Revista do Instituto Adolfo Lutz
https://doi.org/10.53393/rial.2023.v.82.39242
ISSN: 1983-3814 (on-line)
Original Article/Artigo Original

Factors interfering with the production of *Histoplasma capsulatum* antigens

Fatores que interferem na produção de antígenos de *Histoplasma capsulatum*

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Received/Recebido: 06.04.2023 – Accepted/Aceito: 22.09.2023

ABSTRACT

Histoplasma capsulatum causes systemic mycosis that depends on host susceptibility, fungal virulence, and factors associated with the infectious process. We evaluated the possible interference of the phenotype of 12 samples of *H. capsulatum* isolated from HIV-positive and negative patients in obtaining antigens, aiming at the serological diagnosis through the gender-specific recognition of the H and M fractions. The antigens were evaluated by double immunodiffusion against *H. capsulatum* anti-antigen polyclonal antibody and serum samples from patients with histoplasmosis. The phenotypic evaluation revealed differences in the identification of the fungal agent and in the expression of H and M antigens, considered serological markers of the disease, associated with pigmentation and the production of conidia. It was found that antigenic preparations obtained from *H. capsulatum* isolated from HIV-positive patients may have satisfactory antigenic capacity. The patient's immune status does not seem to interfere with the expression of antigenic proteins secreted by *H. capsulatum*. However, we suggest that prolonged use of antiretrovirals drugs or steroids can cause important phenotypic alterations. We showed that some fungal samples from patients with a long history of immunosuppressive drugs produced atypical cellular elements and low reactivity against the H and M fractions.

Keywords. Histoplasma, Histoplasmosis, Phenotypic Variation, Antigens, Antiretroviral Therapy.

RESUMO

Histoplasma capsulatum causa micose sistêmica endêmica que depende da suscetibilidade do hospedeiro, da virulência fúngica e de fatores associados ao processo infeccioso. Avaliamos a possível interferência do fenótipo de 12 amostras de *H. capsulatum* isolados de pacientes HIV positivos e negativos na obtenção de antígenos, visando o diagnóstico sorológico por meio do reconhecimento gênero-específico das frações H e M. Os antígenos foram avaliados por imunodifusão dupla, frente a anticorpo policlonal anti-antígeno de *H. capsulatum* e frente a amostras de soro de pacientes com histoplasmose. A avaliação fenotípica revelou diferenças, não só na identificação do agente fúngico, mas também na expressão dos antígenos H e M, considerados marcadores sorológicos da doença, associados à pigmentação e produção de conídios. Verificou-se que preparações antigênicas obtidas de *H. capsulatum* isoladas de pacientes HIV positivos podem ter capacidade antigênica satisfatória. O estado imunológico do paciente parece não interferir na expressão de proteínas antigênicas secretadas por *H. capsulatum*. No entanto, sugerimos que o uso prolongado de antirretrovirais e/ou esteróides pode causar alterações fenotípicas importantes. Verificou-se que algumas amostras fúngicas isoladas de pacientes com longo histórico de uso de imunossupressores produziram elementos celulares atípicos e baixa reatividade sorológica contra as frações H e M de *H. capsulatum*.

Palavras-chave. Histoplasma, Histoplasmose, Variação Fenotípica, Antígenos, Terapia Antirretroviral.

INTRODUCTION

Histoplasma capsulatum, the etiologic agent of classical histoplasmosis (HP), is a widespread dimorphic fungus causing a mycosis that principally affects the lungs and other organs of the endothelial reticulum system[1,2]. Sahaza et al[3] reported that this mycosis has different clinical presentations depending on the host susceptibility, fungal virulence, and other factors associated with the infection process. HP is the most common systemic mycosis in North America, with an evident increase in the incidence of cases in the Americas. This is the first or second most prevalent mycosis in areas of Central and South America, causing progressive infections, particularly in individuals who are immunocompromised by hematologic malignancies, cytotoxic therapy, and especially among patients with acquired immunodeficiency syndrome (AIDS)[1,2]. Disseminated HP affects up to a quarter of all HIV-1 patients. The majority of HP cases occur when HIV-positive individuals have CD4 T-cell counts < 100 cells/mm^3[3,4]. In Brazil, there were cases of HP in all regions of the country[5]. Human infection results primarily from inhalation of aerosolized microconidia, the mold form of *H. capsulatum*, which transforms into the yeast form in the lungs[6]. The clinical spectrum of HP ranges from asymptomatic to severely disseminated forms of the disease[1]. The majority of the exposed population has a mild self-limiting or subclinical form of infection[1]; however, infants and immunocompromised individuals may contract acute symptomatic or progressive life-threatening disseminated forms[1,5,7]. The definitive diagnosis of HP relies on the isolation of *H. capsulatum* by culture from clinical specimens[8,9].

