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SEROLOGICAL STUDIES ON SCHISTOSOMIASIS MANSONI IN THE NORTHEAST BRAZIL (I)

Masanobu TANABE (1), Mitsu OKAZAKI (2), Masaichi OKAZAKI (2), Seiki KOBAYASHI (1), Nobuaki KANEKO (1), Tsuneari SEKIGUCHI (1), Seiki TATENO (3), Severa R. N. MOTTA (1, 2, 3) & Tsutomu TAKEUCHI

SUMMARY

Sera from the patients (N = 10) with schistosomiasis mansoni of the hospital of Federal University of Pernambuco, the **Schistosoma mansoni** egg-positive (N = 51) and -negative (N = 452) inhabitants in Cabo City area, out-patients (N = 37) of the IMIP hospital and Japanese immigrants (N = 127) in Petrolina City area of northeast Brazil as well as Japanese healthy subjects (N = 30) were examined by serological tests including an enzyme-linked immunosorbent assay with antigens prepared from eggs (ELISA-egg) and adult worms (ELISA-adult). The ELISA with egg or adult antigen correctly identified 100% of the uninfected individuals lived in non-endemic area of schistosomiasis. Moreover, when examined cross-reactivity of our ELISA with sera isolated from 78 subjects infected with various intestinal parasitic infections, only one of these sera reacted with the egg and adult antigens. On the examination of 51 sera from the egg-positive subjects, the ELISA-egg revealed the highest sensitivity (98.0%), whereas a large number of false negative reactions of ELISA-adult, Ouchterlony method using adult antigen, circumoval precipitation and immediate intradermal skin test were observed. A low sensitivity of these serologic tests except for ELISA-egg appears to be primarily due to their inability to detect antibody in the sera from egg-positive infantiles. There was no positive correlation between the absorbance values of these two types of ELISA among the sera isolated from ELISA-positive subjects. Rather, by the reactivity of these sera to egg or adult antigen, they could be divided into two subgroups; one reacted more positively with egg antigen and the other with adult antigen. Moreover, it was confirmed that the sera from young subjects (under 20 years old) appear to be highly reactive to the egg antigen than did aged ones.

These data suggest that the ELISA with egg antigen, but not with the adult antigen, appears to be useful for the serological survey of schistosomiasis mansoni in the endemic area of northeast Brazil.

KEY WORDS: Schistosoma mansoni; Schistosomiasis mansoni; Intradermal skin test; Ouchterlony; Circumoval precipitation test; Enzyme-linked immunosorbent assay.

(1) Department of Parasitology, School of Medicine, Keio University, Tokyo, Japan.
(2) Secretaria de Saúde, Pernambuco, Brazil.
(3) Laboratório de Imunopatologia Prof. Keizo Asami, Universidade Federal de Pernambuco, Pernambuco, Brazil.
(4) Department of Biophysics and Radiobiology, Universidade Federal de Pernambuco, Pernambuco, Brazil.
Address for correspondence: Masanobu Tanabe, Ph. D. Department of Parasitology, School of Medicine, Keio University. 35 Shinanomachi, Shinjuku-ku, Tokyo, 160, Japan.

TANABE, M.; OKAZAKI, M.; OKAZAKI, M.; KOBAYASHI, S.; KANEKO, N.; SEKIGUCHI, T.; TATENO, S.; MOTTA, S. R. N. & TAKEUCHI, T. — Serological studies on schistosomiasis mansoni in the northeast Brazil (I). **Rev. Inst. Med. trop. S. Paulo, 32(2)**: 121-131, 1990.

INTRODUCTION

Our previous study on the inhabitants of rural sector around Cabo City, an endemic area for schistosomiasis mansoni in northeast Brazil, demonstrated a high prevalence of intestinal parasitic infections, and also that approximately 10% of these inhabitants were positive for **Schistosoma mansoni** eggs[23]. In the epidemiological study on schistosomiasis in São Lourenço, Pernambuco in 1975 and 1979, ASAMI et al.[1] detected schistosome eggs from over 40% of primary school pupils. A high prevalence of **S. mansoni** infection was also reported in the northeast Brazil[2, 16]. Moreover, by the migration of unskilled labor from the endemic areas in the northeast to non-endemic areas in accordance with national projects of the engineering and agriculture, the endemic foci was expanded in Brazil[4]. Schistosomiasis, therefore, seems to be still one of the most serious public health hazards in Brazil.

