Clinical and laboratorial evaluation of urinary schistosomiasis in Brazilians after staying in Mozambique

Avaliação clínica e laboratorial de esquistossomose urinária em brasileiros após permanência em Moçambique

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ABSTRACT

We examined 87 Brazilian individuals of a group of 132 that, on July and November 1994, participated in a peace mission in Mozambique. They served in an endemic area for haematobic schistosomiasis, where they swum in Licungo river during leisure time. Their arithmetic mean age was 31 year and all of them were male. Their urine test showed that 30 (34.5%) eliminated S. haematobium eggs and 55 (63.2%) presented positive serology by the enzime-linked immunoelectrotransfer blot test with purified microsomal antigen of S. haematobium adult worms. Eosinophilia was found in 30 (34.5%), haematuria in 26 (29.9%), dysuria in 32 (36.8%) and lombar pain in 36 (41.4%). All of those that eliminated eggs through urine had positive serology. Among the 25 patients with positive serology and without S. haematobium eggs in the urine test, 13 were symptomatic and 12 assymptomatic. The treatment with praziquantel for the 30 patients, with urine positive to S. haematobium eggs, presented 70% of parasitological cure. **Key-words:** Schistosomiasis. Enzyme-linked immunoelectrotransfer blot assay. Brazilians. Mozambique.

RESUMO

Nós examinamos 87 brasileiros de um grupo de 132 que, entre julho e novembro de 1994, participaram de um missão de paz em Moçambique. Eles serviram em uma área endêmica de esquistossomose haematóbica e nadaram no rio Licungo em períodos de lazer. A idade aritmética deles era 31 anos e todos eram do gênero masculino. O exame de urina revelou que 30 (34,5%) eliminavam ovos de *S. haematobium* e 55 (63,2%) tinham sorologia positiva pelo teste *enzyme-linked immunoelectrotransfer blot* com antígeno microsomal purificado de vermes adultos de *S. haematobium*. Eosinofilia foi encontrada em 30 (34,5%), haematuria em 26(29,9%), disúria em 32(36,8%) e dor lombar em 36(41,4%). Todos que eliminavam ovos pela urina tiveram sorologia positiva. Entre os 25 pacientes com sorologia positiva e sem ovos de *S. haematobium* no exame de urina, 13 eram sintomáticos e 12 assintomáticos. O tratamento pelo Prazinquantel nos 30 pacientes com urina positiva para ovos de *S. haematobium* apresentou 70% de cura parasitológica.

Palavras-chaves: Esquistossomose. Enzyme-linked immunoelectrotransfer blot assay. Brasileiros. Moçambique.

The habitat of *S. haematobium* is the urinary tract where the host response to the eggs retained in the tissues results in lesions and hematuria.

Urinary schistosomiasis is found in 53 countries on the African continent and in the Middle East^{33 34}; however, no autochthonous case has been reported in Brazil thus far. A

number of the 132 Brazilian military and ex-military servicemen, who served 4 months in Mozambique (Africa) on a United Nations peace mission in 1994 and had swum in the Licungo River, Zambezia province, during their leisure time, were infected with *Schistosoma haematobium*. The enzyme-linked immunoelectrotransfer blot assay (EITB)

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with purified antigens²⁵ has been used for the diagnosis of several infectious diseases. EITB has been important in elucidating epidemiological information in neurocysticercosis and schistosomiasis^{1 4 5 8 15 17 19 22 29}. EITB was used to solve problems with cross reactivity in the serological diagnosis of parasitic infections, aiding in the selection of specific diagnostic antigens^{3 10 18 20 23 25 26 27}; in the evaluation of immunodiagnostic techniques for various infectious diseases^{2 5 8 9 11 13 15 21 24 35} and in acquired immunity studies after the use of specific antigens¹¹. EITB can be highly specific and sensitive in the detection of antibodies for many infectious agents^{7 14 31 33}.

The aim of this study was to evaluate the signs and symptoms, laboratorial exams and clinical usefulness of EITB serology for the identification of *Schistosoma haematobium* infections in Brazilian returnees after exposure in Mozambique.

PATIENTS 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 S. haematobium (HAMA) or S. mansoni (MAMA), was performed as previously described^{27 32}.

The patients avoiding *S. haematobium* 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.

RESULTS

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 *S. haematobium* 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 *S. haematobium* eggs in their urine had serum EITB positive for HAMA. Among the 55 EITB positive individuals 30 (54.5%) presented *S. haematobium* eggs in their urine.

Table 1- Clinical and laboratorial evaluation in the 87 individuals at	ter
exposure to Schistosoma haematobium infection in Licungo Riv	ver
(Mozambique - Africa).	

