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ORIGINAL ARTICLE

USEFULNESS OF AN ELISA KIT FOR THE DETECTION OF *Histoplasma capsulatum* ANTIGEN IN PATIENTS WITH AIDS

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ABSTRACT

Histoplasmosis is a systemic mycosis frequently affecting patients infected with HIV, appearing as acute or subacute disseminated forms. Early diagnosis is simple when muco-cutaneous lesions are present; but in their absence the use of non-culture based methods is usually required presenting a fundamental challenge for the management and prognosis of this infection. The aim of this study was to analyze the sensitivity and specificity of an Elisa kit for the detection of the galactomannan antigen of *Histoplasma capsulatum* in different clinical samples. A total of 98 clinical samples obtained from different organic fluids were analyzed: 66 sera, 28 urine samples, 3 bronchoalveolar lavages and one cerebrospinal fluid. They corresponded to a total of 61 patients: 27 with histoplasmosis associated with AIDS, 7 histoplasmosis in non-reactive HIV individuals and 27 patients with other diseases but which were clinically similar to histoplasmosis. The sensitivity of the detection of the galactomannan antigen in serum of patients with histoplasmosis and AIDS was 76% and the specificity was 56%. In urine samples of this group of patients the sensitivity was 75%.

KEY WORDS: Histoplasmosis; *Histoplasma capsulatum*; *H. capsulatum* antigen

INTRODUCTION

Histoplasmosis is a systemic mycosis that frequently affects HIV-infected individuals and is the third most frequent mycosis in Argentina in this population. This group of patients usually suffers acute or sub-acute disseminated forms. When cutaneous or muco-cutaneous lesions are present (> 60% of cases in our country) early diagnosis is facilitated since it is possible to visualize yeasts in direct examinations of samples obtained by scraping these lesions. However, there is a percentage of patients without muco-cutaneous manifestations and whose symptoms are similar to those of many other AIDS-related infections. In such cases the diagnosis is often reached from the blood cultures, but this requires considerable time and early mortality in untreated patients is very high (30%) (Cáceres et al. 2016; Colombo et al. 2011; Negroni 2008).

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Recently, ELISA techniques for the detection in urine or serum of circulating antigens of *Histoplasma capsulatum* have been developed with promising results (Connolly et al. 2007; Hage et al. 2011; Wheat et al. 1986). However, they are only available in the USA. Several researchers have developed other immunoenzymatic techniques and the Center for Disease Control (CDC) has standardized a kit which was validated both in Guatemala and Colombia (Cáceres et al. 2014; Gómez et al. 1997; Gómez et al. 1999; Guimarães et al. 2004; Guimarães et al. 2010; Lindsley et al. 2007a; Lindsley et al. 2007b; Scheel et al. 2009; Scheel et al. 2014; Swartzentruber et al. 2009a; Theel et al. 2013; Theel et al. 2015; Zhang et al. 2013).

The aim of this study was to analyze the sensitivity and specificity of an ELISA kit for the diagnosis of histoplasmosis by detection of the galactomannan antigen of *H. capsulatum* in different clinical samples of patients with histoplasmosis, especially when this mycosis was associated with AIDS.

MATERIAL AND METHODS

A total of 98 samples of different organic fluids were analyzed: 66 sera, 28 urine samples, 3 bronchoalveolar lavages and one cerebrospinal fluid. They corresponded to a total of 61 patients: 27 with histoplasmosis associated with AIDS, 7 cases of progressive histoplasmosis not related to AIDS and 27 patients with other diseases that were clinically similar to histoplasmosis and which were evaluated to study the specificity of the test (control group).

Histoplasmosis has been confirmed in all cases by direct examination or cultures of different clinical samples according to the methodology routinely used in the Mycology Unit (Arechavala et al. 1993; Bianchi et al. 2000; Guelfand et al. 2015) (Tables 1 and 2).

Serological tests (immunodiffusion and counter immunoelectrophoresis) for detection of anti- *H. capsulatum* antibodies were applied to all the sera used for the detection of the galactomannan antigen (Guelfand et al. 2015).

