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Histoplasmosis in HIV-positive patients in Ceará, Brazil: clinical-laboratory aspects and in vitro antifungal susceptibility of *Histoplasma capsulatum* isolates

Raimunda S.N. Brilhante[a,b,c,*], Maria A.B. Fechine[a,c], Jacó R.L. Mesquita[f], Rossana A. Cordeiro[a,b,c], Marcos F.G. Rocha[a,b,d], André J. Monteiro[c,e], Rita A.C. Lima[a,b], Érica P. Caetano[a,b], Juliana F. Pereira[a,b], Débora S.C.M. Castelo-Branco[a,b], Zoilo P. Camargo[g], José J.C. Sidrim[a,b,c]

[a] *Specialized Medical Mycology Center, Federal University of Ceará, Fortaleza, Ceará, Brazil*
[b] *Postgraduate Program in Medical Microbiology, Federal University of Ceará, Fortaleza, Ceará, Brazil*
[c] *Postgraduate Program in Medical Sciences, Federal University of Ceará, Fortaleza, Ceará, Brazil*
[d] *Postgraduate Program in Veterinary Science, State University of Ceará, Fortaleza, Ceará, Brazil*
[e] *Department of Statistics and Applied Mathematics, Federal University of Ceará, Fortaleza-CE, Brazil*
[f] *Hospital São José, Ceará, Brazil*
[g] *Federal University of São Paulo, São Paulo, São Paulo, Brazil*

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ABSTRACT

This study contains a descriptive analysis of histoplasmosis in AIDS patients between 2006 and 2010 in the state of Ceará, Brazil. Additionally, the in vitro susceptibility of *Histoplasma capsulatum* isolates obtained during this period was assessed. We report 208 cases of patients with histoplasmosis and AIDS, describing the epidemiological, clinical, laboratory and therapeutic aspects. The in vitro antifungal susceptibility test was carried out by the microdilution method, according to Clinical and Laboratory Standards Institute, with *H. capsulatum* in the filamentous and yeast phases, against the antifungals amphotericin B, fluconazole, itraconazole, voriconazole and caspofungin. In 38.9% of the cases, histoplasmosis was the first indicator of AIDS and in 85.8% of the patients the CD4 cell count was lower than 100 cells/mm^3. The lactate dehydrogenase levels were high in all the patients evaluated, with impairment of hepatic and renal function and evolution to death in 42.3% of the cases. The in vitro susceptibility profile demonstrated there was no antifungal resistance among the isolates evaluated. There was a significant increase in the number of histoplasmosis cases in HIV-positive patients during the period surveyed in the state of Ceará, northeastern Brazil, but no antifungal resistance among the recovered isolates of *H. capsulatum*.

1. Introduction

Histoplasmosis is the most prevalent systemic mycosis in the Americas. Its etiological agent is the dimorphic fungus *Histoplasma capsulatum*, which is widely distributed throughout the world.[1]

Since the 1980s, extrapulmonary histoplasmosis has been considered an important marker disease of AIDS,

* Corresponding author. Present address: Rua Barão de Canindé, 210; Montese. CEP: 60.425-540. Fortaleza, CE, Brazil. Fax: +55 85 3295 1736.
E-mail address: brilhante@ufc.br (R.S.N. Brilhante).

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occurring in up to 5% of patients infected with HIV in endemic areas.[2] It is the most frequent opportunistic systemic infection found in this group,[3] with high mortality rates, and is often confused with other diseases such as tuberculosis and pneumocystosis.[4]

The treatment of histoplasmosis depends on the severity of the infection, clinical manifestations and individual risk factors. The drugs used to treat patients are amphotericin B, fluconazole, itraconazole, voriconazole, posaconazole or caspofungin.[5]

Various outbreaks of histoplasmosis have been reported in the Americas, such as in the city of Indianapolis, United States, where 155 cases were reported between 1988 and 1995 (average of 22.14 cases a year),[2] and in French Guiana, with 200 cases from 1982 to 2007 (yearly average of 8 cases).[3] In Brazil, outbreaks of histoplasmosis have been described in several regions,[6–8] with up to 10 cases a year. Specifically in the northeast region, Daher et al.[9] reported 164 cases of disseminated histoplasmosis in HIV-positive patients in the state of Ceará from January 1995 to January 2004 (average of 18 cases a year).

This article contains a descriptive analysis of the histoplasmosis cases in HIV-positive patients from 2006 to 2010 in the state of Ceará, northeastern Brazil. We also report in vitro susceptibility tests of the isolates of *H. capsulatum* recovered from these cases against the most commonly used antifungals.

2. Materials and methods

A survey was conducted of cases of histoplasmosis and AIDS, diagnosed in a tertiary reference hospital for infectious diseases in the state of Ceará, between January 2006 and December 2010. The study protocol was reviewed and approved by the hospital's ethics committee, under number 029/2007.