Evaluation	Positive		Negative	
	n°	%	n°	%
Symptomatology	65	74.7	22	25,3
Hematuria	26	29.9	61	70.1
Dysuria	32	36.8	55	63.2
Pollakiuria	13	28.6	42	76.4
Lumbar pain	36	41.4	51	58.6
Eosinophilia	30	34.5	57	65.5
Eggs in urine exam	30	34.5	57	65.5
EITB (HAMA)	55	63.2	32	36.8

Among the 25 individuals who were negative for the urine examination, but positive for serology, 13 (52%) were symptomatic and 12 (48%) asymptomatic. Among these 25 individuals, 6 (24%) reported dysuria, 6 (24%) lumbar pain 5 (20%), hematuria and 2 (8%) pollakiuria.

In the follow up of 30 patients with *S. haematobium* eggs in urine exams, treated by praziquantel, 21 presented parasitological cure by urine exams and cystoscopy, biopsy and histopathological examination. Nine patients continued with viable eggs in the cystoscopy examination, in spite of negative urine examination. After the second treatment, five became negative for cystoscopy, one positive and the last three without information.

DISCUSSION

According our findings, signs and symptoms, *S. haematobium* 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 *S. haematobium* infection⁷. The presence of eggs in urina confirm the infection. It is considered the golden standard of the diagnosis and indicate the specific treatment.

The EITB positive in all our cases with *S. haematobium* eggs in urine enhances the intrinsic value of this serological method. Otherwise 25 individuals who were negative 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.

REFERENCES

 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. American Journal of Tropical Medicine and Hygiene 60: 960-966, 1999.

- Andriansimahavandy A, Esterre P, Auzemery A, Godinaud P. Particularities in the immune response in ocular cysticercosis. Archive of Institute Pasteur Madagascar 63: 34-37, 1996.
- Cho SY, Kang SY, Kim SI. Analysis of antigen specificity using monoclonal and polyclonal antibodies to *Cysticercus cellulosae* by enzyme-linked immunoelectrotransfer blot technique. The Korean Journal of Parasitology 25: 159-167, 1987.
- 4. Correa D, Sarti E, Tapia-Romero R, Rico R, Alcantara-Anguiano I, Salgado A, Valdez L, Flisser A. Antigens and antibodies in sera from human cases of epilepsy or taeniasis from an area of Mexico where *Taenia solium* cysticercosis is endemic. Annals of Tropical Medicine and Parasitology 93: 69-74, 1999.
- Cruz ME, Preux PM, Debrock C, Cruz I, Schantz PM, Tsang VC, Dumas M. Epidemiology of cerebral cysticercosis in an Andean community in Ecuador. Bulletin of Society Pathology Exotic 92: 38-41, 1999.
- Da Silva AJ, Piuvezam MR, de Moura H, Maddison S, Peralta JM. Rapid competitive enzyme-linked immunosorbent assay using a monoclonal antibody reacting with a 15-kilodalton tegumental antigen of Schistosoma mansoni for serodiagnosis of schistosomiasis. Journal of Clinical Microbiology 31: 2315-2319, 1993.
- El-Gendy SD, Osman AM, Al-Sherbiny MM. Epidemiology and immunodiagnosis of *schistosomiasis heamatobium* in low endemic area in Egypt. Journal of Egyptian Society Parasitology 29: 229-246, 1999.
- Garcia HH, Gilman RH, Tsang VCW, Gonzalez AE and Cysticercosis Working Group in Peru. Clinical significance of neurocysticercosis in endemic villages. Transaction of the Royal Society of Tropical Medicine and Hygiene 91: 176-178, 1997.
- Gomez-Priego A, Crecencio-Rosales L, de-La-Rosa JL. Serological evaluation of thin-layer immunoassay-enzyme-linked immunosorbent assay for detection in human trichinelosis. Clinical Diagnostic Laboratory Immunology 7: 810-812, 2000.
- Gottstein B, Tsang VC, Schantz PM. Demonstration of species-specific and cross- reactive components of *Taenia solium* metacesto de antigens. American Journal of Tropical Medicine and Hygiene 35, 308-313, 1986.
- Hyllier GV, Galanes MS. Identification of a17-kilodalton *Fasciola hepatica* immunodiagnostic antigen by the enzyme-linked immunoelectrotransfer blot technique. Journal of Microbiology 26; 2048-2053, 1988.
- Hillyer GV, Galanes MS, Garcia Rosa MI, Montealegre F. Acquired immunity in Schistosomiasis with purified *Fasciola hepatica* cross-reactive antigens. Veterinary Parasitology. 29: 265-280, 1988.
- Hillyer GV, Nieves-Frau LL, Vasquez G. Identification of a genus-specific Schistosoma mansoni soluble egg antigen reactive with the serum of infected patients. American Journal of Tropical Medicine and Hygiene 35: 1198-1204, 1986.
- Hillyer GV, Pacheco E. Isolation and characterization of *Schistosoma haematobium* egg antigens. American Journal of Tropical Medicine and Hygiene 35: 777-785, 1986.
- Hillyer GV, Tsang VC, Vivas-Gonzalez BE, Noh J, Ahn LH, Vorndam V. Agespecific decrease in seroprevalence of schistosomiasis in Puerto Rico. American Journal of Tropical Medicine and Hygiene 60: 313-318, 1999.
- Mansour WA, Kaddah MA, Shaker ZA, al Assal FM, Derbala MF. A monoclonal antibody diagnoses active Fasciola infection in humans. Journal of Egyptian Society Parasitology 28: 711-727, 1998.
- Migliani R, Rasolomaharo M, Ravaoalimalala VE, Rabarijaona L, Andriantsimahavandy A. Cystiscercosis in the port of Mahajanga: more frequent than we thought!. Archive of the Institute Pasteur of Madagascar 66: 39-42, 2000.
- Mousa WM. Evaluation of cercarial antigen for the serodiagnosis of fascioliosis in experimentally and naturally infected sheep. Vet. Parasitol 97: 47-54, 2001.
- 19. Qiu L, Liu S, Xue H, Zhang Y, Li H, Hu Y, He Y. SDS-PAGE and EITB analysis of the protein components of different isolates of *Schistosoma japonicum*