Although many serological tests have been utilized for diagnostic and epidemiological study on schistosomiasis[17, 18], evaluation of these tests suggested that circumoval precipitation test (COPT)[11, 12, 28], enzyme-linked immunosorbent assay (ELISA) with egg antigen[8, 20, 21, 29] and radioimmunoassay with purified egg antigen[12, 25, 29] were generally reliable. However, parasitological and serological studies on the primary school pupils, who were proven infection with **S. mansoni**, in the northeast Brazil demonstrated a large number of false negatives by all of the serologic tests examined[24, 35].

In our preliminary study using some serological tests in the northeast Brazil, a large number of false negatives were also found in the sera isolated from egg-positive young subjects. Therefore, we tried to establish an ELISA to detect a low level of antibody against schistosome egg or adult antigens so that such false-negative reactions are ruled out. This communication deals with 1) evaluation of the sensitivity and specificity of ELISA using egg (ELISA-egg) or adult antigen (ELISA-adult), 2) evaluation of reliability of the ELISA when applied for the field study in the northeast Brazil, and 3) the difference of the ELISA reactivity of the sera isolated from various age populations to schistosome egg or adult antigen.

MATERIAL AND METHODS

Human subjects

In our previous study[23], approximately 10% of the 503 inhabitants of several villages around Cabo City, 50 km southeast of Recife, Pernambuco, Brazil, were positive for **Schistosoma mansoni** eggs. These inhabitants primarily received general health examination, and then their blood and stool samples were collected. On the examination of primary school pupils in these areas, all subjects were examined by intradermal skin test after general health examination, and then blood and stool samples were collected. Virtually all of the egg -positive subjects dit not show any clinical symptoms due to schistosomiasis except for occasional diarrhea and malnutrition. They usually shedded a small number of the eggs in the stool (30 to 100 eggs per g of stool). Moreover, preliminary ultrasonographic examination revealed no appreciable change in the liver and other organs as previously reported[5]. Accordingly, they seemed to be light cases of **S. mansoni** infection.

The out-patients of the hospital of Instituto Materno Infantil de Pernambuco (IMIP), located in the center of Recife, were also examined by serological tests. Approximately 71% of these patients were positive for at least one species of intestinal parasite. Only 2 patients were positive for **S. mansoni** eggs by stool examination. Virtually all of these patients lived in the suburban area of Recife. Preliminary characterization of 56 lyophilized sera, which had carried to Japan, indicated that only 2 of these sera were positive by COPT. Eventually, thirty-seven sera were selected on the basis of parasitological and serological examinations, and used for the evaluation of specificity of our ELISA as described below.

One hundred and twenty-seven sera isolated from Japanese immigrants in Petrolina (non-endemic area for schistosomiasis), 800 km west from Recife, were used to evaluate the specificity and sensitivity of the ELISA. Virtually all Japanese immigrants were farmers and their prevalence rate of intestinal parasites were 19.8%. De-

tected species were **Ascaris lumbricoides**, hookworm, **Trichuris trichiura, Hymenolepis nana, Entamoeba histolytica, Giardia lamblia** and **Entamoeba coli.** Approximately one-fifth of them had moved from high endemic area of schistosomiasis in Bahia 6 to 10 years before our present examination. Half of these immigrants from Bahia revealed positive reaction to the skin test, but no schistosome egg was found by stool examination. All immigrants except for 2 cases (one case with gallstone and another with chronic nephritis) did not showed any clinical signs and abnormalities on physical and ultrasonographic examinations.

The sera from 10 patients of the hospital of Federal University of Pernambuco (UFPE) were also examined in this study. All of them were clinically diagnosed to have schistosomiasis with mild to severe symptoms.

Thirty sera from Japanese healthy subjects were also examined to evaluate the specificity of the ELISA. They were negative for parasitic infection and had abnormal findings by periodic health examination.

To evaluate the cross-reactivity of our ELISA with the sera isolated from human subjects with various parasitic infections, we examined 78 sera isolated from two groups of human subjects.

The first group of sera were obtained from 11 Japanese patients with amebic liver abscess. The second group of sera were isolated from 37 out-patients of the IMIP hospital and 30 Japanese immigrants in Petrolina who were negative for intradermal skin test, but positive for single or a few species of intestinal parasitic infections.

Sex and age distribution of all subjects examined in this study were summarized in Table 1.