Microscopic identification of yeast forms of fungi in clinical materials such as bone marrow, sputum, and tissue is usually difficult[8]. Isolation of *H. capsulatum* by culturing clinical specimens is a standard method of microbial identification; however, the isolation of *H. capsulatum* is costly and time-consuming[1,2,9]. If a biopsy is possible and less harmful to the patient, Gomori-Grocot staining can assist in confirmatory diagnosis through the observation of intracellular *H. capsulatum* yeast cells[1,8-11]. Many cases of HP have been serologically diagnosed[2,8-11]. Antigens from mycelial-form cultures of *Histoplasma* species consistently appear to produce either H or M antigens or both and can be identified using the double immunodiffusion (DI) assay[11-13]. Several molecular in-house assays have also been developed and presented encouraging results. Nonetheless, none of these assays are commercially available[11]. In addition, these assays require standardization and validation processes[11]. Antigen detection assays have high sensitivity in disseminated pulmonary HP cases and are of great value in the follow-up of patients with HP. A limitation of *Histoplasma* antigen testing is its significant cross-reactivity with other fungal antigens, including *Blastomyces dermatitidis*, *Paracoccidioides brasiliensis*, *Penicillium marneffei*, and, less commonly, *Coccidioides immitis* and *Coccidioides posadasii*. Although useful, antigen detection is expensive and is only performed in a few laboratories[9-11].

This study evaluated the genus-specific interference of antigenic products obtained from 12 different *H. capsulatum* isolates for recognition of H and M fractions through DI assay.

MATERIAL AND METHODS

H. capsulatum isolates

Twelve *H. capsulatum* samples, molecularly confirmed by Nested PCR using primers against *H. capsulatum* 18S rRNA (HC18S), 5.8S rRNA ITS (HC5.8S-ITS) and a 100 kDa protein (HC100)[14], isolated

from different clinical specimens (bronchoalveolar lavage fluid, blood, cerebrospinal fluid, skin, bone marrow, and urine) from patients with HP co-infected with or without HIV were selected for this study. The isolates (**Table 1**) were maintained in the Culture Collection of the Mycology Laboratory of the Institute of Tropical Medicine of São Paulo (LIM-53) Brazil. These isolates were maintained in potato agar medium (Difco Laboratories, Detroit, MI, USA) at room temperature (27 °C), and sub-cultured every three months, in the mycelial form, respecting biosafety standards.

Phenotypic Analysis

Mycelial cells from *H. capsulatum* samples were cultured at 27 °C on Sabouraud dextrose agar (Difco Laboratories, Detroit, MI, USA) for 90 days to evaluate their macromorphological characteristics. The micromorphological aspects were analyzed according to Riddell[15]. Fungal samples were cultured at 27 °C in agar potato (Difco Laboratories, Detroit, MI, USA) for 45 days.

Preparation of *H. capsulatum* antigen

To prepare the antigen of each isolate of *H. capsulatum*, we employed the Kaufman and Standard's method[12], with certain modifications[16,17]. The *Histoplasma* DI antigen (H50110) was used as a reference antigen (IMMY, Norman, OK, USA). Briefly, mycelial cells of all isolates were grown in solid Sabouraud-dextrose (Difco Laboratories, Detroit, MI, USA) medium at 27 °C during 15 and 33 days. Following the incubation period, the cultures were treated with an aqueous solution of thimerosal-borato 1:5,000 (Sigma Chemical Co. St. Louis, MO, USA) and incubated at room temperature for 24 h. Afterward, the supernatants were filtered through Whatman® no. 1 paper (Whatman, Brentford, UK). The antigen solutions were concentrated 10- to 20-fold using a lyophilization procedure. After protein dosage using the Bradford method[16], antigenic preparations were stored at -20 °C until use. The *Histoplasma* DI antigen (H50110) was used as a reference antigen (IMMY, Norman, OK, USA).