in China and Japan. Zhongguo Ji Sheng Chong Xue Yu Ji Sheng Ching Bing Za Zhi 17: 367-369, 1999.

- Rajshekhar V, Oommen A. Utility of the cysticercus immunoblot in a patient with an atypical solitary cerebral cystiscercus granuloma. Neurology India 49: 75-77, 2001.
- Ramadan NI, Abel Aaty HE, Mahmoud MS, El Nori A. An enzyme-linked immunoelectrotransfer blot assay for diagnosis of human cystic echinococcosis. Journal of Egyptian Society Parasitology 29: 849-857, 1999.
- Sanchez AL, Ljungstrom I, Medina MT. Diagnosis of human neurocysticercosis in endemic countries: a clinical study in Honduras. Parasitology International 41: 81-89, 1999.
- Santiago N, Hillyer GV. Antibody profiles by EITB and ELISA of cattle and sheep infected with *Fasciola hepatica*. Journal of Parasitology 74: 810-818, 1988.
- 24. Shaker ZA, Demerdash ZA, Mansour WA, Hassanein HI, el Baz HG, el Gindy HI. Evaluation of specific Fasciola antigen in the immunodiagnosis of human fascioliasis in Egypt. Journal of Egyptian Society of Parasitology 24: 463-470, 1994.
- Shen DW, Li YL, Hang JJ, Shi YE. Purification of 31/32 kDa proteins of adult Schistosoma japonicum and studies on their protective immunity. Zhongguo Ji Sheng Chong Xue Yu Ji Sheng Chong Bing Za Zhi 11: 241-243, 1993.
- 26. Silva IM, Thiengo R, Conceição MJ, Rey L, Lenzi HL, Pereira Filho E, Ribeiro PC. Therapeutic failure of praziquantel in the treatment of *Schistosoma haematobium* infection in Brazilians returning from Africa. Memórias do Instituto Oswaldo Cruz 100: 445-449, 2005.
- Tian M, Yi X, Zeng X, Zeng Q. Identification of antigens shared between Schistosoma japonicum and Trichinella spiralis. Hunan Yi Ke Da Xue Bao 23: 225-228, 1998.
- 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). Journal of Immunology 132: 2607-2613, 1984.
- 29. Tsang VC, Hancock K, Maddison SE, Beatty AL, Moss DM. Isolation and partial characterization of shared antigens of *Biomphalaria glabrata* and *Schistosoma mansoni* and their evaluation by the ELISA and the EITB. Journal of Parasitology 71: 547-555, 1984.
- Tsang VC, Hillyer GV, Noh J, Vivas-Gonzales BE, Ahn LH, Pilcher JB, Hightower AW, Deseda C, de Melecio CF. Geographic clustering and seroprevalence of schistosomiasis in Puerto Rico. American Journal of Tropical Medicine and Hygiene 56: 107-112, 1997.
- 31. Tsang VC, Tsang KR, Hancock K, Kelly MA, Maddison SE. Schistosoma mansoni adult microsomal antigens, a serologic reagent. I. Systematic fractionation, quantitation, and characterization of antigenic components. Journal of Immunology 130: 1359-1365, 1983.
- Tsang VCW, Peralta JM, Simons AR. Enzyme-Linked Immunoelectrotransfer Blot Techniques (EITB) for studying the specificities of antigens and antibodies separated by electrophoresis. Methods in Enzymology 92: 377-391, 1983.
- World Health Organization, CEGET-CNRS. Atlas of the global distribution of schistosomiasis28: 223-230, 1987.
- World Health Organization. Home Page WHO: http://www.who.int/ctd/ schisto/burdens.htm, 2004.
- Xue H, Liu S, Ren H, Qiang H, Xiao S, Feng Z, Hotez P. Enzyme-linked immunoelectrotransfer blotting analysis of human serologic responses to infective hookworm larval antigen. Chinese Medical Journal 112: 249-250, 1999.
- 36. Zeng X, Wang Y, Yi X, Wang S. Identification of antigens in circulating immune complexes of schistosomiasis japonica. Hunan Yi Ke Da Xue Xue Bao 22: 377-380, 1997.