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DIFFICULTIES IN THE DIAGNOSIS OF SCHISTOSOMIASIS IN PATIENTS OF THE DEPARTMENT OF TROPICAL AND PARASITIC DISEASES OF THE MEDICAL UNIVERSITY OF GDAŃSK

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Between 2002 and 2006 in the Department of Tropical and Parasitic Diseases of the Medical University of Gdansk 40 hospitalized patients were suspected of schistosomiasis on the basis of clinical manifestations, epidemiological data and positive serology tests (ELISA IgG).

In spite of multiple tests, schistosoma eggs were not identified neither in stool nor in urine of the patients.

Histopathological examinations of liver and colon or bladder mucosal biopsy have not revealed schistosoma eggs in chosen patients.

Diagnosis confirmation in case of negative parasitic tests requires serologic tests for schistosomiasis. ELISA serology tests for antibodies class G were performed in all 40 patients. In some cases the results were dubious – index in the upper limit or only slightly elevated. In those cases, cross reactions with *Plasmodium* spp. were taken into account. In 10 patients, serologic index for schistosomiasis was elevated during or a few weeks after treatment for malaria. In control tests, 4-8 weeks after the first examination,

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serologic indexes for schistosomiasis were significantly lower or normal without specific treatment with praziquantel (Biltricide, Cesol). Seven patients were lost from follow up.

Because of diagnostic difficulties confirmation tests with Immuno-Blot IgG were introduced to verify ELISA. After final clinical and serologic analysis, human schistosomiasis was diagnosed in 23 patients who were treated with success.

INTRODUCTION

Parasitic diseases are a major health hazard for people in tropical countries. Malaria is the most prevalent parasite disease, schistosomiasis is second. According to World Health Organization reports about 200 million people in 74 tropical and subtropical countries are infected with schistosoma worms, of these 170 million live in Africa (1). About 120 million are symptomatic, with 20 million having severe clinical disease. Number of deaths is not known but it is assumed that from hundreds of thousands to one million people die from schistosomiasis each year.

Human schistosomiasis is caused by a few species: *Schistosoma mansoni, S. haematobium*, *S. japonicum, S. mekongi* and *S. intercalatum*. The main cause of infection is exposure to fresh water in lakes, ponds, pools or slow rivers infected with snails *Biomphalaria* and *Oncomelania* which are natural hosts of *Schistosoma* spp. cercariae.

Cercariae develop into adult flukes in 4-8 weeks and then produce eggs for the first time. Adult *S. mansoni* produces 300 eggs per day, *S. japonicum* around 3000 eggs per day. Adult flukes are 10 to over 12 mm long and live 5-10 years.

Most infections are asymptomatic but in some cases a few weeks after infection high fever may develop (Katayama fever) accompanied by muscle pain and fatigue. Rarely acute infection may cause headache, abdominal pain, diarrhea, cough, skin rush, generalized limphadenopathy, hepatosplenomegaly and eosinophilia. Sometimes focal neurological defects may develop (2).

In many cases symptoms develop many years after infection causing chronic schistosomiasis. Late signs like granulomas and fibrosis that develop in human tissue and are caused by schistosoma eggs. Infection with *S. mansoni* or *S. japonicum* is characterized by periportal fibrosis which leads to portal hypertension, gastrointestinal hemorrhage, splenomegaly, hypersplenism and intestinal polyposis with diarrhoea and the passage of blood and mucus. Typical signs of *S. haeamatobium* infection in the late stage of the disease are bladder polyposis, hematuria, bladder scarring, urinary tract

calcifications, secondary renal disorders and increased frequency of bladder cancer. Infection with any *Schistosoma* species can cause pulmonary hypertension and focal changes in the central nervous system (http://www.emedicine.com/med./topic2071.htm, Nov 26,2007)

OBJECTIVE

The objective of this study was to:

- draw attention to a disease rarely diagnosed in Poland,
- analyze diagnostic procedures in patients suspected of schistosomiasis,
- asses diagnostic value of serologic tests performed in patients who stayed in regions endemic for schistosomiasis.

