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ORIGINAL PAPER

Epidemiological, Clinical and Outcome Aspects of Patients with Cryptococcosis Caused by *Cryptococcus gattii* from a Non-endemic Area of Brazil

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Abstract Cryptococcosis by *Cryptococcus gattii* occurs mainly in immunocompetent hosts, however, during the last decades, a growing number of cases in immunocompromised individuals have been noticed around the world. This report presents epidemiological, clinical and outcome aspects of patients with cryptococcosis caused by this species from a non-endemic area in Brazil. Of 278 *Cryptococcus* spp. clinical isolates recovered during the same period, 267 (96%) were molecularly identified as *Cryptococcus neoformans* VNI genotype and 11 (4%) as *C. gattii* VGII genotype by *URA*-5 RFLP. Of the 11 *C. gattii* patients, eight were male, mean age of 47.5 years. Of these, four were HIV-infected, one was kidney transplanted, one presented low $CD4^+$ T cells values of unknown cause, another presented chronic liver disease meanwhile the remaining four were apparently immunocompetent. Disseminated disease and cryptococcal meningitis were present in four patients each. Most patients received amphotericin B plus fluconazole. Seven out of the 11 patients cured and four died before or during the therapy. The increased number of individuals with cryptococcosis by this species during the last decades needs to be carefully evaluated specially those who are HIV-infected. Nevertheless, *Cryptococcus* species differentiation is currently relevant in order to better know their relation with geographical, clinical host preference and outcome particularities.

Keywords Cryptococcosis · *Cryptococcus gattii* · *URA*5-RFLP · HIV infection · VGII genotype · Cryptococcal meningitis · AIDS · Immunocompetent host

Introduction

Cryptococcus neoformans is worldwide distributed and causes cryptococcal meningitis in immunocompromised hosts, mostly HIV-infected who present advanced immunodeficiency and severe fungal disease at admission. Consequently, high mortality rates of 60–70% are still reported from limited-resource settings [1, 2]. Furthermore, 17–22% of diagnosed cases have no predisposing factors and develop severe pulmonary or extra-pulmonary cryptococcosis [3]. Otherwise, *Cryptococcus gattii* formerly restricted to tropical and subtropical regions causes disease predominantly in immunocompetent individuals.

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During the last decades, several outbreaks of cryptococcosis by this species occurred in temperate regions from North America which involved nearly 40% of immunocompromised individuals [4–7]. This fact supports the geographical expansion of *C. gattii* and could suggest changes in its host preference as evidenced by the increased rates of cryptococcosis in HIV-infected patients reported from several regions around the world during the last years [8–10].

Several authors have pointed out differences on clinical and outcome features between patients with *C. gattii* infection and those with *C. neoformans* infection [11, 12]. Patients with cryptococcosis by *C. gattii* can evolve with more morbidity and poor outcome and this fact would be associated with more severe pulmonary and cerebral cryptococcomas which even require surgery resection and longer periods of antifungal therapy [11, 13]. Otherwise, different authors have suggested that the host immune status is more pivotal to define the clinical picture and outcome than the cryptococcal species involved [14, 15]. In immunocompetent individuals, *C. gattii* infection often presents a less subacute picture during several weeks or months and can be mistaken with other infectious or non-infectious diseases especially when lung and brain cryptococcomas are seen on computerized tomography (CT) or magnetic resonance (MRI) images [16, 17].

Usually, cryptococcal meningitis by *C. gattii* in HIV-infected patients presents more subacute evolution similar to that observed in *C. neoformans* despite, there are scarce available data and few differences related to clinical picture and in-hospital mortality rates [9, 18]. This study aims to present some epidemiological, clinical and outcome features of patients with cryptococcosis by *C. gattii* diagnosed at the teaching hospital from a non-endemic area in Brazil focusing on HIV-infected cases.