Determinations to detect *H. capsulatum* galactomannan antigen were performed by ELISA, IMMY ALPHA® (Immunomycologics, Norman, Ok. USA). The total number of determinations was very limited as we only had a single kit. This was provided by the manufacturers to test the methodology, as it is not commercially available in our country yet. Determinations in serum were performed in the same way as in urine samples, EDTA or heat were not used to pre-treat the serum samples to break immune complexes, as has been reported in other papers (Swartzentruber et al. 2009a).

Table 1. Results of the microbiological, serological and *Histoplasma capsulatum* antigen detection studies in HIV-negative patients with histoplasmosis

Patient	Microbiological diagnosis	Histoplasmosis serology		Antigen detection		Time at which antigen determination was performed
	Material	ID* titer	Nr. of bands in CIE#	Sample	Result ng/ml	
1	Nostril biopsy	1:8	3	Serum	0.46	At diagnosis
2	Urine culture	Pure serum	1	Serum	0	At diagnosis
				Urine	0	
		Neg	1	Serum	0	Treatment control
3	Subcutaneous nodules	1:8	3	Serum	0	At diagnosis (kidney transplant)
4	Palate biopsy	1:32	2	Serum	4.54	At diagnosis
5	Skin biopsy	Neg	Neg	Serum	0	At diagnosis
6	Cutaneous scraping	1:4	2	Serum	0.82	At diagnosis
				Urine	0.57	
7	Cutaneous scraping and sputum	Pure serum	1	Serum	0	Treatment control

*ID: immunodiffusion test; #CIE: Counterimmunoelectrophoresis; Neg: negative

Table 2. Results of the microbiological, serological and *Histoplasma capsulatum* antigen detection studies in 27 patients with AIDS-associated histoplasmosis

Patient	Microbiological diagnosis	Histoplasmosis serology		Antigen detection		Time at which antigen determination was performed
	Material	ID titer	Nr. of bands in CIE	Sample	Result ng/mL	
1	Face scraping	1:4	0	Serum	0.7	At diagnosis
				Urine	20.5	
		1:4	0	Serum	4.3	First control
		s. p.	2	Serum	2.3	Second control
				Urine	0.57	
2	Blood culture, bronchoalveolar lavage	1:32	3	Serum	3.96	At diagnosis
				Urine	2.78	
				CSF	0.40	
3	Palate scraping	Neg^	Neg	Serum	0	At diagnosis
				Urine	0	
4	Skin scraping, blood culture	Neg	Neg	Serum	11.60	At diagnosis
				Urine	42.30	
5	Skin scraping	Neg	Neg	Serum	0.25	Inflammatory reconstitution immune syndrome
6	Skin scraping	Neg	Neg	Serum	31.2	At diagnosis
				Urine	14.3	
		Neg	Neg	Serum	3.39	Control
				Urine	0	
7	Skin scraping	Pure serum	1	Serum	0	At diagnosis
				Urine	5.65	
8	Skin scraping	Neg	0	Serum	4.47	At diagnosis
				Urine	29.78	
9	Skin scraping	Neg	0	Serum	1.74	At diagnosis
				Urine	0.46	

10	Skin scraping, blood culture, bronchoalveolar lavage	Neg	0	Serum	21.9	At diagnosis
				Serum	16.6	Control
				Urine	66.7	
11	Perianal ulcer	Neg	1	Urine	0	At diagnosis
12	Skin scraping, blood culture	Neg	0	Serum	20.9	At diagnosis
				Urine	55.0	
13	Blood culture, sputum, skin scraping	1:4	2	Serum	3.93	At diagnosis
14	Skin scraping	Pure serum	2	Serum	9.4	At diagnosis
15	Blood culture	Pure serum	2	Serum	0	At diagnosis
		BAL		Urine	0.2	
				0.2		
16	Blood culture, skin scraping	Neg	0	Serum	0	At diagnosis
				Urine	22.58	
17	Skin scraping	Neg	0	Serum	29.1	At diagnosis
				Urine	62.93	
18	Skin scraping	1:4	2	Serum	10.7	At diagnosis
				Urine	40.8	
				BAL	46	
19	Palate scraping, bronchoalveolar lavage	1:8	2	Serum	2.8	At diagnosis
				Urine	22.9	
20	Tongue biopsy	Neg	0	Urine	0	At diagnosis
21	Bone marrow aspiration	Neg	0	Serum	0.07	At diagnosis
				Urine	6.7	
22	Blood culture, skin scraping, bronchoalveolar lavage	Neg	0	Serum	17.8	At diagnosis
				Urine	33.2	
				BAL	38.9	
23	Blood culture	Neg	0	Serum	2	At diagnosis
24	Face scraping	Neg	0	Serum	27.9	At diagnosis
				Urine	50.4	