H. capsulatum polyclonal antibodies production

Anti-*H. capsulatum* sera were produced in New Zealand with female rabbits immunized subcutaneously with the *H. capsulatum* antigen (culture filtrate of VMF, CFLA, and 879 *H. capsulatum* isolates) using the Mckinney and Parkinson[18] method. We also used H and M *H. capsulatum* reference antisera (IMMY, Norman, OK, USA). The negative control antisera used was anti-*P. brasiliensis* and anti-*Aspergillus fumigatus*[16].

Double immunodiffusion assay (DI)

The DI assay was performed according to Freitas et al[16,17].

RESULTS

H. capsulatum isolates

Fifty percent of *H. capsulatum* isolates were obtained from patients with AIDS and co-infected with *H. capsulatum*. In samples 49, 802, 879, and 2030, the anatomical site of infection was the kidneys. Half of

these patients had AIDS, and the other three had renal failure. Isolates 200 and 361 were obtained from immunocompetent individuals. Sample 268 was isolated from a patient without HIV/AIDS. This patient had chronic meningitis and intracranial hypertension, and was treated for 17 days with high doses of corticosteroids. Of the patients with AIDS, 66.7% presented with irregular use of antiretroviral treatment, while 33.3% reported abandonment or absence of antiretroviral treatment (**Table 1**). Antifungal treatment of HP with itraconazole occurred in 66.7% of patients, while 33.3% used liposomal amphotericin B. Four patients used liposomal amphotericin B (75%), and three of these patients died. Fifty percent (6) of the patients died, and of these, five cases were from patients with HIV/AIDS (**Table 1**).

Phenotypic analysis of *H. capsulatum* samples

The macromorphological aspects of *H. capsulatum* cultures revealed that 75% had a cottony texture, 25% were velutinous, and 83.3% had a membranous border. White pigmentation was observed in 58.3% of these cultures, whereas 41.7% demonstrated pigmentation ranging from beige to ocher. The central surface of the culture revealed areas of elevation and depression (33.3%) and protrusion (33.3%); however, a flat texture was observed in 50% of the median surface (**Table 2** and **Figure**). According to the methodology described by Ridell[15], 91.7% of micromorphology from hyphae was typical, except for isolate 268, which presented thick hyphae and absence of conidia, which is unusual for this genus. This sample was isolated from the cerebrospinal fluid of a patient treated with high doses of corticosteroids (**Table 2** and **Figure**).

We observed the presence of a germ tube in isolates 299 and 584 (from HIV/AIDS patients). Seventy-five percent of these isolates had typical microconidia, except for isolates 212, 268, and 340. Macroconidia with a club-shaped ornamentation was observed in 75% of the isolates. Absent, smooth, and degenerate macroconidia occurred in isolates 212, 268, and 340, respectively. When relating colony pigmentation to conidia production, we found that isolates 200, 299, 361, and 406 expressed macroconidia with club-shaped ornamentation, pigmentation ranging from beige to ocher. Additionally, we observed microconidia and macroconidia with club-shaped ornamentation, except for isolate 340, which, despite having an ocher colony, did not produce conidia (**Table 2** and **Figure**). In contrast to certain atypical cultures from HIV/AIDS patients, the other cultures were found to be in intense sporulation, including the 879 sample, had undergone 13 years of maintenance in the LIM-53 Culture Collection (**Table 2** and **Figure**).

