Cerebellar cryptococcoma due to Cryptococcus gattii VGI; a rare and first report from India

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Introduction: Cryptococcus gattii is an emerging global pathogen. Recent reports suggest that C. gattii cryptococcosis is more common in immunocompetent as well as HIV-infected patients than earlier estimated. We report the first case of cerebellar cryptococcoma from India due to C. gattii in an immunocompetent individual.

Case Presentation: A 73-year-old immunocompetent male, presented with a 3 month history of severe headache, neck stiffness, walking difficulty, bilateral papilloedema and slight cerebellar signs including gait imbalance and giddiness. Magnetic resonance imaging (MRI) revealed a well-defined enhancing lesion in the cerebellum, suggestive of abscess and mild hydrocephalus. Blood parameters and liver function tests were within normal limits. The patient underwent paramedian suboccipital craniotomy, and the aspirated purulent material grew Cryptococcus neoformans. Histologically, the lesion was a cryptococcoma. The isolate was further identified as C. gattii VGI, serotype C, mating type alpha, and produced extracellular phospholipase enzyme. It was sensitive to amphotericin B, 5-flucytosine and azoles. The patient was treated with intravenous amphotericin B (0.7 mg kg\(^{-1}\) day\(^{-1}\)) for 15 days along with a maintenance dose of oral fluconazole (400 mg day\(^{-1}\)) for 6 months. The patient’s symptoms recovered while on treatment but he was lost to follow up.

Conclusion: Cryptococcus gattii is an emerging pathogen in India that affects otherwise healthy, immunocompetent individuals and requires rapid identification and treatment in order to prevent severe neurological sequelae.

Keywords: cerebellar cryptococcoma; Cryptococcus gattii; immunocompetent; antifungal susceptibility.

Introduction
Cryptococcus neoformans (anamorph) is an encapsulated fungus that belongs to the phylum Basidiomycota. Although the genus Cryptococcus contains more than 50 species, only Cryptococcus neoformans and Cryptococcus gattii are considered human pathogens. Infection of the central nervous system (CNS) caused by Cryptococcus neoformans usually presents with either meningeal or parenchymal manifestation (Li et al., 2010). Meningitis is the most common presentation, accounts for up to 85% of the total and is more common in immunocompromised patients (Morãnovã et al., 2013). Cryptococcoma is a rare entity, seen more frequently in immunocompetent patients, and the symptoms are consistent with those of an intracranial mass (Li et al., 2010; Gültash et al., 2007). Among all the cases of intracranial cryptococcoma reviewed, formation of lesions has been described in ventricles, basal ganglia, pons, spinal cord, frontal-, parietal-, temporal-, and occipital-lobes, pituitary gland and cerebellum (Li et al., 2010) caused by both C. neoformans and C. gattii (Li et al., 2010; Gültash et al., 2007).

Due to the importance of the C. neoformans/C. gattii species complex as a human fungal pathogen, several research groups are currently focusing on the molecular determination of the number of genetically diverged subgroups within each species (Cologni, 2013). Based on multi-locus sequence typing (MLST) and amplified fragment length polymorphism (AFLP) analysis, C. neoformans and C. gattii

Abbreviations: AFLP, amplified fragment length polymorphism; CNS, central nervous system; MLST, multi-locus sequence typing; PAS, periodic acid-Schiff.
have been further classified into several distinct genotypes: AFLP1/VNI and AFLP1A/1B/VNI (C. neoformans var. grubii), AFLP2/VNIV (C. neoformans var. neoformans), AFLP3/VNIII (hybrid serotype AD) and AFLP4/VGII, AFLP6A/VGIIa, AFLP6B/VGIIb, AFLP6C/VGIIc, AFLP5/VGIII, AFLP7/VGIV and AFLP10/VGIV (C. gattii, serotype B/C) (Cogliati, 2013). Cryptococcus gattii AFLP4/VGII and AFLP6/VGII have been reported to infect immunocompetent hosts causing meningitis and pulmonary cryptococcosis, whereas AFLP5/VGIII, AFLP7/VGIV and AFLP10/VGIV appear to infect primarily immunocompromised patients (Cogliati, 2013).