25	Blood culture, Skin scraping	Neg	0	Serum	8.3	At diagnosis
		Neg	0	Serum	11.45	First control
				Urine	48	
		Neg	0	Serum	9.3	Second control
26	Cheek biopsy	Neg	0	Serum	0.03	At diagnosis
27	Node biopsy, blood culture	Pure serum	2	Serum	19.56	At diagnosis
				Urine	25.3	

*ID: immunodiffusion test; #CIE: counter immunoelectrophoresis; ^ Neg: negative. BAL: bronchoalveolar lavage

In the group of histoplasmosis in HIV negative patients the detection of the antigen was done at the moment of the diagnosis in 6 cases and in a post-therapeutic control in 2 patients (Table 1). In all cases the determination was performed in serum and in 2 patients it was also fulfilled in urine.

In the group of patients with histoplasmosis and AIDS the determinations were made at the time of diagnosis in 26 cases and in one case it was performed in a patient who had suffered the disease two years earlier. This individual was undergoing an immune reconstitution inflammatory syndrome with presence of yeasts compatible with *H. capsulatum* in a lymph node sample obtained by puncture but whose culture was negative. In 4 cases further determinations were made during follow-up and in one case only in a treatment control.

RESULTS

Histoplasmosis in HIV-negative patients

Serological tests were positive in 6/7 cases at the moment of diagnosis of histoplasmosis but the detection of antigenemia was positive only in 2/6 sera (33.3%) and in 1/2 of the analyzed urine samples. The concentration of galactomannan antigen in the positive samples was low and ranged from 0.57 to 4.54 ng/ml. The results of the studies performed on HIV-negative patients with histoplasmosis are shown in Table 1.

The results were positive in 25/33 sera, 18/24 urine samples, and 2/3 bronchoalveolar lavages. Some negative antigenemia results corresponded to sera from patients with positive antigenuria and others to post-treatment controls and only 3 patients were negative at the time of diagnosis. Eight out of 10 patients with positive serology results showed the presence of galactomannan antigen in serum or urine. The antigen concentration in the 2 positive bronchoalveolar lavages was very high (46 and 38.9 ng/ml). The data corresponding to the group of patients with histoplasmosis associated with AIDS is presented in table 2.

Controls

Fourteen serum and two urine samples were negative. In the 11 positive samples antigen levels varied between 0.75 and 2.65 ng/ml except in one case of chronic pulmonary aspergillosis where the value was 14.59 ng/ml. The results of antigen detection in serum and urine of patients suffering from other conditions different from histoplasmosis used as controls are presented in Table 3.

Table 3. Results of antigenemia and antigenuria of patients without histoplasmosis. Control group

Number of patients	Antigen in serum	Antigen in urine	Serology	Antigen level ng/ml
	Positive results/Analized samples			
27	11/25	0/2	0/25	0.75-2.65*

* Except from a patient with aspergilosis whose antigen level was 14.6 ng/ml

DISCUSSION

AIDS-associated histoplasmosis usually appears as an acute or subacute disseminated disease, with a poor prognosis if not diagnosed rapidly. In our country the circulating clade of *H. capsulatum* (LAmB) (Kasuga et al. 2003) causes muco-cutaneous lesions in a high proportion of patients (> 60%), in such cases the diagnosis can be made quickly by scarification or biopsy of the lesions. However, there is a group of patients who are diagnosed from the development of the fungus in blood or respiratory samples cultures, which

require about 15-20 days and on several occasions patients die before the results of the mycological exams are available. Moreover serological tests for antibody detection have low sensitivity in this group of patients in contrast with what is often seen in chronic forms of histoplasmosis in HIV-negative individuals (Guimarães et al 2006; Hage et al. 2010; Kauffman 2007; Kurowski & Ostapchuk 2002; Scheel & Gómez 2014).