Double immunodiffusion analysis

Antigenic preparations obtained from 12 samples of *H. capsulatum* yielded improved results after they were concentrated 20 times. Analysis of the precipitation lines showed 100% specificity when evaluated against genus-specific antibodies[14]. Among the samples of *H. capsulatum* (49 and 2030) isolated from patients with HIV/AIDS, it which if observed that isolates that presented typical morphology showed serological reactivity for polyclonal antibody anti-*H. capsulatum*, polyclonal antibody anti-fractions H and M, serum samples from patients with HP disease, and strong reactivity for patients with HP infection. Among the *H. capsulatum* samples (200, 406, 802, 879) isolated from patients without immunosuppression caused by HIV it was observed that only sample 200 had a wavy edge and the other membranous edges, samples 200 and 802 had a velvety texture, but samples 406, 802 and 879 had a cottony texture, of which the isolate 200 and 406 had ocher and beige pigmentation, respectively, while

Table 1. Isolation source profiles of the evaluated isolates

Isolates With HIV/AIDS	Site of isolation	Co-infection AIDS	Antiretroviral treatment	HP treatment	CD4	Outcome
49	Kidney and lung	Pneumocystosis, tuberculosis and cryptococcosis	Abandonment	Liposomal Amphotericin B	77	Death
212	Nervous system	Absent	Irregular	Itraconazole	Unrealized	Death
299	Disseminated	Absent	Irregular	Itraconazole	1	Clinical and mycological cure
340	Disseminated	Absent	Irregular	Itraconazole	70	Death
584	Disseminated	Neurotoxoplasmosis, Human papillomavirus, and ganglionic tuberculosis	Irregular	Itraconazole	12	Death
2030	Kidney and lung	Acute kidney failure, staphylococcal disease, pulmonary cryptococcosis, and disseminated intravascular coagulation	Absent (1)	Liposomal Amphotericin B	Unrealized	Death
Non-HIV/AIDS isolate	**Site of isolation**	**Other Pathologies**		**HP treatment**		**Outcome**
200	Cutaneous	Absent		Itraconazole		Clinical and mycological cure
268 (2)	Nervous system	Hydrocephalus with Intracranial hypertension, and cytomegalovirus		Corticosteroids and Liposomal Amphotericin B		Death
361	Cutaneous	Absent		Itraconazole		Clinical and mycological cure
406	Nervous system	Hepatitis		Liposomal Amphotericin B		Clinical and mycological cure
802	Kidney	Diabetes mellitus type II, renal failure and kidney transplant		Itraconazole		Clinical and mycological cure
879	Kidney	Addison's syndrome, diabetes mellitus type II, and failure renal		Fluconazole and itraconazole		Clinical and mycological cure

Note: (1) HIV Diagnosis during hospitalization
(2) Late diagnosis of HP

Table 2. Phenotypic aspects of *H. capsulatum* isolates used for antigenic production

MACROMORPHOLOGY					MICROMORPHOLOGY		
Surface	Edge	Colony texture	Pigment	Hyphae	Germ tube	Microconidia	Macroconidia
Isolates from HIV/AIDS patients							
49	Central: grooves Median: flat	Membranous	Cottony	White	Typical	Absent	Typical	Club-shaped ornamentation
212	Central: Elevation and depression areas Median: flat	Smooth	Cottony	White	Typical	Absent	Absent	Smooth and generation
584	Central: protruding Median: grooves	Membranous	Cottony	White	Typical	**Present**	Typical	Club-shaped ornamentation
299	Central: Elevation and depression areas Median: flat	Membranous	Velvet	Beige	Typical	**Present**	Typical	Club-shaped ornamentation
340	Central: Elevation and depression areas Median: flat	Membranous	Cottony	Ocher	Typical	Absent	Absent	Absent
2030	Central: protruding Median: grooves	Membranous	Cottony	White	Typical	Absent	Typical	Club-shaped ornamentation
Isolates from Non-HIV/AIDS patients							
200	Central: concentric bands Median: radial grooves	Wavy	Velvet	Ocher	Typical	Absent	Typical	Club-shaped ornamentation
268	Central: Elevation and depression areas Median: flat	Membranous	Cottony	White	**Atypical**	Absent	Absent	Absent
361	Central: grooves Median: Elevation and pleated	Membranous	Cottony	Beige	Typical	Absent	Typical	Club-shaped ornamentation
406	Central: grooves Median: Elevation and pleated	Membranous	Cottony	Beige	Typical	Absent	Typical	Club-shaped ornamentation
802	Central: protruding Median: flat	Membranous	Velvet	White	Typical	Absent	Typical	Club-shaped ornamentation
879	Central: protruding Median: grooves	Membranous	Cottony	White	Typical	Absent	Typical	Club-shaped ornamentation