Cryptococcus gattii was originally thought to be geographically restricted to tropical and subtropical regions. Since the outbreak of C. gattii infection in immunocompetent individuals in the east coast of Vancouver Island and British Columbia, Canada (Cogliati, 2013), it has been isolated from clinical and environmental samples from different parts of the world including India, Australia, Oregon, Washington and Southern California in the USA and sub-Saharan Africa. In north and north-western regions of India, VGII, VGIII and VGIV appear to be more prevalent in the environment (Chowdhary et al., 2012). From 2005 to 2013, a total of 16 cases of C. gattii meningitis have been reported from Bangalore and other parts of India (Cogliati, 2013; Gurung et al., 2012; Cogliati et al., 2012), whereas CNS cryptococcoma by C. gattii had not yet been reported. To the best of our knowledge, this is the first report of cerebellar cryptococcoma from India, an extremely rare site of isolation, which was microbiologically analysed.

Case

A 73-year old man from the north-east region of India presented to our tertiary neuro-care centre with a 3 month history of severe throbbing holocranial headache, projectile vomiting, loss of appetite and gait imbalance. He was bed bound and had no history of fever, seizures, hypertension, diabetes mellitus, blood transfusion, organ transplantation or surgery. He was apparently immunocompetent with an unremarkable physical examination. He was an alcoholic and a smoker but had quit a few months before the onset of clinical symptoms. He was conscious and oriented with normal vital parameters. There was bilateral papilloedema, with giddiness and neck stiffness but no sensory or motor deficits.

Investigations

Complete haemogram, serum electrolytes and liver function tests were within normal limits, and the patient was seronegative for HIV1 and HIV2 antibodies tested as per National AIDS Control Organization (NACO) guidelines. His CD4 count was 517 cells mm\(^{-2}\).

MRI of the brain revealed a well-defined thick ring enhancing lesion with an irregular inner wall in the right cerebellar hemisphere, hypo-intense on T1, hyper-intense on T2 with restricted diffusion and no bleed. Perilesional oedema was minimal, causing mild hydrocephalus (Fig. 1).

Diagnosis and treatment

The patient underwent right paramedian suboccipital craniotomy, purulent material was aspirated, and the lesion was excised. The thick, yellow-coloured non-foul-smelling pus admixed with blood was considered for microbiological analysis. A wet-mount preparation revealed numerous degenerated polymorphonuclear cells, red blood cells, and round yeast-like cells. Gram-stain revealed Gram-positive, round budding yeast cells suggestive of Cryptococcus with no bacteria. On Sabouraud dextrose agar (SDA) incubated at 37 °C following 72 h, cream coloured, round, smooth, moist colonies were seen which was further identified as var. gattii by canavanine glycine bromothymol blue agar (Casali et al., 2003).

Antifungal susceptibility by broth microdilution (according to CLSI guidelines M27-A3) and VITEK2C (bioMérieux) showed sensitivity to amphotericin B, fluconazole, 5-flucytosine and voriconazole. Extracellular phospholipase \(\beta\) production on egg yolk agar plates demonstrated production of virulent enzyme by the isolate (Casali et al., 2003).

Genomic DNA isolation and mating type determination was performed by the method described by Casali et al. (2003). The isolate was detected as serotype C by the Lac1 multiplex PCR method as described by Ito-Kuwa et al. (2007). PCR fingerprinting by M13 minisatellite primer confirmed it as C. gattii VGI (Casali et al., 2003).

Histologically the cerebellar lesion was composed of focal necrosis, lobules of capsulated yeast forms walled by inflammatory tissue and infiltrated by lymphocytes, macrophages, plasma cells and occasional multinucleate giant cells (Fig. 2). The round, medium-sized budding forms of the fungi with wide, pale and transparent capsules were positive for polysaccharide on periodic acid-Schiff (PAS) and mucicarmine staining.

Thus, the patient was diagnosed to have intracranial cryptococcoma of cerebellar origin by C. gattii VGI, serotype C, which was a sensitive strain.

Following surgery, the patient was started on intravenous amphotericin B (0.7 mg kg\(^{-1}\) day\(^{-1}\)), which was increased gradually from lower to higher doses for 15 days continuously, in combination with oral fluconazole 400 mg day\(^{-1}\). The symptoms resolved, and maintenance therapy with fluconazole (400 mg day\(^{-1}\)) was advised on discharge, but the patient was lost to follow up.

Discussion

Patients infected with C. gattii are likely to develop a cerebral mass and eventually abscess (Gültaslı et al., 2007). The organism releases two novel metabolites, acetoin and...
dihydroxyacetone, along with polyols, which are pro-inflammatory factors. These factors inhibit neutrophil function, promoting the survival of extracellular organisms and localized multiplication to form cryptococcomas. (Wright et al., 2002; Colombo et al., 2014). Almost 53% of intracranial cryptococcomas have been misdiagnosed as vascular lesions, tuberculomas or tumours. Posterior fossa mass lesions are rarely diagnosed as Cryptococcoma; hence reports on cerebellar cryptococcoma are limited. In the late stages, it may mimic brain abscess (Li et al., 2010; Harris et al., 2013).