Collection of blood samples

All blood samples except those from Japanese healthy subjects were collected during April to August, 1987. The sera were separated by centrifugation in the Laboratorio de Imunopatologia Prof. Keizo Asami (LIKA) within 4 hours after bleeding. Half volume of each serum was lyophilized and the other half was stored at — 20°C until use.

The lyophilized sera were carried to Japan, and reconstituted by adding distilled water containing 0.02% NaN_3, and then examined by COPT and Ouchterlony test.

Preparation of antigens from S. mansoni eggs and adult worms

Veronal buffered saline (VBS)-extracted antigen was prepared from lyophilized adult worms according to the method of CHAFFEE et al.[6].

Viable eggs were separated by filtration method[7] from the mouse liver infected with **S. man-**

TABLE 1

Sex and age distribution of several groups of human subjects examined by serological tests for schistosomiasis mansoni

	Japanese healthy subjects	Japanese immigrants (Petrolina)	Out-patients of IMIP hospital	Cabo inhabitants		Patients with schistosomiasis (UFPE hospital)
				Egg (+)	Egg (−)	
Sex						
Male	30	65	23	19	163	3
Female	0	62	14	32	289	7
Age distribution						
0-10	0	7	27	17	165	0
11-20	0	25	10	14	131	0
21-30	30	22	0	4	52	0
31-40	0	32	0	6	30	0
41-50	0	22	0	3	32	5
51-	0	19	0	7	42	5
Mean (range)	22.1 ± 2.0 (21 − 28)	32.4 ± 15.7 (4 − 82)	5.4 ± 3.1 (1 − 11)	30.3 ± 21.3 (1 − 73)	26.8 ± 18.2 (5 − 78)	50.1 ± 8.4 (41 − 62)
Total	30	127	37	51	452	10

soni for 9 weeks. The soluble egg antigen was prepared from freshly isolated eggs acording to the slightly modified method of BOROS & WARREN[3].

Both egg and adult antigens were lyophilized, carried to Brazil, and stored at — 20°C until use. Each of these lyophilized antigens was dissolved in 20 mM sodium phosphate buffer, pH 7.2, immediately before use, and stirred for 30 min at 4°C. The supernatant fluid was separated by centrifugation at 15,000 g for 30 min at 4°C and sterilized by filtration with Millex GC filter (Millipore Co., Bedford, MA, USA) at 0.2 μm porosity. The protein concentration of egg and adult antigens were measured by Lowry's method[15] using bovine serum albumin (fraction V) as a standard.

Serological tests

The immediate intradermal skin test was performed according to YOKOGAWA et al.[35]. The protein concentration of VBS-extracted antigen prepared from **S. mansoni** adult worms was adjusted to 50 μg per ml. Approximately 0.01 to 0.02 ml of the VBS antigen solution, corresponding to 0.5 to 1 μg protein per site, was intradermally injected into the forearm of the subjects; which was enough to raise a wheal of 3 mm in diameter. The diameter of the reaction wheal exceeded 8 mm 15 min after the injection was judged positive. One hundred and fifty-six primary school pupils and 127 Japanese immigrants were examined by skin test in this study.

Ouchterlony immunodiffusion test using the VBS-extracted adult antigen (3 mg protein per ml) was performed according to HILLYER & FRICK[9]. The sera from 51 egg-positive subjects were only examined in this study.

For the COPT, the purified eggs were fixed with 2% paraformaldehyde in 100 mM sodium phosphate buffer, pH 7.4, according to the previous method[13] and stored at 4°C until used. The eggs were repeatedly washed with ice-cold 10 mM sodium phosphate buffered saline, pH 7.2, to remove aldehyde, and finally suspended in 50 mM sodim phosphate buffer, pH 7.2, containing 0.45% NaCl, 15 mM NaN_3 and 5 mM EDTA according to the modified method reported previously[13]. The COPT reaction was conducted using 96 wells microtiter flat-bottomed plate (NUNC Co. Denmark). Each well was added with 30 μl of the egg suspension (7,000 eggs per ml) and with the same volume of test serum. After incubation at 37°C for 72 hours in a moist chamber, the reaction product was examined by inverted light microscope (Diaphot-TMD; Nikon, Tokyo, Japan). The sera from 51 egg-positive subjects and 56 out-patients of IMIP hospital were examined by COPT in this study.