Population and Methods

In order to identify cryptococcosis cases admitted at the teaching hospital of Triângulo Mineiro Federal University in Uberaba, Minas Gerais, Brazil, from 1998 to 2017, the micological diagnosis records were retrospectively reviewed. Cryptococcosis case definition was based on the clinical picture (pulmonary, meningeal or cutaneous involvement) plus a *Cryptococcus* sp. positive culture on Sabouraud dextrose Agar, with or without India ink positive stain or a positive Latex-crypto antigen test (IMMY Mycologics Inc, OK, USA). Among the confirmed cases, those who presented *C. gattii* infection diagnosed by L-Canavanine-glycine-bromothymol blue (CGB) agar and orotidine monophosphate pyrophosphorylase (*URA5*) gene restriction fragment length polymorphism (RFLP) analysis were selected [19, 20]. Demographic, epidemiologic, clinical and outcome data of these patients were obtained from their medical records, registered and analyzed through descriptive statistics. This study was approved by the institutional ethical board on protocol number 2365.

Results

Along the last 20 years, 278 patients with cryptococcosis were admitted at the teaching hospital. Of these, 271 were HIV-infected and presented cryptococcal meningitis. Of 278 clinical isolates recovered from these cases, 267 (96%) were *C. neoformans* VNI genotype and 11 (4%) were *C. gattii* VGII genotype (Fig. 1). The 11 *C. gattii* patients were from the Triângulo Mineiro, Southeast region from Brazil and eight (72.7%) out of the 11 were male, median age of 47.4 years. They had no history of trips to known endemic areas. The average time between the onset of symptoms until the diagnosis was of 37 days, and the median time of hospitalization was 35 days. Fever, headache and weight loss were the predominant claims and most of them presented underlying medical conditions favoring this infection (Table 1). Four out the 11 patients presented cryptococcal meningitis, four disseminated fungal infection, two cutaneous primary cryptococcosis and one pulmonary involvement. The cerebrospinal fluid (CSF) analysis showed: mononuclear pleocytosis, low glucose level, and high protein level in most cases. Diagnosis was based on: positive culture in 100% of the cases, India ink stain in 63%, biopsy of skin fragments stained with Grocott and Mucicarmine in 27% and Latex-*Cryptococcus* antigen test in 45% of cases. Granulomatous inflammatory lesions at CT and MRI images of the lungs and CNS were seen in four (36%) patients, respectively, of whom three were apparently immunocompetent.

Among these patients, four were HIV-infected of whom, three were male, median age of 34.2 years and

in two cases, HIV infection and cryptococcosis were concomitantly diagnosed. The median time since the onset of symptoms until diagnosis was 8.5 days, and the median time of hospital staying was 47 days. Despite the antifungal therapy, two patients died and a disseminated fungal infection to five or more organs was evidenced at necropsy. Of the two survivors, one presented pulmonary cryptococcosis and the other cryptococcal meningitis.

The kidney-transplanted patient died before the antifungal therapy, and a disseminated cryptococcosis was seen at necropsy. The patient with low $CD4^+$ T cells count of unknown cause developed cryptococcal meningitis and evolved with good outcome after antifungal therapy. The patient with chronic liver failure had history of thrombosis of the cava vein 11 years before and referred skull traumatism three months before the onset of neurological picture. At admission, he was severely sick, with mental confusion and symptoms of intracranial increased pressure. Brain and pulmonary cryptococcomas were seen at CT and MRI image which were confirmed by histopathology and CSF positive culture. Due to the evidence of severe hydrocephalus, a ventriculo-peritoneal shunt was placed and amphotericin B and fluconazole were administered during 6 months. His clinical picture progressively improved, and currently, he is followed as an outpatient.

The two patients with primary cutaneous cryptococcosis were farmers with history of direct traumatism with *Eucalyptus* logs. They presented chronic atypical inflammatory lesions in their arms which did not improve despite several antibiotic prescriptions. Underling immunosuppression and asymptomatic pulmonary or meningeal involvement were also discarded. They received fluconazole during 10 weeks, and both had a good outcome. One of these cases had been already reported. Several attempts to isolate *C. gattii* from different trees and sites on their farms and neighborhood were unsuccessful. In the remaining four patients, apparently immunocompetent and despite an adequate clinical investigation, no underlying medical conditions were found.

