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Research Article

Co-Detection of Schistosoma Mansoni and schistosoma Haematobium infections by enzyme-Linked Immunoelectrotransfer Blot Test in Brazilians after Staying in Mozambique

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Abstract

A group of 132 Brazilians was in Mozambique (Africa) on July and November 1994, participated in peace a mission by United Nations Organization (UNO), in an endemic area for *Schistosoma mansoni* (***Sm***) and *Schistosoma haematobium* (***Sh***) and they swum in Licungo river during leisure time. We examined 87 individuals, all of them were male and their arithmetic age was 31 year. They presented signals and symptoms such as lumbar pain, dysuria, hematuria and pollakyuria and, they did not present gastrointestinal complaints. Urine test Showed that 30 (34.5%) eliminated *Sh* eggs and all of them presented eosinophilia. The *Enzyme-linked immunoelectrotransfer blot assay* (EITB) detected positive IgM for ***Sm*** and ***Sh*** in 26 (29.9%); only for ***Sm*** in 11 (12.6%); only for ***Sh*** in 24 (27.5%) and negative for both in 14 (16.1%). The diagnosis of them is commom after exposition in the region (Africa). Some regions of Brazil are endemics to ***Sm*** but not to ***Sh***, however there is not described autochthonous infection for ***Sm*** in Brazil. All of them with urine positive for ***Sh*** presented 100% of parasitological cure after first, second or third treatment with praziquantel.

ABBREVIATIONS

EITB: Enzyme-linked Immunoelectrotransfer *Blot assay*; UNO: United Nations Organization; HAMA: Microsomal antigens from *S. haematobium*; MAMA: microsomal antigens from *S. mansoni*; *Sh*: *Schistosoma haematobium; Sm*: *Schistosoma mansoni*

INTRODUCTION

Schistosomiasis is described as an acute and chronic disease caused by parasitic worms. There are two major forms of schistosomiasis: intestinal which ***Sm*** has infected people in Africa, Middle East, Caribbean, Brazil, Venezuela and Suriname and urogenital which ***Sh*** has infected people in Africa, Middle East and Corsica (France). Estimates Show that at least 258 million people required preventive treatment for schistosomiasis in 2014. More than 61.6 million people were reported to have been treated for schistosomiasis in 2014 [1]; 249 million people are infected in 78 countries, and more than 650 million live in endemic areas [2]. Estimates therefore vary widely between 20 000 and 200 000 deaths per year [3,4]. In Africa, it is estimated 85% of the world's cases of schistosomiasis, where prevalence rates can exceed 50% in local populations. ***Sm*** and ***Sh***, both are distributed throughout Africa [3]. It mostly affects poor and rural communities, particularly agricultural, fishing populations, children with inadequate hygiene and contact with infested water and women doing domestic chores in infested water, such

as washing clothes and, eco-tourism, in travelers and migrants [1,5-8].

Acute schistosomiasis is characterized by fever, headache, myalgia, diarrhea, and respiratory symptoms. Eosinophilia is present, as well as often painful hepatomegaly or splenomegaly in ***Sm***. The clinical manifestations of chronic schistosomiasis are the result of host immune responses to schistosome eggs. Eggs secreted by adult worm pairs enter the circulation and lodge in organs and cause granulomatous reactions. Chronic inflammation can lead to bowel wall ulceration, hyperplasia, and polyposis and, with heavy infections, to periportal liver fibrosis in ***Sm***. ***Sh*** eggs typically lodge in the urinary tract and can cause dysuria and hematuria [3]. In co-infection (***Sm and Sh***), the ectopic elimination of schistosome eggs results from sexual interactions between the two species of schistosomes, and from a spillover of high infection loads. The clinical study Showed that the morbidity was lower in individuals with mixed infections and high loads of ***Sh*** than in those with ***Sm*** infections only, suggesting a possible lowering effect of ***Sh*** infection on ***Sm*** morbidity [9,10].