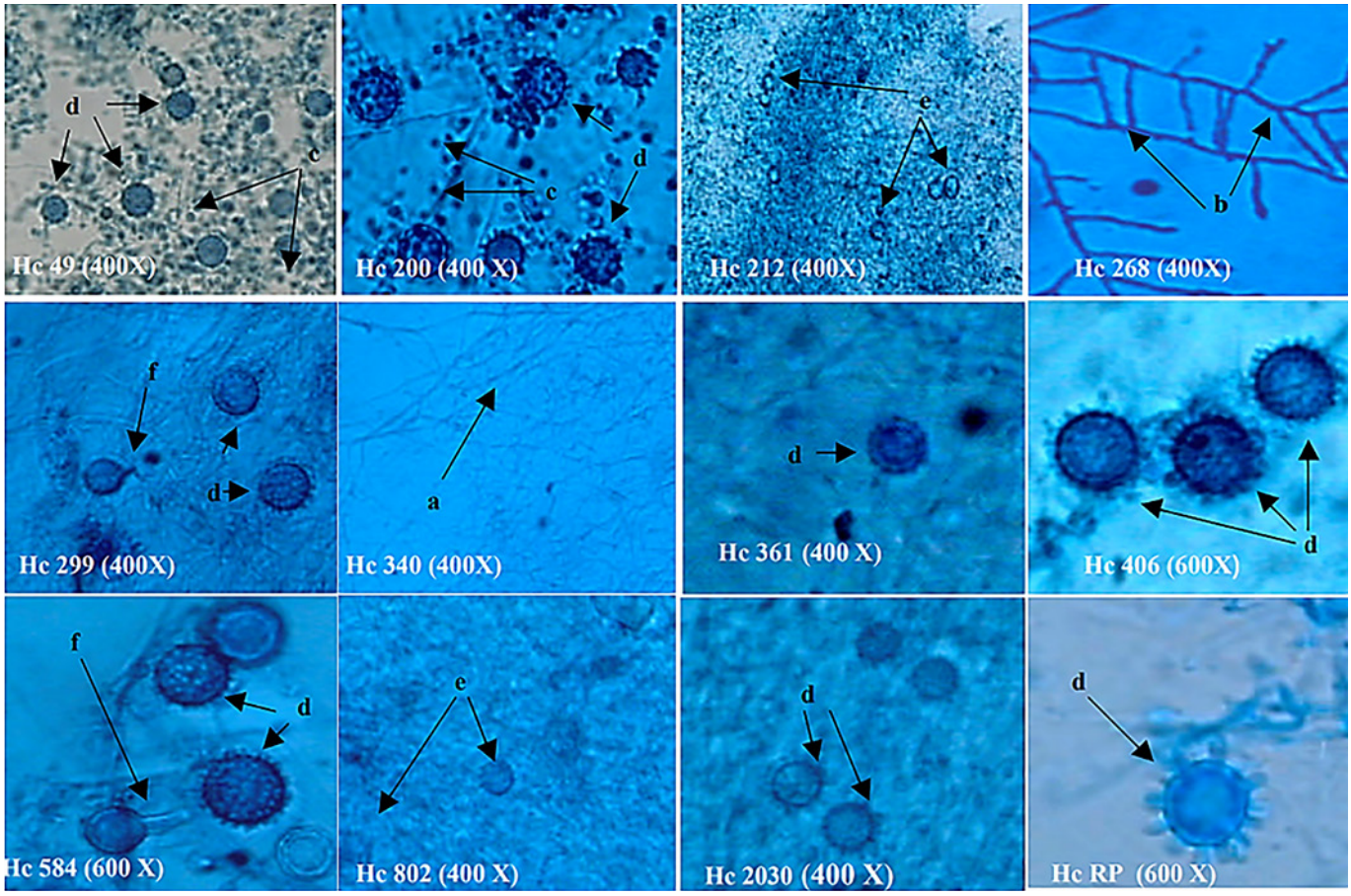

Figure. Micromorphological aspects of *H. capsulatum* isolates used for antigenic production