Discussion

In accordance with other authors, the burden of human disease due to *C. gattii* is unrecognized as many laboratories around the world do not undertake detailed species of *Cryptococcus* clinical isolates [21–23]. Previously, *C. gattii* was considered a primary pathogen restricted to tropical and subtropical areas with remarkable preference by immunocompetent hosts. The growing number of *C. gattii* cryptococcosis reports in HIV-infected patients from Africa together with data obtained from outbreaks in temperate regions from North America, where 40% of the affected individuals had underlying immunosuppression, called the attention of the scientific community to this fungus species [24–26]. Currently, the evidence confirms the *C. gattii* geographical expansion around the world and could permit to arise the hypothesis that changes in its host preference could be on going.

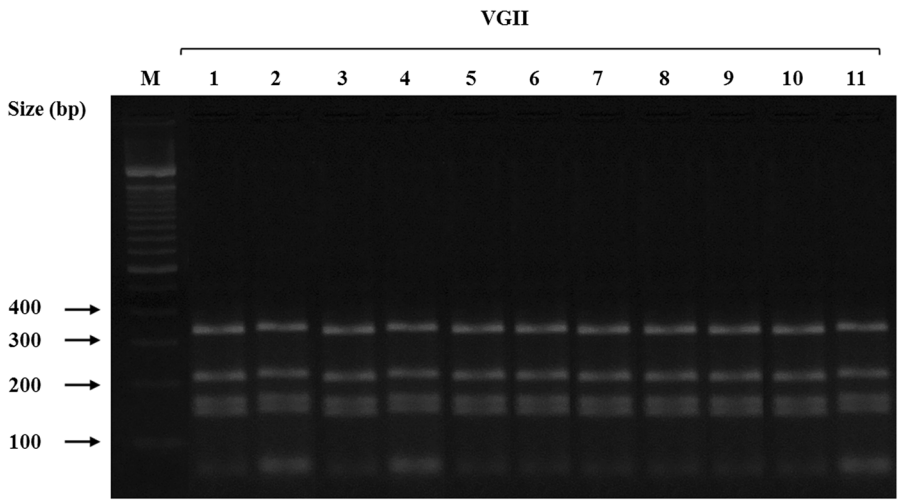

Fig. 1 Agarose gel electrophoresis of *URA*5-RFLP after double digestion with *Sau*96I and *Hha*I from the *Cryptococcus gattii* clinical isolates. Lanes 1–11 *C. gattii* (VGII genotypes). M-100 bp DNA ladder (Bionner, USA)

Table 1 Main epidemiological, clinical and outcome data of 11 patients with cryptococcosis by *Cryptococcus gattii*

Patient	Year of diagnosis	Age	Gender	Underlying disease	Symptoms onset (days)	Hospital stay (days)	Treatment	Outcome
01	2001	58	M	Apparently immunocompetent	37	39	AMB + FLZ	Death
02	2001	24	M	HIV infection	7	8	AMB	Death[a]
03	2001	31	M	HIV infection	9	78	AMB	Cure
04	2005	36	M	HIV infection	10	39	AMB + FLZ	Death[a]
05	2006	46	F	HIV infection	8	55	AMB	Cure
06	2006	47	M	Apparently immunocompetent	70	35	AMB + FLZ	Cure
07	2007	46	F	Lymphopenia of unknown cause	92	26	AMB + FLZ	Cure
08	2008	35	F	Renal transplant	8	8	NONE	Death[a]
09[b]	2009	76	M	Apparently immunocompetent	36	11	AMB + FLZ	Cure
10	2012	68	M	Apparently immunocompetent	95	21	AMB + FLZ	Cure
11	2017	54	M	Chronic liver disease	120	60	AMB + FLZ	Cure

M male, *F* female, *AMB* amphotericin B, *FLZ* fluconazole

[a]Disseminated cryptococcosis at necropsy

[b]Case already reported [44]

However, this requires additional studies and a critical review of the cases already reported. [8–10, 27–29].

