CASE REPORT

CRYPTOCOCCUS HUMICOLUS MENINGITIS: FIRST CASE REPORT IN MALAYSIA

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Abstract. We report a case of *Cryptococcus humicolus* meningitis complicated by communicating hydrocephalus in an apparently immunocompetent 49-year-old psychiatric patient from a nursing home. He presented with a history of poor oral intake, weight loss, headache, vomiting, blurred vision, frequent falls and unsteady gait for the previous three months. He had a history of chronic cough, productive of whitish sputum for the previous month but no hemoptysis. Cerebrospinal fluid culture was positive for *Cryptococcus humicolus*. He was treated with intravenous amphotericin B and oral fluconazole and had clinical and microbiological improvement after three weeks of treatment. Unfortunately, the patient acquired nosocomial methicillin-resistant *Staphylococcus aureus* infection and died due to overwhelming sepsis.

Keywords: *Cryptococcus humicolus*, non-neoformans *Cryptococcus*, meningitis

INTRODUCTION

Non-neoformans *Cryptococcus* species are less commonly reported as a cause of human disease and have usually been considered non-pathogenic, ubiquitous and saprophytic. There have been reports of non-neoformans cryptococcal infections over the past few decades due to *C. albidus, C. curvatus, C. laurentii, C. luteolus* and *C. uniguttulatus. Cryptococcus laurentii* and *C. albidus*, together, are responsible for 80% of non-neoformans *Cryptococcus* reported cases (Khawcharoenporn *et al*, 2007). We report a cryptococcal humicolus meningitis, the first reported case in Malaysia.

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CASE REPORT

Mr TMF was a 49-year-old Chinese male who stayed at a nursing home. He was admitted to Kuala Lumpur Hospital on 28 March 2010 with a history of poor oral intake, weight loss, headache, vomiting, blurred vision, frequent falls and unsteady gait for the previous three months. There was no history of loss of

consciousness, fever, seizures, hallucinations, abnormal behavior, weakness or limb numbness. He had a history of chronic cough productive of whitish sputum for the previous month but no hemoptysis. He had been diagnosed with schizophrenia and epilepsy in 1990 and received care at the psychiatric clinic of Kuala Lumpur Hospital. He was treated with clozapine 100 mg twice daily, diazepam 10 mg twice daily and phenytoin 300 mg before bed.

On examination his blood pressure was 126/86 mmHg, pulse rate 87/min and temperature 38ºC. His oxygen saturation was 98% on room air. He was confused, disorientated and had a Glasgow Coma Scale score of 10/15 (E2, M5, V3). His pupils were 3 mm equal bilaterally with sluggish reaction to light. Fundoscopy showed bilateral papilledema. He had neck stiffness and a positive Kernig's sign. He had no cranial nerve palsies and his upper and lower limbs strength was 4/5 with normal deep tendon reflexes and down-going plantar reflexes.

A chest radiograph showed multiple soft fluffy shadows with pleural thickening (Fig 1). A computed tomography (CT) of the thorax showed multiple ill-defined air space opacities of varying sizes involving both lung fields, with air bronchograms and a pleural effusion on the left. There were cavitating lesions in the right middle lobe, in the apicoposterior segment of the left upper lobe and subcentimeter preaortic, aortopulmonary lymph nodes in the lungs. His sputum for acid-fast bacilli (AFB) smear was negative, His erythrocytes sediment rate (ESR) was 63 mm/hr, His Mantoux test was negative and his retroviral screening was negative. His white blood cell count ranged from 4 to 7 X 10^9/l, ruling out clonazapine induced agranulocytosis.

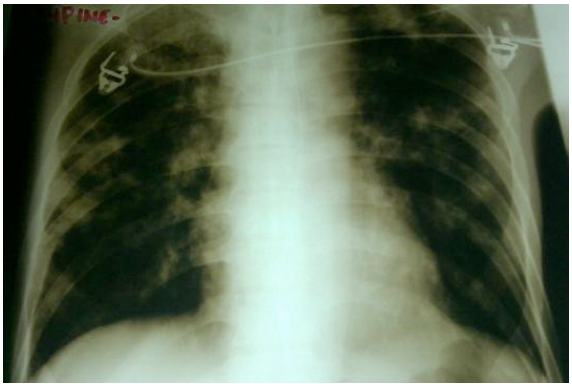

Fig 1–Chest x-ray showing multiple soft fluffy shadows with pleural thickening.

A CT of the brain showed a communicating hydrocephalus with a septic end-arteritic infarction in the left external capsule and the posterior limb of the left internal capsule (Fig 2). A lumbar puncture showed an elevated opening pressure of 36 cm. A Gram stain of the cerebrospinal fluid (CSF) showed 5 polymorpholeukocytes per high powered field. An India ink stain of the CSF showed encapsulated yeast cells. A cryptococcal antigen test (Pastorex™ *Crypto* Plus, Bio-Rad, Hercules, CA) was positive, with a titer of greater than 1:512. A latex agglutination test for bacteria, an acid-fast bacilli (AFB) smear and a mycobacterial culture were negative. The CSF protein was elevated at 1.09 g/l, and the glucose was low at 0.1 mmol/l (random blood sugar was 5.6 mmol/l).

