Case report

Cryptococcus neoformans var. *gattii* meningitis in Egypt: a case report

A. Mansour,[1] I. Nakhla,[1] M. El Sherif,[1] Y.A. Sultan[2] and R.W. Frenck[1]

Introduction

Cryptococcus neoformans is an encapsulated yeast ubiquitous in nature which, when it infects humans, typically causes pulmonary disease and occasionally meningitis [*1*]. Serotyping of the organism has identified 3 main varieties: *C. neoformans* var. *grubii* (serotype A), *C. neoformans* var. *neoformans* (serotype D) and *C. neoformans* var. *gattii* (serotypes B and C) [*2*]. Serotypes A and D have been isolated throughout the world and have been associated with birds [*1*]. While *C. neoformans* var. *gattii* has been more frequently associated with disease in tropical and subtropical areas, infections throughout the world, including the United States and Europe, have been reported [*3,4*]. Eucalyptus trees appear to be the principle reservoir for the organism but the infection has been diagnosed in areas lacking eucalyptus, indicating that other environmental sources exist [*3,5*]. However, prolonged incubation periods can make it difficult to identify the true sources of exposure [*6*].

Cryptococcosis has rarely been reported from Egypt although a number of factors would predict the country to be an area where the fungus would be common. In the current report, we describe the first case of *Cryptococcal neoformans* var. *gattii* serotype (B) from Egypt, in a patient positive for human immunodeficiency virus (HIV). Future work should be performed to better understand the epidemiology and prevalence of cryptococcal disease within the region.

The case

A 38-year-old Egyptian male was reported to be in excellent health until 2 weeks before hospitalization when he developed fever and confusion which, over the ensuing 48 hours, progressed to visual hallucinations along with urinary incontinence. The day prior to admission, the patient developed a cough, neck stiffness and tonic–clonic seizures leading him to seek medical attention. The patient had no known pet or animal exposures but many pigeons were reported to live near his home. The patient lives in the Nile river delta, an area where eucalyptus trees are common. The patient reported no recent travels, denied any high-risk behaviour and had no history of blood transfusions.

The patient was thin, moderately ill with an altered mental state, oriented only to person. He had an oral temperature of 39 ºC, pulse of 120 beats/minute and a blood pressure of 110/80 mmHg. The patient had

[1]*US Naval Medical Research Unit, Abbassia, Cairo, Egypt (Correspondence to R.W. Frenck: rfrenck@uclacvr.labiomed.org).*
[2]*Abbassia Fever Hospital, Cairo, Egypt.*
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marked nuchal rigidity and diminished deep tendon reflexes but the physical examination was otherwise unremarkable.

The peripheral white blood cell count was 2600 cells/mm^3 with 89% polymorphs, 10% lymphocytes and 1% monocytes. Analysis of the cerebral spinal fluid (CSF) revealed a white cell count of 30 cells/mm^3 (46% polymorphs), a protein level of 92 mg/dL and glucose of 13 mg/dL. Gram stain and Ziehl–Nielsen stain of the CSF were negative. A tuberculin skin test was non-reactive and 3 induced sputum samples were normal. A serum HIV enzyme-linked immunosorbent assay (ELISA) test was positive and confirmed by Western blot. A computerized tomography scan of the brain revealed mild ventricular dilatation along with a hypodensity in the deep left parietal region.

A diagnosis of bacterial meningitis was made and parenteral ampicillin and chloramphenicol were administered. Two days later, due to lack of clinical improvement, an aliquot of the admission CSF was tested and found to have a cryptococcal antigen titre of 1:8192 (Crypto-LA, Wampole Laboratories, Cranbury, New Jersey, USA). The following day, the laboratory notified the clinicians that *C. neoformans* was isolated from the CSF. Using the Crypto Check kit (Iatron Laboratories, Japan), the isolate was sub-grouped and found to be *C. neoformans* var. *gattii* (serotype B).

Therapy was changed to amphotericin B (0.5 mg/kg/day), and over the ensuing 3 weeks the patient become afebrile, his mental status improved significantly and his CSF culture became sterile. In addition, the CSF cryptococcal antigen titre decreased to 1:4096. At this time, the patient left the hospital against medical advice and, despite numerous attempts to contact him, was lost to further follow-up.

Discussion

C. neoformans var. *gattii* has usually been isolated from patients without obvious immune problems, unlike other varieties of *C. neoformans*, which more commonly cause disease in immunocompromised patients, especially patients with HIV [*7*]. The rarity of *C. neoformans* var. *gattii* in immunocompromised patients remains unexplained. One possibility is that people have a relative lack of exposure to *C. neoformans* var. *gattii* as compared to *C. neoformans* var. *neoformans*. However, in a report from Australia where both varieties of the fungus are common, *C. neoformans* var. *gattii* was still principally isolated from patients with apparently intact immune systems [*8*]. This suggests an alternative explanation in which disease with *C. neoformans* var. *gattii* is in part related to an intact immune system reaction to the fungus [*2*]. However, as demonstrated by the present case, where the patient is infected with HIV, the fungus can cause an infection regardless of the immune status of the patient.

C. neoformans has been isolated from the environment in Egypt including from the flower of the *Eucalyptus camaldulensis* variety [*9,10*]. However, reports of infections in humans in Egypt, particularly meningitis, are rare. The largest reported outbreak to date was an autopsy series which isolated *C. neoformans* var. *neoformans* from the meninges of 4 patients who had died of meningitis [*11*]. To our knowledge, the present case is the first isolate of *C. neoformans* var. *gattii* known to have been associated with human disease in Egypt.