ELISA was performed according to the previous method[21] using 96 wells microtiter flat-bottomed plate (NUNC) except for the protein concentration of antigens and labelled antibody. The optimum antigen concentration was determined by checker board titration with serially diluted antigens against positive and negative reference sera. The wells were sensitized with 100 μl of the egg or adult antigen, prepared to yield 10 μg (egg antigen) and 20 μg protein (adult antigen) per ml of 50 mM carbonate buffer, pH 9.6, in a moist chamber at 37°C for 2 hours, and the sensitized plates were kept at — 70°C until use. All test sera were diluted 200-times and applied to the sensitized well. Horseradish peroxidase (HRP)-labelled anti-human Ig G rabbit immunoglobulin (Miles Scientific, Naperville, IL, USA) was diluted 2,000-times (ELISA-adult) and 10,000-times (ELISA-egg), respectively, and applied to all wells. The antibody titer of ELISA was expressed as the absorbance at 405 nm which was measured by EIA Reader (Bio Rad Lab., USA).

Statistics

Statistical analysis of data were done with Student t-test. Difference with probability (P) less than 0.05 were judged significant.

RESULTS

Sex and age distribution of all subjects examined in this study were summarized in Table 1.

To evaluate the specificity and sensitivity of our ELISA using **Schistosoma mansoni** egg and adult antigens, serum samples isolated from 30 Japanese healthy subjects, 40 Japanese immi-

grants and 41 egg-positive inhabitants in Cabo were examined. The mean absorbance values of the sera from Japanese healthy subjects were 0.037 ± 0.018 and 0.030 ± 0.015 using egg and adult antigens, respectively. Accordingly, the cut-off values of the ELISA with egg and adult antigens, calculated by the method reported previously[19], were 0.073 and 0.061, respectively. None of the 40 sera from Japanese immigrants, who had never lived in the endemic area of schistosomiasis, and were negative for parasitic infections and intradermal skin test, revealed positive reaction by the ELISA irrespective of the antigens employed. In contrast, the absorbance of the sera isolated from 41 egg-positive inhabitants in Cabo were far more than the cut-off value except for 5 cases for ELISA-adult and 1 case for ELISA-egg. Accordingly, the ELISA using egg antigen seemed to have a sufficient sensitivity. However, the ELISA with adult antigen appeared to be less sensitive.

The cross-reactivity of ELISA with the egg and adult antigens was also examined 11 serum samples of the Japanese patients with amebic liver abscess and 67 sera obtained from out-patients of the IMIP hospital and Japanese immigrants in Petrolina, who were positive for single or a few species of intestinal parasitic infections, were examined by our ELISA. As shown in Fig. 1, none of these sera except for one case with trichuriasis reacted with the egg and adult antigen. Thus, our ELISA seemed to have a sufficient specificity irrespective of the antigen employed.

Utilizing sera from the 51 egg-positive subjects, the sensitivity of our ELISA was compared with that of other serological tests such as Ouchterlony test, COPT and intradermal skin test (Table 2). The skin test was not carried out with aged subjects. With respect to the sensitivity of the skin test, 13 (72.2%) out of 18 young subjects (under 20 years old) were negative, although all of them had proven infection with **S. mansoni**. Among the 31 egg-positive young subjects, 15 (48.3%) and 19 (61.3%) were positive by Ouchterlony and COPT, respectively, which were much lower than those of aged subjects (older than 21). Using ELISA-adult, 22 (71.0%) out of the 31 egg-positive young subjects revealed positive reaction, which was also less than that of aged subjects (100%). In contrast to these serological

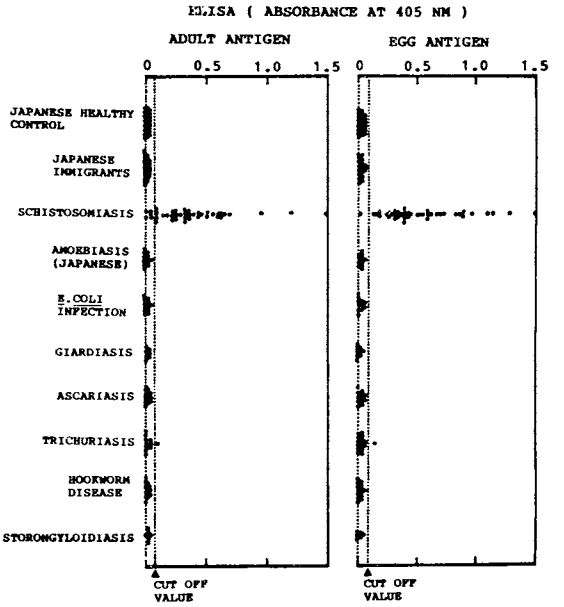

Fig. 1 — Evaluation of sensitivity and cross-reactivity of enzyme-linked immunosorbent assay (ELISA) with **Schistosoma mansoni** egg and adult antigens