The CSF specimen was cultured on Sabouraud's dextrose agar at 25ºC and yielded glistening light yellow colonies, with a slightly wrinkled texture after 3 day incubation in a Bactec blood bottle (Fig 3). Further identification with ID 32C (bioMérieux, Marcy L'Etoile, France) revealed *C. humicolus*, with a 99.5% specificity. The carbohydrate assimilation reactions were

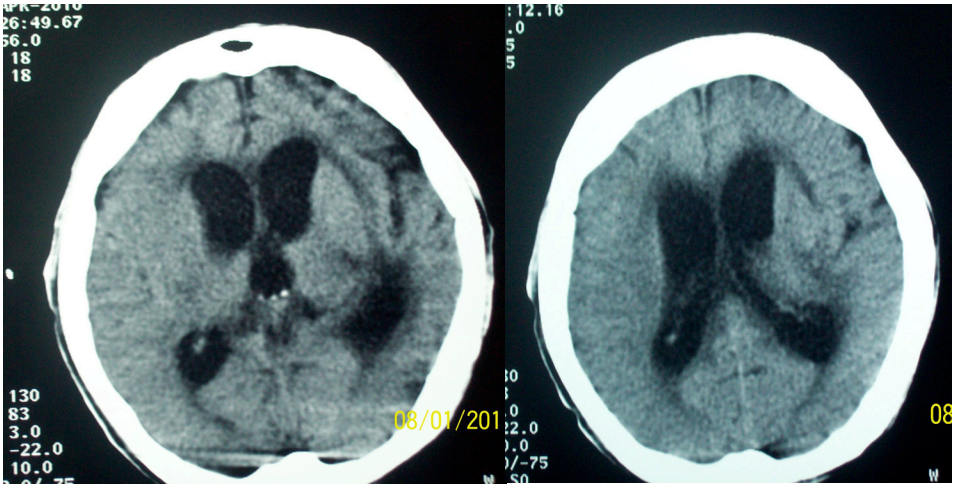

Fig 2–A CT of the brain showing communicating hydrocephalus in the left external capsule, and the posterior limb of the internal capsule.

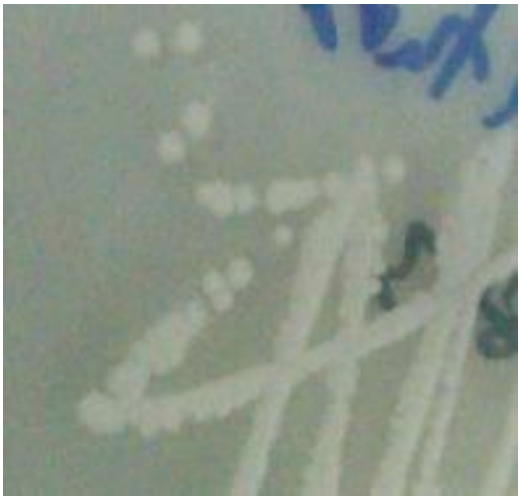

Fig 3–Colonies of *Cryptococcus humicolus* on Sabouraud dextrose agar.

positive for D-galactose, D-saccharose (sucrose), N-acetyl-glucosamine, lactic acid, D-cellobiose, D-maltose, D-trehalose, potassium-2-ketogluconate, methyl-αD-glucopyrasonide, D-sorbitol, D-xylose, D-ribose, glycerol, L-rhamnose, palatinose, erythritol, D-melibiose, sodium glucuronate, potassium gluconate, D-mannitol, D-lactose, inositol, D-glucose, glucosamine and esculin ferric citrate.

The isolated strain had high minimum inhibitory concentrations (MIC) against amphotericin B, fluconazole and itraconazole and was resistant to caspofungin when tested using the E-Test (AB-Biodisk, Solna, Sweden).

The patient was diagnosed as having pulmonary cryptococcosis and *C. humicolus* meningitis complicated with communicating hydrocephalus. He was started on intravenous amphotericin B 35 mg daily (0.7 mg/kg/day) as induction therapy for 6 weeks, followed by fluconazole 400 mg twice daily for eight weeks as consolidation therapy and then fluconazole 200 mg daily for one year as maintenance therapy.

He was referred to the neurosurgeon for insertion of an extra ventricular drain (EVD) to manage the hydrocephalus. Another culture of the CSF, India ink stain and Gram's stain revealed the continuing presence of encapsulated yeast cells. A cryptococcal-antigen latex agglutination test was still positive with the same titer. Repeat blood and sputum cultures for fungus were negative.

Clinical improvement was seen by three weeks of treatment along with a drop in the cryptococcal antigen titer. Three consecutive CSF cultures were sent during the third and fourth weeks of treatment with intravenous amphotericin B which showed no fungal growth. Despite negative fungal culture results, the patient deteriorated throughout his stay in the hospital due to multiple bouts of nosocomial infections which include *Enterococcus* sp and *Pseudomonas aeruginosa* in the urinary tract, *Klebsiella* sp pneumonia (extended spectrum beta-lactamase positive) and methicillin-resistant *Staphylococcus aureus* (MRSA) infection of the CSF and external ventricular shunt.