Histoplasma capsulatum antigens that are released from the fungal cells can be detected in several body fluids (serum, bronchoalveolar lavage, pleural fluid, cerebrospinal fluid and urine) (Scheel & Gómez 2014). In order to improve and accelerate the diagnosis of histoplasmosis, especially in HIV-positive patients, techniques for the detection of circulating antigens were implemented many years ago. The first method applied was a radioimmunoassay technique described in 1986, which was only performed in Indianapolis (USA) (Wheat et al. 1986). This reference center then implemented immunoenzymatic methods (Connolly et al. 2007; Gutiérrez et al. 2008; Hage et al. 2010; Hage et al. 2011; Wheat 2007; Wheat et al. 1997; Wheat et al. 2007).

The CDC and some groups of researchers have developed *H. capsulatum* galactomannan antigen detection kits by capture ELISA with polyclonal or monoclonal antibodies (Cáceres et al. 2014; Cloud et al. 2007; Gómez et al. 1999; Guimarães et al. 2004; Guimarães et al. 2010; Gutierrez et al. 2008; Lindsley et al. 2007a; Lindsley et al. 2007b; Scheel et al. 2009; Theel et al. 2013).

Thus, this methodology might be available to diagnostic laboratories in some countries with a high prevalence of histoplasmosis (especially AIDS-associated histoplasmosis) and with few resources (Cáceres et al. 2016; Gutierrez et al. 2008; Muñoz et al. 2010; Scheel & Gómez 2014; Zhang et al. 2013).

Numerous studies have demonstrated that the ELISA technique has a high sensitivity to detect antigens in urine and also in serum. However cross-reactions may occur, especially when tested on materials from patients with other systemic mycoses (specificity in that group ranges between 10 and 31%) (Assi et al. 2011; Wheat et al. 1997; Wheat et al. 2006; Wheat et al. 2007). There are differences in the levels of sensitivity and specificity between the different kits that use this technique, and some of them are not commercially available (Cáceres et al. 2014; Cloud et al. 2007; Guimarães et al. 2010; Hage et al. 2011; Lindsley et al. 2007b; Scheel et al. 2009; Swartzentruber et al. 2009a; Theel et al. 2013; Theel et al. 2015; Wheat 2007).

In this study, the sensitivity of the detection of the galactomannan antigen in serum of patients with histoplasmosis and AIDS was 76% and in urine was 75%, and the specificity in serum was 56%. Only 2 determinations were made in urine in the control group that resulted negative.

The sensitivity of the technique with serum samples was lower in HIV-negative patients with chronic forms of histoplasmosis. In 6/7 cases

antibodies were detected by immunodiffusion. Some authors demonstrated that the sensitivity of the detection of circulating antigen might be increased by breaking the immune complexes with EDTA and/or heat (Swartzentruber et al. 2009a). Since we only had one kit to perform the technique, it was not simultaneously tested in sera treated with EDTA.

The technique was only tested in 3 bronchoalveolar lavage samples in patients with AIDS-associated histoplasmosis. In two, a very high value of antigen was detected which is in agreement with that reported in other publications (Hage et al. 2010; Scheel & Gómez 2014; Swartzentruber et al. 2009b).

As in other publications some cross-reactions were detected, the highest value was for a patient with chronic pulmonary aspergillosis (14.6 ng/ml). Another case with pneumocystosis had positive antigenemia as well as one with leishmaniasis and in a patient with lung cancer. One patient with a urinary candidiasis showed a negative result.

Theel et al. (2013) have evaluated this equipment and compared it with the one developed in Indianapolis (MiraVista) used as reference. 1003 samples of urine were processed in parallel and the results were concordant in 939 samples that were negative and 40 samples that were positive. Two samples were false positive with the IMMY kit and 45% of the remaining 22 that had been positive only with the MiraVista kit had values <0.4 ng/ml (Theel et al. 2013; Theel et al. 2015).

Despite the fact that the number of patients is low, we observed that this rapid technique contributes to the diagnosis of AIDS-related histoplasmosis. Galactomannan antigen levels were very high in urine although in some cases the determination was positive in serum and not in urine. It would be of great interest to be able to increase the number of samples to present more consistent data, especially referring to the specificity. It would also be of value to test with sera treated with EDTA and heat to observe if breaking the immune complex increases the sensitivity of this technique.

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