others being white. Micromorphologically, all were typical, and they presented serological reactivity for polyclonal antibody anti-*H.capsulatum* and anti-H and M fractions, serum from patients with HP disease, and strong reactivity for patients with HP infection. Among *H. capsulatum* samples (212, 299, 340, and 584) isolated from patients with HIV/AIDS; and from sample 361, from non-HIV/AIDS patients who did not show reactivity against anti-H and M polyclonal antibodies, and from serum samples from patients with HP disease. Alterations on micromorphological analysis of isolates 299 and 584 (tube germ), smooth edge, and alterations from micromorphology of conidia in the isolates 212, 340, and 268, in addition, isolate 268 had atypical hyphae. Isolate 268, which despite showing reactivity against species-specific polyclonal antibodies, did not show reactivity against serum samples from patients with infection and disease by *H. capsulatum* (**Table 3**).

DISCUSSION

In a previous study, Freitas et al[16] used the same 12 *H. capsulatum* isolates to obtain antigens for diagnostic purposes and demonstrated the sensitivity, specificity, and stability of the *H. capsulatum* antigens. However, in this study, we sought to analyze the factors that could interfere with obtaining them. According to Hussain et al[19], early and accurate diagnosis is critical for successful treatment, of

Table 3. Screening of antigen obtained within 33 days against polyclonal antibody, and serum positive patients with infectious disease caused by *Histoplasma*

Isolate	Polyclonal anti-exoantigen antibody of *H. capsulatum*	Anti-*Histoplasma* polyclonal antibody, H and M fraction(1)	Sera from patients with HP diseases	Sera from patients with HP infection
49	**Strong positive**	**Positive**	**Positive**	**Strong positive**
212	Weak positive	Negative	Positive	Strong positive
299	Strong positive	Weak positive	Negative	Weak positive
340	Weak positive	Negative	Negative	Weak positive
584	Strong positive	Positive	Negative	Weak positive
2030	**Weak positive**	**Positive**	**Positive**	**Strong positive**
200	**Strong positive**	**Positive**	**Positive**	**Strong positive**
268	Strong positive	Strong positive	Negative	Weak positive
361	Strong positive	Positive	Negative	Weak positive
406	**Strong positive**	**Strong positive**	**Positive**	**Strong positive**
802	**Weak positive**	**Positive**	**Positive**	**Strong positive**
879	**Weak positive**	**Positive**	**Positive**	**Strong positive**
Reference (2)	Positive	Positive	Positive	Positive

Note: (1) H and M *H. capsulatum* reference antisera (IMMY, Norman, OK, USA)
(2) DI Antigen (H50110) (IMMY, Norman, OK, USA)

infections caused by fungal pathogens. Diagnosis allows the implementation of adequate antifungal therapy, reducing the unnecessary use of toxic drugs and minimizing the emergence of multidrug-resistant fungal strains[19]. The presence of α- and β-glucans in the cell walls of different fungal species is related to distinct biological functions and changes during fungal morphogenesis[20]. β-Glucans are found predominantly during the filamentous phase of *H. capsulatum*; at room temperature, there is an increase in the synthesis of polysaccharide β-(1,3) glucans[21-23]. β-Glucans have antigenic properties[21], and they are found predominantly during the filamentous phase of *H. capsulatum*; at room temperature, there is an increase in the synthesis of polysaccharide β-(1,3) glucans[22-25]. In the yeast form, at 37 °C, the fungus increases the synthesis of α-(1,3) glucans and decreases the synthesis of β-(1,3) glucans. Studies have shown that in murine models, in respiratory infection, avirulent strains of *H. capsulatum* are deficient in α-(1,3) glucans in the cell wall[25,26]. This fungal morphogenesis is considered relevant to *H. capsulatum* virulence. Considering this characteristic of the cell wall, we produced agent antigens in the filamentous phase. This antigen is produced in the early stages of fungal development and is

detectable by DI assay or enzyme immunoassays. The methodology showed specific results during the exponential growth phase of the fungus[12,16,17].