MATERIAL

Between 2002-2006, in the Department of Tropical and Parasitic Diseases 127 patients were hospitalized and examined for tropical diseases. Among them malaria was the most prevalent disease (32 patients) and schistosomiasis was in the second place.

During this period of time, 40 patients were suspected of schistosomiasis on the basis of clinical manifestations, epidemiological data and positive results in ELISA IgG tests. Final diagnosis of schistosomiasis was made in 23 patients – 22 men aged 22-64 years (mean age 37 years) and one woman 31 years old. All patients were Polish citizens.

Most patients contracted the disease in Africa (Cameroon, Nigeria, Ghana, Ivory Cost, Gabon, Togo, South Africa, Zambia, Kenya, Senegal, Sierra Leone). Only three patients were infected in South America (Argentina, Bolivia, Peru, Chile, Mexico). Patients stayed in tropical countries from 2 months to 25 years.

METHODS

The following diagnostic tests were used:

1. parasitic stool examination:
   - microscopic stool smear in saline and Lugol solution,
   - Kato method examination,

- flotation by Fuelleborn,
- decantation.

2. parasitic urine analysis for Schistosoma spp. eggs

3. histopathological examination of liver biopsy and biopsy of colon mucosa (performed in the Department of Anatomopathology of the Medical University of Gdansk)

4. Serologic analysis

- ELISA IgG (NovaTec Immunodiagnostica GmbH, Germany) – positive index > 2,3
- Immuno-Blot IgG with "Schistosoma Western blot IgG" LDBIO Diagnostics, France
- IFA tests (Indirect Fluorescent Antibody) for the presence of Plasmodium falciparum antibodies (3)

Patients were treated with praziquantel 40 mg per kg given once or in two doses with 6-8 hours interval. All patients who came up for control examination after 4-8 weeks were again treated with praziquantel (4).

RESULTS

ELISA serology tests for IgG were positive in all 40 patients (index 2,4-22,4). On the other hand parasitic stool and urine tests did not reveal Schistosoma spp. eggs or eggs and cysts of other parasites in any of the patients. The following clinical and laboratory parameters raised high suspicion of schistosomiasis:

- high level of class E antibodies in 13 patients,
- eospinophilia in 7 patients,
- hepatomegaly in almost all patients (33),
- splenomegaly without thrombocytopenia in 19 patients or with thrombocytopenia in 5 patients (hypersplenism)
- blood in urine of unknown etiology in 3 patients.

None of the patients had signs of the acute schistosomiasis with fever.

All patients had a history of exposure to fresh water reservoir in regions endemic for schistosomiasis.

In presented cases positive results of serologic tests for schistosomiasis (ELISA IgG) were the main reason for diagnosing schistosomiasis despite of negative results of parasitic urine and stool examinations.

Table 1
Serologic tests in patients suspected of schistosomiasis, with high titers of antibodies against *Plasmodium falciparum* (untreated)

No.	Patient age	Country/ Duration of stay	Serology for schistosomiasis		Serology for malaria IFA IgG
			ELISA IgG	Immuno-Blot IgG	
1.	W.P. 51	Togo 8 years	I – 9,2 II - 3,2	Negative	1:2560
2.	M.A. 28	Nigeria 6 months	I – 2,5 II – negative	„	1:2560
3.	B.E. 42	Cameroon 13 years	I - 2,7	„	1:2560
4.	B.T. 39	Kenya 13 years	I – 3,3 II – 2,5	„	1:1280
5.	K.F. 36	Gabon 1 year	I – 2,6 II – negative	„	Malaria P. falc.
6.	S.M. 45	South Africa, 7 years	I – 4,09 II – 2,01	„	1:2560
7.	P.J. 22	Bolivia, Chile 3 months	I - 2,8 II – 2,1	„	1:640
8.	A.Z. 44	Sierra Leone 6 months	I – 2,8 II – negative	„	1:1280
9.	O.S. 33	Cameroon 2,5 years	I – 2,4 II – 2,0	„	1:1280
10.	H.A. 52	Zambia 4 years	I – 11,6 II – 3,3	„	1:1280