The diagnosis of histoplasmosis was confirmed by identification of *H. capsulatum* through mycological examination of samples obtained from peripheral blood smear, bone marrow aspirate, blood and bone marrow culture, and histopathology of different tissues,[10] while HIV infection was confirmed according to the criteria of CDC.[11]

We gathered information on demographics (sex, age and place of residence), clinical aspects (date of histoplasmosis diagnosis, period of hospitalization, symptoms, signals, treatment and evolution of the patient), mycological diagnosis (direct examinations and fungal culture) and other laboratory data (complete hemogram, serum creatinine and hepatic function tests, CD4 cell count and chest x-ray).

To measure the antifungal susceptibility in vitro, the microdilution method was used, according to protocol M27-A2,[11,12] with 68 clinical isolates of *H. capsulatum* var. *capsulatum* (*H. capsulatum*) in filamentous form, obtained from patients surveyed in this study and stored in the collection of the Specialized Medical Mycology Center of Ceará Federal University, as well as with eight isolates chosen at random in yeast form. The assays were conducted in a Level 3 biosecurity laboratory.

The fungal inocula were prepared according to earlier reports.[11,13] The final concentrations were obtained by serial dilutions, with intervals varying from 0.002–1.0, 0.98–500, 0.0001–0.06, 0.002–1.0 and 0.016–64 $\mu g\ ml^{-1}$ for amphotericin B, fluconazole, itraconazole, voriconazole and caspofungin, respectively. The strain of *Candida parapsilosis* ATCC 22019, as recommended by the Clinical and Laboratory Standards Institute,[12] were used as controls. The MICs of the drugs were read as described in the protocol.[12]

The data were subjected to Student's t-test for variables with approximately normal distribution, the Mann-Whitney test for variables with the presence of extreme values, and the χ^2 test for comparison between the categorical variables and for independence analysis.

3. Results

There were a total of 208 cases of patients with histoplasmosis and AIDS from January 2006 to December 2010. Of these, 81.7% (170/208) of the individuals were men and 18.3% (38/208) were women, a ratio of 4.5: 1, with average ages of 35.8 and 35.0 years, respectively.

In 38.9% (81/208) of the cases, histoplasmosis was the first indicator of AIDS, and at the moment of histoplasmosis diagnosis, 80.3% (167/208) of the patients were not being treated with highly active antiretroviral therapy (HAART). The clinical aspects of the patients are shown in Table 1.

In 51.4% (107/208) of the cases there was a positive reaction in the direct examinations, while *H. capsulatum* was isolated in 92.3% (192/208) of the clinical sample cultures. The CD4 count was performed on blood samples from 120 patients. As can be seen in Table 2, 85.8% (103/120) of these had CD4 counts less than or equal to 100 cells/mm^3. The hemoglobin levels were lower than 8 g/dl in 43.3% (90/208)

Table 1
Clinical manifestations of histoplasmosis in patients with AIDS in Ceará, between January 2006 and December 2010

Clinical Aspects	n (%)
General symptoms (n = 208)	
Fever	195 (93.8)
Weight loss	181 (87.0)
Anorexia	150 (72.1)
Adynamia	117 (56.3)
Headache	39 (18.8)
Lymphadenopathy	32 (15.4)
Pulmonary symptoms (n = 208)	
Cough	158 (76.0)
Dyspnea	104 (50.0)
Chest pain	16 (7.7)
X-ray examination (n = 185)	
Interstitial infiltrate	123 (66.5)
Pleural effusion	23 (12.4)
Reticulonodular infiltrate	4 (2.2)
Mixed infiltrate	4 (2.2)
Alveolar infiltrate	1 (0.5)
Fibrocavitary disease	1 (0.5)
Digestive symptoms (n = 208)	
Diarrhea	111 (53.4)
Vomiting	49 (23.6)
Hepatomegaly (n = 208)	126 (60.6)
Splenomegaly (n = 208)	102 (49.0)
Cutaneous lesions (n = 36)	
Erythema	15 (41.6)
Papula	12 (33.4)
Erythema + papula	9 (25.0)

Table 2
Laboratory tests of patients with histoplasmosis and AIDS in Ceará, between January 2006 and December 2010