Each point represent the absorbance value of the ELISA with egg or adult antigen. The dotted line represents the cut-off value, calculated according to the method described in the text, of each ELISA method. The sera isolated from Japanese healthy subjects, Japanese immigrants, **S. mansoni** egg-positive inhabitants in Cabo, Japanese patients with amoebiasis and the out-patients of IMIP hospital were examined by the ELISA.

tests, all sera except for one showed positive reaction by ELISA-egg. The over-all positive rate of the ELISA using egg antigen was 98.0%, which was markedly higher than those of Ouchterlony (58.8%), COPT (72.5%) and ELISA-adult (82.4%). Thus, ELISA-egg seemed most sensitive method.

To evaluate the reliability of both ELISA for the field study, the sera isolated from primary school pupils in Cabo and from Japanese immigrants in Petrolina were examined (Table 3). Of 127 sera from Japanese immigrants, 16 and 10 sera were positive by the ELISA with egg and adult antigens, respectively. All of the positive sera were obtained from the individuals who had moved from high endemic areas in Bahia and were positive by the skin test. Moreover, no positive reaction of both ELISA was found in the sera from the individuals who had never lived in the endemic area. As also shown in Table 3, 121 (73.8%) and 59 (36.0%) out of 164 primary school pupils in Cabo, an endemic area for schis-

TABLE 2

Results of intradermal skin test, Ouchterlony immunodiffusion, circumoval precipitation and enzyme-linked immunosorbent assay in the **Schistosoma mansoni** egg positive inhabitants in Cabo area, northeast Brazil

Serological tests		Cabo inhabitants (egg positive)				Total
		0-10	11-20	21-40	41 - (yrs)	
Intradermal skin test	+	4 (30.8%)	1 (20.0%)	1	—	6 (31.6%)
	–	9 (69.2%)	4 (80.0%)	0	—	13 (68.4%)
	Total	13	5	1		19
Ouchterlony	+	7 (41.2%)	8 (57.1%)	9 (90.0%)	8 (80.0%)	30 (58.8%)
	–	10 (58.8%)	6 (42.9%)	1 (10.0%)	2 (20.0%)	21 (41.2%)
	Total	17	14	10	10	51
Circumoval precipitation	+	10 (58.8%)	9 (64.3%)	9 (90.0%)	9 (90.0%)	37 (72.5%)
	–	7 (41.2%)	5 (35.7%)	1 (10.0%)	1 (10.0%)	14 (27.5%)
	Total	17	14	10	10	51
ELISA (adult antigen)	+	11 (64.9%)	11 (78.6%)	10 (100%)	10 (100%)	42 (82.4%)
	–	6 (35.3%)	3 (21.4%)	0	0	9 (17.6%)
	Total	17	14	10	10	51
ELISA (egg antigen)	+	17 (100%)	14 (100%)	9 (90.0%)	10 (100%)	50 (98.0%)
	–	0	0	1 (10.0%)	0	1 (2.0%)
	Total	17	14	10	10	51

TABLE 3

Incidence of positive reaction on the enzyme-linked immunosorbent assay (ELISA) using **Schistosoma mansoni** egg or adult antigen of the sera from Japanese immigrant in Petrolina and primary school pupils in Cabo

		Japanese immigrants in Petrolina	Primary school pupils in Cabo
ELISA (adult Ag)	+	10 (7.9%)[a]	59 (36.0%)
	–	117 (92.1%)	105 (64.0%)
	Total	127	164
ELISA (egg Ag)	+	16 (12.6%)	121 (73.8%)
	–	111 (87.4%)	43 (26.2%)
	Total	127	164
S. mansoni eggs	+	0	17 (10.4%)
	–	127	147 (89.6%)
	Total	127	164

a; Values in parentheses represent the incidence (%) of sero-positives in each group.