Cryptococcal meningitis is typically a sub-acute infection with patients complaining of 2–4 weeks of headache, fever, lethargy, coma or memory loss prior to

diagnosis [*2*]. Definitive differentiation between the varieties of *C. neoformans* is not possible using only clinical criteria. However, certain features may suggest *C. neoformans* var. *gattii* is the causative agent. Intracerebral cryptococcomas appear to be more commonly associated with *C. neoformans* var. *gattii* [*7*]. Additionally, a more severe and aggressive clinical course than expected may lead the clinician to suspect *C. neoformans* var. *gattii* [*12*]. While response to therapy may be slower and morbidity greater in patients infected with *C. neoformans* var. *gattii*, mortality is higher in patients infected with *C. neoformans* var. *neoformans* [*13*]. The reason for the discrepancy between morbidity and mortality caused by the 2 varieties of *C. neoformans* is unclear, but may be because *C. neoformans* var. *gattii* occurs more commonly in otherwise healthy patients while *C. neoformans* var. *neoformans* occurs more often in patients with underlying immune problems.

C. neoformans can be diagnosed from stains, cultures or latex agglutination tests but speciation requires specialized growth media or capsular serotyping. *C. neoformans* var. *gattii* typically produce green-pigmented colonies on Staib niger seed (*Guizotia abyssinica*) agar media and if grown in tryptophan-containing media will produce a brown pigment. Additionally, *C. neoformans* var. *gattii* can use glycine as a sole carbon source, whereas cryptococcal serotypes A and D generally cannot [*14*]. When *C. neoformans* var. *gattii* is grown on agar containing L-canavanine, glycine and bromothymol blue, alkalinization of the medium results from the ammonia released during glycine degradation and causes the agar to turn blue in contrast to cryptococcal serotypes A and D where the medium remains yellow [*14,15*]. At the molecular level, *C. neoformans* var. *gattii* can be differentiated from other cryptococci by analysis of DNA base composition and sequence homology [*5*].

The Crypto Check kit (Iatron) was used for speciation of the isolate recovered from our patient. The test is a simple and practical method to differentiate cryptococci using 5-factor sera. The sensitivity and specificity of the test can be further increased by using it in conjunction with the phenol oxidase test and nitrate reduction test [*16*].

In conclusion, we report the first known human case of *C. neoformans* var. *gattii* in Egypt. Cryptococcal meningitis in Egypt is rarely diagnosed but this may be due to inadequate investigation rather than absence of the organism. *C. neoformans* should be considered as a cause of meningitis in Egypt, especially in a patient with unexplained, chronic meningitis that is not responding to conventional therapy.

References

1. Levitz SM. The ecology of *Cryptococcus neoformans* and the epidemiology of cryptococcosis. *Reviews of infectious diseases*, 1991, 13:1163–9.
2. Perfect JR, Casadevall A. Cryptococcosis. *Infectious disease clinics of North America*, 2002, 16:837–74.
3. Kwon-Chung KJ, Bennett JE. Epidemiologic differences between the two varieties of *Cryptococcus neoformans*. *American journal of epidemiology*, 1984, 120:123–30.
4. Kwon-Chung KJ et al. Virulence, serotype, and molecular characteristics of environmental strains of *Cryptococcus neoformans* var. *gattii*. *Infection and immunity*, 1992, 60:1869–74.

5. Chen SC et al. *Cryptococcus neoformans* var. *gattii* infection in northern Australia: existence of an environmental source other than known host eucalypts. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1997, 91:547–50.

6. Hajjeh RA, Brandt ME, Pinner RW. Emergence of cryptococcal disease: epidemiologic perspectives 100 years after its discovery. *Epidemiologic reviews*, 1995, 17:303–20.

7. Sorrell TC. *Cryptococcus neoformans* variety *gattii*. *Medical mycology*, 2001, 39: 155–68.

8. Chen S et al. Epidemiology and host- and variety-dependent characteristics of infection due to *Cryptococcus neoformans* in Australia and New Zealand. Australasian Cryptococcal Study Group. *Clinical infectious diseases*, 2000, 31:499–508.

9. Soliman A et al. Cryptococcal meningitis in Cairo, Egypt: report of five cases. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1995, 89: 410.

10. Abdel-Salam HA. Characterization of *Cryptococcus neoformans* var. *neoformans* serotype A and A/D in samples from Egypt. *Folia microbiologica*, 2003, 48:261–8.

11. Girgis NI et al. Fatal cryptococcal meningitis in four Egyptian patients. *Ain Shams medical journal*, 1985, 36:93–9.

12. John R, Perfect AC. Cryptococcosis. *Infectious disease clinics of North America*, 2002, 16:837–74.

13. Speed B, Dunt D. Clinical and host differences between infections with the two varieties of *Cryptococcus neoformans*. *Clinical infectious diseases*, 1995, 21: 28–34; discussion 35–6.

14. Min KH, Kwon-Chung KJ. The biochemical basis for the distinction between the two *Cryptococcus neoformans* varieties with CGB medium. *Zentralblatt fur Bakteriologie, Mikrobiologie, und Hygiene. Series A*, 1986, 261:471–80.

15. Kwon-Chung KJ et al. Urease inhibition by EDTA in the two varieties of *Cryptococcus neoformans*. *Infection and immunity*, 1987, 55:1751–4.

16. Kabasawa K et al. Evaluation of a new method for identification of *Cryptococcus neoformans* which uses serologic tests aided by selected biological tests. *Journal of clinical microbiology*, 1991, 29: 2873–6.