The diagnosis is through the detection of parasite eggs in stool or urine specimens but antibodies and/or antigens detected in blood or urine samples are also indications of infection. For urogenital schistosomiasis, a filtration technique using nylon, paper or polycarbonate filters is the standard diagnostic technique. ***Sm*** infections approximately have microscopic blood in their urine and chemical reagent strips can detect this. The eggs of intestinal schistosomiasis can be detected in faecal specimens through a technique using methylene blue-stained cellophane soaked in glycerin or glass slides, the Kato-Katz technique. For people living in non-endemic or low-transmission areas, serological and immunological tests may be useful in showing exposure to infection and the need for thorough examination, treatment and follow-up [3].

Serologic tests are useful to diagnose light infections where egg shedding may not be consistent in travelers and in others who have not had schistosomiasis previously [11]. Antibody tests do not distinguish between past and current infection. Test sensitivity and specificity vary, depend on the antigen preparation used and how the test is performed [3,12-15].

EITB in *S. japonicum* had acceptable performance characteristics in China [16], in ***Sh*** of Brazilians after staying in Africa [17], in epidemiological studies in Puerto Rico [18], in *S. bovis* permits the confirmation of diagnosis in chronic and acute phases of the disease [19]. The combination of urine CCA and serum CAA for detecting circulating antigens and the combination of the ***Sh*** adult worm microsomal antigens (HAMA) FAST-ELISA and the HAMA EITB for detecting antibodies significantly improved the sensitivity on detecting ***Sh*** circulating antigens and antibodies [12].

MATERIALS AND METHODS

The protocol of this study was approved by the Research Ethics Committee of the Army Biology Institute (Instituto de Biologia do Exército), Rio de Janeiro. Informed consent was obtained from all patients and the guidelines for human experimentation of the National Health Council were followed in the conduct of clinical research. Among the 132 Brazilian men who participated in a United Nations Peace Mission in Mozambique, Africa, 87 accepted our offer of clinical and serological evaluation for schistosomiasis. The principal signs and symptoms were evaluated together with laboratory exams. Three 24-hour urine samples were collected at a minimum interval of one week from all patients. Helminth eggs were recovered by sedimentation (24hr) and centrifugation (3,500g/5 min) of the urine sample. One-hundred µL of the centrifuged material was examined with a microscope at 100X and 400X magnifications. EITB assay, with purified adult worm microsomal antigens from ***Sh*** (HAMA) or ***Sm*** (MAMA), was performed as previously described. The patients avoiding ***Sh*** eggs by urine were treated by praziquantel in a single dose (40mg/kg body weight) by oral route. The parasitological control of cure was done by urine, cystoscopy and histopathology. We didn't perform fecal examination by Kato-Katz method because neither of them presented signals and symptoms suggestive of ***Sm*** infection, but the fecal examination by Ritchie, Faust-Willis and Hoffman-Pons-Janer methods were negatives.

RESULTS AND DISCUSSION

The arithmetic mean age of the 87 patients was 31 (median = 32.02) years old. The clinical and laboratory evaluations are listed in Table (1). Among them 30 (34.5%) presented ***Sh*** eggs in their urine and 55 (63.2%) were serologically positive for EITB. Eosinophilia was observed in 30 (34.5%), dysuria in 32 (36.8%), hematuria in 26 (29.9%) and lumbar pain in 36 (41.4%). All individuals with ***Sh*** eggs in their urine had serum EITB positive for HAMA. Among the 55 EITB positive individuals 30 (54.5 %) presented ***Sh*** eggs in their urine. The *Enzyme-linked immunoelectrotransfer blot assay* (EITB) was positive for microsomal antigens from ***Sm*** (MAMA) in 11 (12.6%), positive for microsomal antigens from ***Sh*** (HAMA) in 24 (27.6%), positive for both (**MAMA and HAMA**) in 26 (29.9%) and negative for both in 14 (16.1%).

