important cause of death in HIV-infected patients. With the advent of AIDS pandemic, a dramatic increase of cryptococcal infections has been observed.⁵ The prevalence of cryptococcal infections among AIDS patients varies from 2-10% in Western Europe and the U.S and up to more than 15% in central Africa and south East Asia.⁶

Cryptococcus neoformans is heterothallic encapsulated yeast. Cryptococcal species are round to oval yeast ranging in size from 3.5-8 µm with single or multiple budding with a narrow neck. Presence of mucopolysaccharide capsule can be seen as a clear halo around the cells in India ink preparation.¹

Cryptococcal infection begins with inhalation of the yeast cells into lungs. Primary infections in lungs are asymptomatic in immunocompetent host. The widespread and acute presentation of cryptococcosis in patients with AIDS shows that the lack of an effective cell mediated immune response results in rapid dissemination of yeasts. Disseminated cryptococcosis in the immunocompromised population can represent either reactivation of latent infection or a primary infection with immediate dissemination.⁴

On some occasions, the diagnosis of cryptococcal meningoencephalitis is made only after *Cryptococcus neoformans* is recovered from another body site. This is especially true with AIDS patients who have a much higher likelihood of having extra neural disease involving the respiratory tract, urinary tract or bloodstream infection at the same time as CNS infection. Blood cultures sent for routine workup in patients with pyrexia of unknown origin in AIDS patients has come positive for *Cryptococcus neoformans* with subsequent lumbar punctures confirming the diagnosis of CNS infection which was relatively asymptomatic. Blood cultures have been positive for cryptococci in about two thirds of AIDS - associated cases of meningoencephalitis.²

In the present case, *Cryptococcus neoformans* was isolated from blood. Like in other bacterial infections, *Cryptococcus* also causes fungaemia before crossing blood brain barrier to cause meningitis. *Cryptococcus* can be isolated from blood in early stage of dissemination.

The incubation of blood culture bottles for seven days at 37°C facilitated the isolation of *Cryptococcus neoformans* in this patient. Care must be taken to look into the morphology of yeast cell. In this case, round budding gram-positive yeast cell gave the first clue to work towards *Cryptococcus* and retrospectively revealed the HIV positive status of the patient. HIV positivity and steroid therapy were the main predisposing factors for cryptococcal infection in our patient.⁷

The probable reason for blood culture positive and repeat CSF culture negative for *Cryptococcus neoformans* in this case could be due to amount of the specimen being submitted for microbiological work-up. In case of CSF, a maximum of 2-3 mL would be available for culture where as for blood culture approximately 8-10 mL of blood is inoculated in biphasic medium (BHI agar and broth). Another possible reason for blood culture being positive for cryptococcosis could be that BHI broth in biphasic medium (not used for CSF) might act as an enrichment medium for *Cryptococcus neoformans*. Furthermore, the period of incubation was longer for blood culture than that for CSF. Primary CSF culture is incubated to a maximum of 48h.

In any case of suspected meningitis, both in adult and paediatric patients, the chance of isolating pathogens would be increased to greater extent if blood culture is sent also for microbiological workup along with cerebrospinal fluid. This case report emphasizes that parallel processing of blood along with CSF in cases of meningitis will increase the yield of the pathogens.

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