Of 127 Japanese immigrants in Petrolina, 24 subjects had moved from high endemic area for schistosomiasis mansoni in Bahia, Brazil.

tosomiasis, revealed positive reaction by the ELISA with egg and adult antigens, respectively, which were much higher than the prevalence (11.0%) of **S. mansoni** infection as estimated by single stool examination.

From these observations, it was pointed out that the ELISA using egg antigen seemed to be highly reactive with the sera from egg-positive, young subjects. To characterize a difference in the sensitivity between two types of ELISA, a correlation between the absorbance values obtained from the ELISA with egg and that with adult antigen was analyzed utilizing the sera isolated from three groups of subjects: 1) egg-positive subjects in Cabo, 2) the patients with confirmed schistosomiasis in UFPE hospital, and 3) egg-negative, but ELISA-positive subjects in Cabo. As shown in Fig. 2, no positive correlation was found between the absorbance values of these two types of ELISA. Rather, this experiment indicated that the serum samples could be devided into two subgroups; one reacted more positively with egg antigen and the other with adult antigen. Moreover, although it is not clear in this figure, the sera from young subjects (under 20 years old) revealed much higher absorbance on ELISA-egg that on ELISA-adult, whereas the sera from more aged subjects showed a higher absorbance on ELISA-adult. To make clear such a difference, the absorbance ratio of the ELISA using egg to that with adult antigen (E / A ratio) of each serum sample was calculated. As shown in Fig. 3 and 4, the E / A absorbance ratios were classified by the age of subjects. The mean E / A ratios of the sera from egg-positive young subjects (age groups; 0-10 and 11-20) were significantly higher than those of the sera from aged groups and the patients of UFPE hospital (Fig. 3). A significantly higher values of the mean E

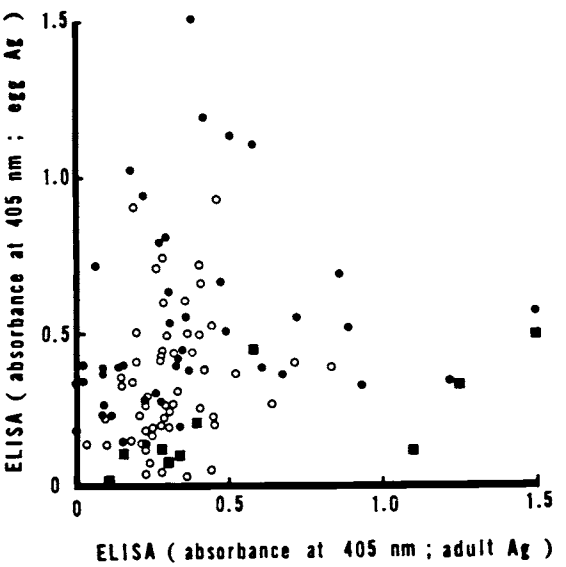

Fig. 2 — Correlation between the absorbance of enzyme-linked immunosorbent assay (ELISA) using **Schistosoma mansoni** egg and adult antigens among the sera from egg-positive and ELISA-positive inhabitants in Cabo

Each point represent the absorbance of ELISA using egg and adult antigens of the serum isolated from (●) **S. mansoni** egg-positive and (○) serologically positive inhabitants in Cabo, and (■) the patients of UFPE hospital.