Hematological results	n	Mean	n (%)	Variation
CD4 cell count, cells/mm^3	120	53 ± 55.1		2–303
1–50			75 (62.5)	
51–100			28 (23.3)	
101–200			14 (11.7)	
>200			3 (2.5)	
Hemoglobin, g/dl	208	8.56 ± 2.03		3.7–15.2
≤8.0			90 (43.3)	
8.0–11.5			105 (50.4)	
>11.5			13 (6.3)	
Leukocytes, cells/mm^3	208	4297 ± 3822		500–32 000
<3500			111 (53.4)	
≥3500			97 (46.6)	
Platelet count, cells/mm^3	208	117 228 ± 100 041		117-642000
<100 000			108 (51.9)	
100 000–150 000			44 (21.2)	
≥150 000			56 (26.9)	
AST, IU/l[a]	205	174 ± 167		8–856
≤34			40 (19.5)	
≥35			165 (80.5)	
ALT, IU/l[b]	203	64.9 ± 63.0		4–493
≤44			67 (33.0)	
≥45			136 (67.0)	
LDH, IU/l[c]	205	3852 ± 3576		164–14 868
Increase <2x			19 (9.3)	
Increase ≥2x			186 (90.7)	
Creatinine, mg/dl[d]	205	1.5 ± 2.03		0.3–12.8
≤0.7			60 (29.3)	
0.8–1.3			94 (45.8)	
≥1.4			51 (24,9)	

[a] Aspartate aminotransferase, normal values 15 a 37U/l.
[b] Alanine aminotransferase, normal values 30 a 65U/l.
[c] Lactate dehydrogenase, normal values ≤225 UI/l.
[d] Creatinine normal values ≤1.3 U/l.

of the cases, while the leukocyte levels were lower than 3500 in 53.4% (111/208) and the platelet counts smaller than 100 000 cells/mm^3 in 51.9% (108/208) of the individuals. The lactate dehydrogenase (LDH) levels were elevated in all the patients assessed, with an average of 3852±3576 U/l (164–14868 U/l). There was impaired hepatic function, demonstrated by high levels of aspartate aminotransferase (AST), in 165 (80.5%) of the 208 patients evaluated, with an average result four times higher than the normal levels, while 67.0% (136/208) of the patients had high levels of alanine aminotransferase (ALT). Finally, impaired renal function was observed in 24.9% (51/208) of the cases, with serum creatinine higher than 1.4 U/L.

The antifungal chosen to treat 86.5% (180/208) of the patients was amphotericin B, and of these 38.3% (69/180) died. No therapy was given in 13.5% (28/208) of the cases and the mortality rate of these patients was 67.9% (19/28).

According to the in vitro susceptibility tests, the *H. capsulatum* isolates in the filamentous phase (n = 68) presented variation of MICs of 0.0078–0.5; 3.9–125; 0.001–0.0312; 0.0078–0.5; 0.016–32 μg/mL, for amphotericin B, fluconazole, itraconazole, voriconazole and caspofungin, respectively. For the *H. capsulatum* isolates in the yeast phase (n = 8), the following MIC results were observed: 0.06–0.5; 3.9–7.8; 0.0039–0.03; 0.002–0.03; 1.0–4.0 μg ml^{-1}, for amphotericin B, fluconazole, itraconazole, voriconazole and caspofungin, respectively (Table 3).

4. Discussion

H. capsulatum can be considered one of the most important opportunistic pathogens at present, mainly because of the relationship with AIDS. It causes serious disseminated infections which are often fatal, particularly in patients

Table 3
Antifungal MIC range against the isolates of *Histoplasma capsulatum*

Antifungals	MIC (μg/mL)			
	Filamentous form (n = 68)		Yeast form (n = 8)	
	Range	Mean	Range	Mean
Amphotericin B	0.0078-0.5	0.12	0.06-0.5	0.16
Fluconazole	3.9–125	36.36	3.9–7.8	5.52
Itraconazole	0.001–0.0312	0.0073	0.0039–0.03	0.016
Voriconazole	0.0078–0.5	0.097	0.002–0.03	0.010
Caspofungin	0.016–32	3.65	1–4	2.0

with CD4 counts lower than 150 cells/mm^3, in the absence of HAART.[14]

In the beginning of the twenty-first century an increase has been noted in the number of histoplasmosis cases, as can be observed in this study: there were 208 cases between 2006 and 2010, vs 164 cases during nine years (1995-2004) in a previous study at the same hospital.[9] These numbers are also higher than those reported in microepidemics in southeastern and southern Brazil, as well as in other South American countries.[8,15]

According to data from the Brazilian Ministry of Health[16] the number of HIV-positive patients in southeastern Brazil is higher than that of the northeastern region. Thus, it was expected that the number of cases of histoplasmosis in HIV-positive patients would follow this tendency. However, in the present study and in the study of Daher et al.,[17] the number of cases of histoplasmosis in the state of Ceará was greater than those from southeastern states of Brazil.[6,8]