Table 2
Patients with diagnosed and treated schistosomiasis

No.	Patient age	Country/ Duration of stay	Serology for schistosomiasis		Serology for malaria IFA and leishmaniasis ELISA IgG Remarks
			ELISA IgG	Immuno-Blot IgG	
1.	R.J. 53	Cameroon 16 years	I - 4,18	+	Malaria 1:640, leishmaniasis (-)
2.	S.M. 64	Zambia 34 years	I - 6,05 II after treatment- 3,1	+	1:640 (-) hematuria
3.	D.R. 36	Togo 9 years	I – 9,72 II – 4,01	+	1:2560 (-)
4.	W.M. 39	Senegal 2 months	I - 9,4 II –10,31	+	(-) (-)
5.	G.K. 57	Ghana 25 years	I - 22,4	+	(-) (-) liver cirrhosis?
6.	K.Z. 36	South Africa 7 years	I - 3,1 II – 4,4	+	1:640
7	M.S. 41	Asia, Africa multiple stays	I -2,9 II -2,3	+	(-) (-) hematuria

8.	J.R. 46	Zambia 13 years	I – 3,4	+	(-) (-)
9.	S.S. 43	Zambia 12 years	I - 2,54	+	1: 1280 (-)
10.	P.M. 36	South America 9 years	I -3,88	+	(-) hematuria
11.	F.A. 52	Angola 18 years	I -2,64	+	1:320
12.	C.B. 36	Ghana 6 years	I - 4,84 II –4,11	+	(-) (-)
13.	G.M. 39	South Africa 10 years	I - 6,7 II –3,66	+	1:2560 (-) Liver cirrhosis
14.	B.E. 51	Cameroon 10 years	I - 3,95	+	1:320 (-) Liver cirrhosis?
15.	C.Ł. 23	South America 6 months	I - 4,7	+	1:640 (-)
16.	Ł.K. 48	Angola 10 years	I - 5,3	+	(-) (-)
17.	W.R. 37	South America 8 years	I - 5,8 II –2,92	+	1:160 (-)
18.	S.H. 31	Cameroon 3 years	I - 2,9	+	1:80 (-)
19.	O.K. 18	South East Asia 4 years	I - 2,7 II –2,07	+	(-) (-)
20.	S.J. 60	Turkey Few months	I - 3,4 II -2,4	+	(-) (-)
21.	S.M. 45	South Africa 9 years	I - 4,09	+	1:2560 (-)
22.	S.J. 42	Chad, South Africa 5 years	I - 9,7 II – 6,6	+	1:320 (-)
23.	J.R. 48	Tanzania 9 years	I - 3,4 II – 3,1	+	(-) (-)

Liver biopsy was performed in two cases, one patient had biopsy of colon mucosa but no parasite eggs were found. In three patients with hematuria urologist ordered cystoscopy but it did not reveal the site of bleeding. No bladder mucosal biopsy was taken. In three patients with severe thrombocytopenia (below 60000/ml) neither liver biopsy nor colon mucus biopsy were made.

In 10 patients with high levels of antibodies against *Plasmodium* spp. minimal or slight elevation of serologic indexes against *Schistosoma* spp. was found (table 1). Specificity of this results was dubious and it could be a result of cross reaction, so those patients were excluded from treatment for schistosomiasis. In control examination after 4-8 weeks, serologic indexes were normal or lowered which might be the result of unspecific reaction. Seven patients with borderline serologic indexes in ELISA test (2,3-2,4) did not show up for control tests. Results of screening test performed with ELISA

were confirmed by Immuno-Blot IgG. This method confirmed *Schistosoma* spp. infection in 23 cases (table 2). Some of those patients were examined retrospectively.