A series of intracranial cryptococcomas by *C. neoformans* and *C. gattii* have been reviewed along with isolated case reports from Brazil, China, Germany, India, Japan, Mexico, Morocco, Spain, Thailand, Uganda and the USA. (Sillero-Filho et al., 2009; Li et al., 2010; Jung et al., 2012; Rai et al., 2012; Okamoto et al., 2010; Hernández et al., 2013; Billah et al., 2014; Colom et al. 2005; Nakwan et al., 2009; Velamakanni et al., 2014; Byrnes et al., 2009). Most patients diagnosed with *C. gattii* intracranial cryptococcoma were immunocompetent; and most of them developed obstructive hydrocephalus as a consequence of the infection.

During our literature review we found only seven English-language case reports of cerebellar cryptococcoma caused by *C. gattii* (Li et al., 2010; Colom et al. 2005; Velamakanni et al., 2014). Among them, three cases were treated with only antifungal drugs and four received surgical intervention along with antifungal treatment. Three of the patients recovered, three died after treatment, and one case was lost to follow up. In the present case the patient recovered after surgery and treatment.

Among the reports of CNS cryptococcoma caused by *C. gattii*, molecular characterization was performed for only three isolates, two of which were VGI (USA, Spain) (Colom et al. 2005; Trilles et al., 2004) and one was VGIIa (Japan) (Okamoto et al., 2010). Our isolate was also analysed.

**Fig. 1.** MRI of brain showing a lesion in the right cerebellar hemisphere, T1 hypo- and T2 hyper-intense, with hyper-dense enhancement on contrast and mild hydrocephalus.
to determine the molecular subtype, hence these data will contribute to the epidemiology of *C. gattii* in India.

Worldwide reports of clinical cases of *C. neoformans* and *C. gattii* showed a higher prevalence of the a-mating type, which was similar to our result. Mating type a of *C. gattii* was also detected in environmental samples collected from north and north-western India (Chowdhary *et al.*, 2012). The north-eastern parts of India have an ideal habitat for a wide variety of vegetation which could be the potential source of infection. In the present case, the acquisition of infection through vegetation is plausible, as the patient had no direct contact or exposure to pigeons.

In most of the case studies, serotype B of *C. gattii* has been reported. The present isolate was serotype C, which was previously found in four patients with cryptococcal meningitis from our hospital in earlier studies (Cogliati *et al.*, 2012).

The limited number of studies available on antifungal susceptibility of *C. gattii* suggests that it is susceptible to amphotericin B, 5-flucytosine and voriconazole like var. *neoformans*, whereas it shows decreased susceptibility to fluconazole unlike var. *neoformans* (Trilles *et al.*, 2004). Our isolate was a strain sensitive to all antifungal drugs mentioned above, which facilitated the patient’s treatment.

It is noteworthy that our earlier reported cases of cryptococcal meningitis due to *C. gattii* were also VGI and resembled African strains (Cogliati *et al.*, 2012).

The availability of whole genome sequences of *C. gattii* strains and the recent progress in molecular analysis at the subspecies level have greatly enhanced the understanding of the epidemiology of this pathogenic yeast.

Identification of this grave pathogen at the subtype level is essential to assess the global occurrence, its evolution and spread of the fungus, as *C. gattii* is more resistant to treatment and could cause long-term sequelae. It appears that the VGI genotype may be emerging as a cause of infection in the Indian population.

**Fig. 2.** Histopathology of cerebellar cryptococcoma. (a) Pale islands of Cryptococci surrounded by inflammatory tissue within the cerebellum, × 12.5. (b) A closer view of the wall reveals dense inflammation with several round capsulated yeasts, × 200. Inset shows the round basophilic wall of the organism surround by a pale to eosinophilic capsule, × 400. (c) A multinucleated giant cell with engulfed yeasts on a background of lymphocytes, × 400. (d) Budding form of the yeast, × 400. (e) PAS staining of the capsules, × 400. Tissue in a–d was stained with haematoxylin and eosin.
Microbiological and molecular analysis should be routinely applied for the diagnosis and treatment of the condition like cryptococcoma, which will in turn relieve clinical and radiological diagnostic dilemmas to distinguish it from other mass lesions.

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The study was approved by Institutional Ethical Committee, NIMHANS, Bangalore, India. Informed consent was given by the patient.

References