The present *C. gattii* case series accounts for 4% of 278 cases of cryptococcosis diagnosed during the last 20 years and resulted from the interest to improve the diagnosis at species and genotype level in a teaching hospital in the Southeast region of Brazil, where most reported cases of cryptococcosis occur in HIV-infected patients and are caused by *C. neoformans* VNI genotype. As possible, the migratory profile of the 11 *C. gattii* patients was investigated. They are from different towns of the Triângulo Mineiro, Southeast region, far from the known endemic areas situated in the North and Northeast regions of Brazil, which include the states of Amazonas, Pará, Bahia and Piauí [30, 31]. They denied having visited or travelled to these areas, where cryptococcal meningitis in immunocompetent hosts is often diagnosed and molecular studies of clinical and environmental *C. gattii* isolates have shown VGII genotype predominance [30–34]. Herein, this fact can be confirmed among the 11 patients whose clinical isolates evaluated by *URA*5-RFLP shown to be VGII genotype (Fig. 1).

Recent studies have shown that the highly recombining VGII genotype ancestors from the native rainforest of the Northern Brazil expanded its boundaries to North America, where it caused outbreaks in Vancouver Island (Canada), mainland of British Columbia and subsequently in the Pacific Northwest region of the USA [4, 35, 36]. Since then, the incidence of *C. gattii* infections in this region was estimated to be approximately 27 times higher than endemic areas of Northern Australia in Aboriginals [27]. In Brazil, cryptococcosis caused by VGII lineages is endemic in the North and Northeast regions, where natural infection occurs early in life [30, 34]. In accordance with this hypothesis, the endemicity of this species in Brazil might be also expanding its limits and could explain the occurrence of cases in non-endemic areas.

Through several molecular tools, four *C. gattii* genotypes: VGI, VGII, VGIII and VGIV were already identified. The VGIIa, b and c subgenotypes were also defined showing the genetic diversity of this species [37, 38]. This fact partially contributes to understand the variability of its geographical distribution, virulence, host preference and somehow the clinical outcome, despite controversial data, which highlight the host immunity instead of the species role as the key factor [11, 14, 34]. The VGII genotype predominated in the Vancouver outbreak and its a subgenotype was considered the most virulent. The VGI predominates in Australia and it has been associated with more central nervous system involvement, whereas VGIII and VGIV are more likely to be found in HIV-infected

patients rather than in immunocompetent hosts. [8–10, 23, 25].

During the last years, reports from several authors pointed out high prevalence of cryptococcosis by *C. gattii* in HIV-infected patients, which contrast with the scarce number of cases reported from Brazil [8, 9, 22]. Here, most HIV-infected patients live in the South and Southeast regions, the most populous and considered non-endemic areas for *C. gattii*, which would explain the small number of cases reported. Four of the 11 cases herein presented were HIV-infected and in accordance with others, no apparent clinical or outcome differences were noticed when compared with those who presented cryptococcal meningitis by *C. neoformans* [14]. In addition, no granulomatous images at CT and MRI in lungs and CNS of these patients were observed, different from non-immunocompromised patients who are more likely to develop pulmonary or cerebral cryptococcomas as observed in four cases of the present series [13]. A previous Brazilian report described three HIV-infected patients from the South region with severe *C. gattii* infection and poor outcome [39]. Another Brazilian case series of 14 patients with cryptococcosis by VGII genotype from the North region, only two were HIV-infected [33]. In one report which evaluated 50 clinical isolates from HIV-infected patients from the West Central region from Brazil, only 3 were *C. gattii* [40]. These data support how *C. gattii* infection in HIV patients in Brazil seems to be less common than in other places [8–10, 25].

The epidemiological and clinical features of patients with cryptococcosis by *C. gattii* herein described are in line with those reported elsewhere to both immunocompetent and immunocompromised hosts and reinforce the severity of clinical pictures with disseminated disease to several organs as evidenced by CT and MRI image or at necropsy [41–43]. Currently, in most places where cryptococcosis occurs, the species differentiation is not routinely performed due to logistical and economic limitations which could underestimate and explain the small number of *C. gattii* cases reported specially in HIV-infected patients.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest. The authors alone are responsible for the content and the writing of the paper.

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