Observation lasting from several months to few years (5 patients) confirmed good results of antiparasitic treatment with regression of clinical symptoms and signs and normalization of laboratory tests except from 3 patients with progressing disease (liver cirrhosis). Gradual normalization of serologic tests was also observed.

DISCUSSION

Parasitic infections might present a diagnostic difficulty because in some patients it is difficult to detect developmental stages of parasites. Such situation is most common in patients traveling to tropical countries, in cases of infections with low parasite burden and in cases of past infections (6).

Lack of positive result of the stool and urine parasitic examinations does not exclude Schistosoma spp. infection because eggs excretion begins, depending on species, 4-8 weeks after infection and afterwards is irregular with intensity depending on parasite burden.

Parasitological diagnosis of Schistosoma spp. requires repeated examinations of stool and urine for eggs of worms and histopathological examinations (http://www.emedicine.com/med/topic1823.htm Aug 23, 2004).

Diagnostic difficulties described above were observed in patients hospitalized in our clinic. Patients had clinical signs suggesting schistosomiasis: splenomegaly, low platelet count, hepatomegaly, portal hypertension and hematuria of unknown etiology. Diagnosis of parasitic infection was also confirmed in other tests: eosinofilia, X-ray, ultrasound, CT, endosocopy and histopathological examinations.

In these cases serological findings are the most important tool for diagnosing schistosomiasis. In endemic regions serologic tests with different sensitivity and specificity are used, that test antibodies as well as antigens of different developmental stages of the parasite (5). In countries where schistosomiasis is rarely diagnosed i.e. in Poland, only chosen tests are used like for example screening test and in case of positive results confirmation test. This type of diagnosis is used by different authors, for example Tsang (7) proposes this kind of diagnosis in people coming from tropical countries.

Most doubts were caused during interpretation of serologic tests positive for schistosomiasis (ELISA IgG) in patients suffering or recovering from malaria, when titers against *Plasmodium falciparum* were high. Results of serologic tests in such cases

may be false positive because of cross reactions with other parasites for example *Plasmodium* spp. as well as *Leishmania* spp. or some helminthiases (8).

Introduction of confirmation test - Immuno-Blot IgG – which detects class G antibodies specific for *Schistosoma* spp. and is a specific and sensitive method, has facilitated serologic diagnosis of schistosomiasis, eliminating nonspecific cross reactions. Because patients in the presented cohort had no acute infections only class G antibodies were identified. Immuno-Blot has the advantage of early detection of schistosomiasis, 3-4 weeks from infection when class M antibodies are produced (9). This test is recommended as serologic marker confirming infection and indicating treatment effectiveness.

Immuno-Blot enabled verification of positive results of current and previous ELISA IgG . Among 40 patients with high ELISA indexes, in 23 cases infection was confirmed with Immuno-Blot IgG. All patients were treated with success. Results showed lower specificity of ELISA IgG with some false positive results.

Clinical picture of schistosomiasis may be modified by other diseases for example mononucleosis or viral hepatitis type B or acute pyelonephritis which was observed in our patients. Symptoms of accompanying diseases masked the parasitic disease and made the diagnosis more difficult.

CONCLUSIONS

1. In 2002-2006, 127 patients with suspected parasitic diseases were hospitalized in our clinic. Out of them, in 40 patients schistosomiasis was suspected . Finally, in 23 of them schistosomiasis was diagnosed, and they were treated accordingly.
2. In 10 patients (25%) with high titer of antibodies against Plasmodium spp. High serologic indexes for schistosomiasis in ELISA IgG were a consequence of cross reactions.
3. In patients with suspected infestation with Schistosoma spp. flukes, with negative parasitic examinations of stool, urine and negative tissue biopsy, positive results of screening serologic tests had to be verified using confirmation test with Western blot method.
4. Because of serious consequences of untreated schistosomiasis, treatment is justified in chosen cases with only indirect evidence of this parasitic infection

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