According our findings, signs and symptoms, ***Sh*** eggs in the urine and eosinophilia, each one of them were frequent in about a third of the patients and the EITB assay positive in two third of them; the serology was twice most sensitive than urine examination. Other authors reported to be three times more sensitive in detecting ***Sh*** infection (1p). The EITB positive in all our cases with ***Sh*** eggs in urine enhances the intrinsic value of this serological method. Otherwise 25 individuals who were negative

Table 1: Clinical evaluation in the 87 individuals after exposure in Licungo River (Mozambique-Africa).

Evaluation	Positive		Negative	
	nº	%	nº	%
Urinary				
symptomatology	65	74.7	22	25.3
Lumbar pain	36	41.4	61	58.6
Dysuria	32	36.8	55	63.2
Hematuria	26	29.9	61	70.1
Pollakiuria	13	28.6	42	76.4
Gastrointestinal				
Symptomatology	0	0	87	100

for the urine examination had positive serology, and among them 12 without symptomatology. That 12 positive patients would not have been identified without EITB. Considering the frequent lacks of symptoms, we deem the EITB an important adjunct to patient identification for schistosomiasis, when its realization is possible. Hematuria, dysuria, lumbar pain, eosinophilia are good indicators of infection, when the individuals come from endemic areas, as happened with some of our patients. The main point is that 11 (12.6%) patients were only with ***Sm*** positive in EITB and the diagnosis of co-infection was detected in 26 (29.9%). It would not be possible with other methods used in these patients. The Kato-Katz method was not performed because signals and symptoms of ***Sm*** infection were not presented. It is possible that when there is co-infection, the ectopic elimination of schistosome eggs results from sexual interactions between the two species of schistosomes, and from a spillover of high infection loads. Our patients went to Mozambique in 1994 and, in 2001, six years after, still eliminated viable eggs in urine; it can be for the high infection load. Moreover, clinical study showed that the morbidity was lower in individuals with mixed infections and high loads of ***Sh*** than in those with ***Sm*** infections only, suggesting a possible lowering effect of ***Sm*** morbidity (1c, 1m). Some patients did not present ***Sm*** complaints, the EITB was positive only to Sm in 12.6%, and it is possible in lower infection load. When patients presented both exams positives (Ms. and she) without symptoms of Ms., It can be because there was a low infection load to Ms. and a high infection load to ***Sh.***

CONCLUSION

The EITB method was an important examination in the patients from endemic area without re-exposition, since it is difficult to get eggs of Ms. and her from patients with low infection loads or after a long time from exposition. We need more studies with patients that come from endemic areas without re-exposition to consider the EITB like a main method to diagnosis, overall in exposition to more than one species. All of them with urine positive for she presented 100% of parasitological cure after first, second or third treatment with praziquantel, so it was effective to these patients.

Table 2: Laboratorial evaluation in the 87 individuals after exposure in Licungo River (Mozambique-Africa).

Evaluation	Positive		Negative	
	nº	%	nº	%
Eggs in urine exam	30	34.5	57	65.5
Eosinophilia	30	34.5	57	65.5
EITB (HAMA/MAMA)	73	83.914	16.1	

Table 3: EITB evaluation in the 87 individuals after exposure in Licungo River (Mozambique-Africa).

Evaluation	Positive		Negative	
EITB (HAMA/MAMA)	nº	%	nº	%
HAMA (only)	24	27.5	63	72.5
MAMA (only)	1112.6		76	87.4
HAMA and MAMA	26	29.9	51	57.1