According to Ehrhard and Pine[27], the antigen obtained from the filamentous phase culture filtrate contains H and M fractions. We verified that the antigens obtained from isolates 299 and 584, even at room temperature, exhibited a germ tube. This characteristic occurs during the parasitic phase and a probable decrease in β-glucan levels. These isolates showed excellent results against polyclonal anti-antigen antibodies of *H. capsulatum* and anti-H and M fractions but did not show reactivity when evaluated against serum samples from patients with HP disease. Additionally, weak reactivity was observed in serum samples from patients with infection by the pathogen. Despite having a typical morphology for the species, isolate 361 yielded an antigen with a low antigenic expression profile, with similar responses to isolates 299 and 584 in patients with infection or disease. The antigenic preparations obtained from isolates 212, 268, and 340, which showed relevant micromorphological alterations, revealed a deficient antigenic response to species-specific polyclonal antibodies, serum samples from patients with HP disease or infection. Nonetheless, the antigen obtained from isolate 268 showed serological reactivity against species-specific polyclonal antibodies. When evaluating the phenotypic aspects and serological reactivity profile against species-specific polyclonal antibodies and sera from patients with HP disease and/or infection, we observed optimal results for the antigenic preparations obtained from 50% of the isolates (49, 200, 406, 802, 2030, and 879). These isolates showed the typical morphology of the genus *Histoplasma*. Samples 49 and 2030 were isolated from patients with HIV/AIDS with irregular and absent antiretroviral drug use, respectively. These isolates revealed the presence of hyphae, microconidia, and macroconidia with club-shaped ornamentation typical of *H. capsulatum*.

Other studies have demonstrated that the culture medium results in phenotypic alterations in terms of the macro-and micromorphology of the isolates. According to Berliner[28], there are two distinct colonies of *H. capsulatum*: albino (type A) and brown (type B). One is characterized by the presence of aerial mycelium, thick hyphae, a large amount of smooth and/or macroconidia with club-shaped ornamentation, and microconidia present or absent; the other is brown or type B, characterized by the presence of many macroconidia with club-shaped ornamentation and a low frequency of microconidia. The author noted that type A cultures with successive subcultures become non-sporulating (mycelial sterilia). Borok[29] evaluated *Histoplasma* isolates maintained by sub-cultivation on Sabouraud agar at 27 °C for at least three years. The authors observed that brown cultures, which become a non-sporulating albino form, could be reversed by manipulating the substrate used in the culture, suggesting that the induction-suppression of this mechanism may be involved in the morphological variations of this species. Our results are consistent with those of Borok[29] regarding the induction-suppression mechanism of the sporulate state and its relationship with the substrate used for cultivation and origin from isolation. This is because there is a difference in conidiogenesis between isolated samples from individuals with and without HIV/AIDS. Isolates from individuals without HIV/AIDS showed intense conidiogenesis, even after a long period of *in vitro* maintenance. This includes the 879 isolate, which had 13 years since isolation and typical micromorphology. However, it appears that samples of *H. capsulatum* isolated from HIV/AIDS individuals tend to lose sporulation capability more quickly. Additionally, degeneration of macroconidia with club-shaped ornamentation occurs, as observed in sample 212, and absence of

conidia as in sample 340, particularly with continuous or irregular use of antiretroviral. In parallel, morphological alterations of hyphae and the absence of conidia occurred in sample 268 from a patient with a late diagnosis of HP, chronic meningitis, and intracranial hypertension treated with high doses of corticosteroids. Treatment with corticosteroids and other immunosuppressive agents that suppress cell-mediated immunity is a major predisposing factor for fungal diseases.