Histoplasmosis is the first opportunistic infection in 22–85% of HIV-positive patients in endemic areas[3] and the state of Ceará is endemic for the disease, as shown by the serological investigation with histoplasmin carried out in the city of Pereiro, Ceará, in individuals of both sexes and age range from 5–50 years. The positive frequency was 61.5%, higher than observed in other Brazilian states, including the northeastern region.[15]

Histoplasmosis was observed as the first manifestation of AIDS in 38.9% of the cases, and 85.8% of the patients presented CD4 counts <100 cells/mm^3, corroborating earlier studies that showed cell immunosuppression to be the most important factor for clinical manifestations of histoplasmosis.[3]

The epidemiological, clinical, laboratory and radiological characteristics of the patients found in the present study are similar to those reported elsewhere in the literature.[17] It should be noted that cutaneous alterations were less frequently observed (17.3%) than in southern and southeastern Brazil, but at similar levels as reported in the midwest region of Brazil and in the United States.[7,18] In the present study gastrointestinal symptoms (diarrhea and vomiting) occurred more often than in the American study[18] and with greater hepatosplenic impairment than previously observed in Ceará,[17] suggesting that different *H. capsulatum* isolates are responsible for different clinical manifestations.[19] Additionally, the presence of certain clinical signs were associated with worse prognosis and death, such as anorexia, dyspnea, elevated serum levels of AST and creatinine, diffuse interstitial pulmonary infiltrate and pleural effusion.

A total of 42.3% (88/208) of the patients died, out of which 78.4% (69/88) were being treated with amphotericin B and the remaining 21.6% (19/88) were not under antifungal therapy. This lethality rate was higher than that observed by Daher et al.[17] (32% of 164 patients), but similar to that observed by Chang et al.[7] (40%). Additionally, Wheat et al.[2] reported that 12% of AIDS patients with disseminated histoplasmosis treated with amphotericin B died, with a lethality rate lower than the one observed in this study. These data show that histoplasmosis is a serious opportunistic infection with high lethality rates among immunocompromised patients.[18]

According to the results of the in vitro tests, the MICs of amphotericin B vs the *H. capsulatum* isolates, in the filamentous phase, were lower than those observed by Andreu et al.,[20] who found an MIC of 0.125–1.0 μg ml^{-1}, and by Li et al.,[21] who reported an MIC$_{90}$ of 1 μg ml^{-1}. Polak and Dixon,[22] in a study of the yeast phase, obtained MIC values of 0.07–0.15 μg/ml, similar to those observed in the present study.

In relation to the azoles, the geometric mean for fluconazole observed in this study was lower than that noted by Andreu et al.[20] (55.7 μg/ml), utilizing the fungus in the filamentous phase. For the yeast phase, we obtained higher MIC values for fluconazole than those reported by Polak and Dixon[22] (0.25–5 μg/mL) and Wheat et al.[23] (0.24–4 μg/mL) versus the yeast form of *H. capsulatum*. It should be mentioned that treatment of histoplasmosis with high doses of fluconazole induces remission in the majority of patients, but many of them suffer relapses.[24] Wheat et al.[25] reported the emergence of resistance to fluconazole as a cause of therapeutic failure in immunosuppressed patients, mainly when in vitro studies demonstrate that the strains need concentrations higher than 5.0 μg ml^{-1} for inhibition.

The MIC values observed in this study for itraconazole were lower than those reported by Andreu et al.[20] (CIM$_{90}$ = 0.125 μg/ml), and Li et al.[21] (CIM$_{90}$ = 0.06 μg/ml), in the filamentous phase, and similar to those reported by Polak and Dixon[22] for the yeast phase, with a geometric mean of 0.019 μg/ml.

Voriconazole is from the triazole class. Studies have demonstrated that it is more active in vitro against dimorphic fungi, such as *Blastomyces dermatitidis*, *Coccidioides immitis* and *H. capsulatum*.[26,27] We observed lower MIC values for voriconazole than those reported by Li et al.[21] (0.03-2 μg/ml) for the fungus in the filamentous phase, but similar to those found by Wheat et al.[23] (0.007–0.25 μg/ml) for the yeast phase.

The results obtained for caspofungin show great variability in the in vitro response of *H. capsulatum*. Kohler et al.[28] demonstrated that caspofungin is not effective against this fungus in vivo, reflecting its low activity in vitro.

Finally, this study revealed a significant increase in the number of histoplasmosis cases related to AIDS, but without noting resistance among the studied *H. capsulatum* isolates.

Authors' Contributions: RSNB, RAC, MFGR, AJM, ZPC and JJCS conceived and designed the study, analyzed the data, critically revised the manuscript for intellectual content and read and approved the final version; MABF, JRLM, RACL, EPC, JFC and DSCMCB conceived and designed the study, interpreted the data, drafted the manuscript and read and approved the final version. RSNB is the guarantor of the paper.

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