Although treated with multiple courses of antibiotics for the nosocomial infections with intravenous cefepime 1 g twice daily for one week, followed by intravenous meropenem 1g three times daily and intravenous vancomycin 1 g twice daily, the patient succumbed to his illness on day 40 of hospitalization as a result of nosocomial sepsis. No autopsy was performed at the request of the family.

DISCUSSION

Cryptococcus humicolus is a non-neoformans *Cryptococcus* species reported to cause significant disease among humans. The first case was described in 2004 by Shinde *et al* (2004) who described the case of a 7-year-old boy with *C. humicolus* fungemia who presented with fever, cough, weight loss and dyspnea. His symptoms and signs mimicked those of tuberculosis and he was treated with anti-tuberculosis medication until his blood culture grew *C. humicolus*. He was successfully treated with intravenous liposomal amphotericin B in combination with fluconazole and had improvement after 3 weeks of the antifungal treatment. In a case reported by Baka *et al* (2007) a 39-year-old man with a history of sigmoidectomy and colostomy developed permeative peritonitis and diverticulitis due to *C. humicolus*. *C. humicolus* was isolated from a blood culture and he was successfully treated with intravenous amphotericin B, voriconazole and fluconazole for three weeks.

Risk factors for *Cryptococcus* infection mainly include newly diagnosed HIV infection, high-dose corticosteroid use, immunosuppressive agents such as alemtuzumab and infliximab, and occasionally non-immunosuppressed patients (Park *et al*, 2009). Thirty-eight percent of non-neoformans cryptococcosis cases were associated with permanent or temporary catheters (vascular or peritoneal) and central nervous system drains or shunts (McCurdy and Morrow, 2003). In this case, the risk factor/s could not be identified.

The clinical presentation of *C. humicolus* meningitis in this patient were similar to *C. neoformans* and other non-neoformans cryptococcus meningitis cases; however the onset of illness was deemed longer, possibly because the patient was immunocompetent (Perfect, 2005). The cryptococcal meningitis in this patient was thought to be a secondary infection with the primary site being pulmonary. The presentation resembled that of tuberculous and was treated as tuberculosis until CSF cultures revealed *C. humicolus*. An India ink smear of the CSF was positive for encapsulated yeasts and cryptococcal antigen was found in the CSF. The sensitivities of these two tests for detecting non-neoforman cryptococcus infection are 60% and 25%, respectively, depending on the extent of disease (Khawcharoenporn *et al*, 2007). Although the manufacturer

of Pastorex™ *Crypto* Plus (Bio-Rad) has stated their product is intended to detect soluble *Cryptococcus neoformans* antigens, occasional cross-reactivity may occur. The capsular antigen, glucuronoxylomannan (GXM), can be found in other non-neoformans *Cryptococcus* spp infections, including *C. humicolus* (Dromer *et al*, 1987, 1993). Polymerase chain reaction (PCR) detection remains the most rapid, sensitive and specific method for detecting all types of *Cryptococcus* infection, but the gold standard for laboratory identification of *Cryptococcus* species is culture (McCurdy and Morrow, 2003; Perfect *et al*, 2005).

Morphologically, *C. humicolus* colonies are cream, yellow or buff in color (Fig 3). It produces true and pseudo-hyphae on cornmeal agar while other *Cryptococcus* species produce only blastoconidia. Phylogenetically, *C. humicolus* has a close relationship to *Cryptococcus curvatus* and *Trichosporon* sp. The diagnosis can be made with a positive culture and standard identification techniques for yeast (Sugita *et al*, 2000; Takashima *et al*, 2001; Mycology Critique, 2003).

Spontaneous recovery of non-neoformans cryptococcal infection may occur in less severe cases with no central nervous system involvement. Non-pharmacologic treatment, such as catheter or infected-tissue removal, may be successful alone or in combination with antifungal agents. Amphotericin B, at a similar dose and duration as that recommended for *C. neoformans* infection, can be effective for the treatment of non-neoformans cryptococcal meningitis, pneumonia, lung abscess and fungemia. Most cases are treated with fluconazole during the maintenance phase (Khawcharoenporn *et al*, 2007). Although patients show clinical and microbiological improvement after three weeks of antifungal treatment, complete eradication of *Cryptococcus* organisms from the CSF may be difficult to ascertain. Although our reported case did not have HIV, his age (≥ 45 years) and CNS involvement are independent predictors of poor prognosis (Khawcharoenporn *et al*, 2007). Since there is lack of data and guidelines for treatment of non-neoformans cryptococcal infection, the guidelines for the treatment of *C. neoformans* meningitis, as specified by the Infection Disease Society of America (IDSA) were adopted (Perfect *et al*, 2010).

In conclusion, with the advent of more advanced laboratory fungal identification techniques, more non-neoformans cryptococcal isolates are being isolated among at-risk patients. In this case, *C. humicolus* infection presented as a subacute infection and clinically resembled TB. Therefore, a high clinical suspicion must be maintained and early institution of appropriate treatment should be made to have a favorable outcome.

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