Freitas et al[30] examined superficial mycosis in cutaneous allergy patients using corticosteroids and verified that treatment with steroids can cause misleading results because the anti-inflammatory effect may attenuate and mask cutaneous lesions, although they are risk factors for the development of superficial mycosis in cutaneous allergy patients. We verified that there is a tendency for morphological alteration due to the use of antiretroviral drugs and corticosteroids. Our results are consistent with those of Cresnar and Zakelj-Mavric[31], who described the action of steroids on the fungal response. The authors observed that dimorphic fungi require a morphogenetic transition to survive and invade the host, and the presence of estrogens affects the morphology of the fungus, including the formation of germ tubes. Cresnar and Zakelj-Mavric[31] reported that corticosteroid intrusion by fungal cells causes stretching of the cell membrane and induction and phosphorylation of protein C-like kinase 2 (pck2), thus altering its integrity. In view of this, the authors emphasized the importance of the cell wall in the growth and morphogenesis of the fungus, as it can modify the interaction of the yeast with the environment or host, acting on adhesion, recognition by the host's immune system, and virulence. Another study observed the presence of mycelial and yeast forms, in addition to that with germ tube formation on histopathologic examination of patients with endovascular infections caused by *H. capsulatum*[32]. The authors did not report the use of corticosteroids and the presence of a germ tube in patients with endovascular infections, whereas in this study, isolates 299 and 584 produced them at room temperature.

A study conducted by Damasceno et al[33] on HP in an HIV patient from northeastern Brazil described the isolation of fifty-one strains. They presented colonies with surfaces ranging from pale white to beige (70.6%) to dark brown (29.4%). In this study, 82.4% of the cultures had cottony texture and 17.6% had a powdery texture. Microconidia were observed in all isolates, whereas macroconidia were identified in 74.5% of *H. capsulatum* isolates. The authors reported that the DI assay yielded only 17.6% positivity for *H. capsulatum* exoantigens. In our study, the antigens obtained from HIV patients, especially those using antiretroviral drugs, demonstrated a low profile of recognition of antigenic fractions H and M on DI. A similar result was observed for antigens obtained from patients using corticosteroids and antiretroviral drugs.

A limiting factor of this study included sampling. However, these data support future studies that relate the immune status of individuals with HIV/AIDS, antiretroviral and corticosteroid treatment, and drug interactions with the dimorphism of these samples, emphasizing atypical morphology. The isolation and identification of *H. capsulatum* is important for the diagnosis of HP.

In conclusion, despite the great variability of existing and available methodologies in the diagnosis of HP, it is of fundamental importance to study the characteristics of *H. capsulatum* isolates. Differences can be demonstrated not only in the identification of the fungal agent but also in obtaining immunobiological agents such as antigens. There is a relationship between the expression of H and M antigens and the morphologies of these isolates. The factor interfering with obtaining immunobiological agents is not the patient's immune status, but the use of drugs such as antiretroviral and/or steroids. These change the

production of atypical cellular elements in culture, leading to a low profile of recognition of antigenic fractions H and M on DI.

CONFLICT OF INTEREST

The authors declare that there are no conflicting interests.

FUNDING

Not apliccable.

ACKNOWLEDGEMENTS

We are grateful to Miss Lucia Cupertino Barreto (*in memorian*), Mr. Almir Robson Ferreira, and Mrs. Maria Iranides Santana de Oliveira for their Technical assistance. We also thank Dr. Adriano Abudd for his assistance in improving the image of the micromorphological aspects of *H. capsulatum*. The study was supported by Instituto Adolfo Lutz (CTC-IAL#06/04), Disease Control Coordination, São Paulo State Health Department , and Institute of Tropical Medicine of São Paulo (#10/03 e # 983/03).

AUTHOR'S CONTRIBUTIONS

All authors have contributed significantly to this study: Roseli Santos de Freitas-Xavier conducted laboratory evaluations and drafted version of the article, Isabel Alves Feitosa Maciel made version of the text, Vera Lúcia Teixeira de Freitas collaborated in the version of the text, Adriana Pardini Vicentini made a critical revision of the text. All authors read and approved the final manuscript.

PRESENTATION NOTE

The results of this article are based on the master's dissertation of Roseli Santos de Freitas-Xavier, entitled "Phenotypic characterization and standardization of *Histoplasma capsulatum* var. *capsulatum* antigens for the diagnosis of histoplasmosis", defended in 2005 by the Graduate Program in Science of the Disease Control Coordination of the São Paulo State Health Department.

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