REFERENCES

1. van der Werf MJ, de Vlas SJ, Brooker S, Looman CW, Nagelkerke NJ, Habbema JD, et al. Quantification of clinical morbidity associated with schistosome infection in sub-Saharan Africa. Acta Trop. 2003; 86: 125-139.
2. PAHO/WHO Schistosomiasis Regional Meeting Defining a road map toward verification of elimination of schistosomiasis transmission in Latin America and the Caribbean by 2020 Venue: Verdanza Hotel, 8020 Tartak Street Isla Verde Carolina, PR 00979 Date: Tuesday, October 21 to Wednesday, October 22, 2014.
3. Parasites- Schistosomiasis. Centers for Disease Control and Prevention. 2012.
4. Drudge-Coates L, EAUN Chair, London (GB) Schistosomiasis. A deadly neglected tropical .disease. http://eaumunich2016.uroweb.org/history-congress.I
5. Clerinx J, Van Gompel A. Schistosomiasis in travellers and migrants. Travel Med Infect Dis. 2011; 9: 6-24.
6. Corachan M. Schistosomiasis and international travel. Clin Infect Dis. 2002; 35: 446-450.
7. Grobusch MP, Muhlberger N, Jelinek T, Bisoffi Z, Corachan M, Harms G, et al. Imported schistosomiasis in Europe: sentinel surveillance data from TropNetEurop. J Travel Med. 2003; 10: 164-169.
8. Meltzer E, Artom G, Marva E, Assous MV, Rahav G, Schwartzt E. Schistosomiasis among travelers: new aspects of an old disease. Emerg Infect Dis. 2006; 12: 1696-1700.
9. Cunin P, TchuemTchuenté LA, Poste B, Djibrilla K, Martin PM. Interactions between Schistosoma haematobium and Schistosoma mansoni in humans in north Cameroon. Trop Med Int Health. 2003; 8: 1110-1117.
10. Ross AG, Vickers D, Olds GR, Shah SM, McManus DP. Katayama syndrome. Lancet Infect Dis. 2007; 7: 218-224.
11. El-Gendy SD, Osman AM, Al-Sherbiny MM. Epidemiology and immunodiagnosis of schistosomiasis haematobium in low endemic area in Egypt. J Egypt Soc Parasitol. 1999; 29: 229-246.
12. Al-Sherbiny MM, Osman AM, Hancock K, Deelder AM, Tsang VC. Application of immunodiagnostic assays: detection of antibodies and circulating antigens in human schistosomiasis and correlation with clinical findings. Am J Trop Med Hyg. 1999; 60: 960-966.
13. Tsang VC, Hancock K, Maddison SE, Beatty AL, Moss DM. Demonstration of species-specific and cross-reactive components of the adult microssomal antigens from Schistosoma mansoni and Schistosoma japonicum (MAMA and JAMA). J Immunol. 1984; 132: 2607-2613.
14. Rivera-Marrero CA, Hillyer GV. Isolation and partial characterization of shared antigens of Biomphalaria glabrata and Schistosoma mansoni and their evaluation by the ELISA and the EITB. J Parasitol. 1985; 71: 547-555.
15. Xue H, Liu S, Ren H, Qiang H, Xiao S, Feng Z, et al. Enzyme-linked immunoelectrotransfer blotting analysis of human serologic responses to infective hookworm larval antigen. Chinese Medical Journal 1999; 112: 249-250.
16. Xu J, Peeling RW, Chen J-X, Wu X-H, Wu Z-D, Wang S-P, et al. Evaluation of Immunoassays for the Diagnosis of Schistosoma japonicum Infection Using Archived Sera. PLoS Negl Trop Dis. 2011; 5: 949.
17. da Silva IM, Tsang V, Noh J, Rey L, Conceição MJ. Clinical and laboratorial evaluation of urinary schistosomiasis in Brazilians after staying in Mozambique. Rev Soc Bras Med Trop. 2006; 39: 272-274.
18. Clerinx J, Van Gompel A. Schistosomiasis in travellers and migrants.

Travel Med Infect Dis. 2011; 9: 6-24.

19. PardoJ, ArellanoJLP, López-Vélez R, CarranzaC, Cordero M, Muro A. Application of an ELISA test using Schistosoma bovis adult worm antigens in travellers and immigrants from a schistosomiasis endemic area and its correlation with clinical findings. Scand J Infect Dis. 2007; 39: 435-440.

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