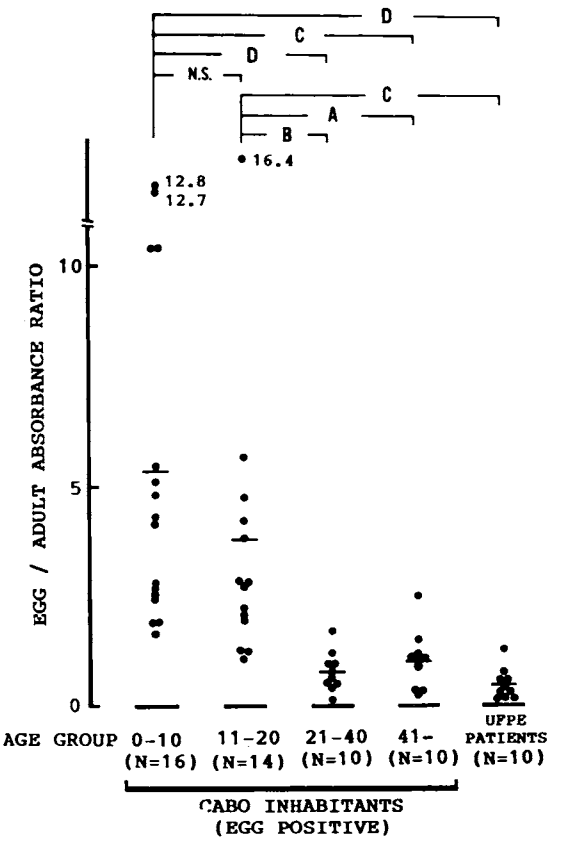

Fig. 3 — The absorbance ratio of enzyme-linked immunosorbent assay (ELISA) using **Schistosoma mansoni** egg and adult antigens among the sera isolated from different age groups of egg-positive inhabitants in Cabo

Each point represents the E/A absorbance ratio of each serum on ELISA. The horizontal line indicates the mean value of E/A ratio of each age group.
A; $P < 0.05$ B; $P < 0.02$ C; $P < 0.01$ D; $P < 0.001$

/ A ratio was also found in the sera obtained from the egg-negative, ELISA-positive young subjects (under 10 years old) as compared with those of aged groups (Fig. 4).

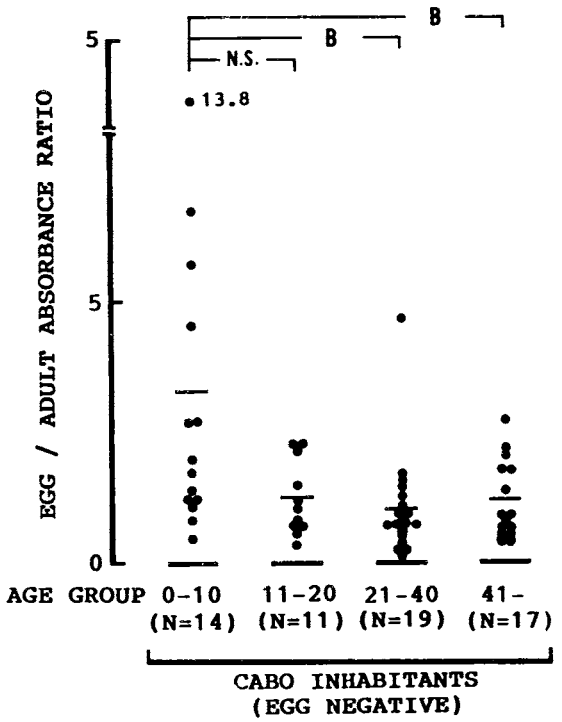

Fig. 4 — The absorbance ratio of enzyme-linked immunosorbent assay (ELISA) obtained by **Schistosoma mansoni** egg and adult antigens among the sera isolated from different age groups of the egg-negative, ELISA-positive inhabitants in Cabo

Each point represents the E/A absorbance ratio of each serum on ELISA. The horizontal line indicates the mean value of E/A ratio of each age group.
B; $P < 0.02$

DISCUSSION

Our present study on the ELISA indicates that ELISA utilizing **Schistosoma mansoni** egg or adult antigen have a high specificity when the "control" serum, which was isolated from individuals who had never been in the endemic area for schistosomiasis, was compared with the sera from infected individuals. The extensive cross-reactivities of ELISA using schistosome antigens have been demonstrated by the examination of the sera from patients with fascioliasis, trichinosis, cysticercosis, and echinococcosis[10, 30]. However, since these parasitic infections have not been reported around the northeast Brazil[26], the influence of these cross-reactivities could be neglected as far as the sera from the inhabitants in the northeast Brazil were concerned. Moreover, there seems no cross reaction of the ELISA with the sera from the inhabitants with various intestinal protozoan and helminthic infections (Fig. 1). Therefore, our ELISA using **S. mansoni** egg or adult antigen in this study seems to have a sufficient specificity for epidemiologic and diagnostic purpose for schistosomiasis in the northeast Brazil.

A small number of false negative were found in the sera of egg-positive subjects by the ELISA with adult antigen; accordingly, its over-all positive rate was estimated to be 82.4%. In contrast, the ELISA with egg antigen indicated the highest sensitivity (98.0%) in this study, which was much higher than that of other serological tests. Although low sensitivity of Ouchterlony and intradermal skin test have been reported previously[12, 17, 27, 34], our finding that a large number of false negatives were demonstrated by the COPT seems much interesting, because HILLYER and his colleagues[11, 12] demonstrated that COPT has a excellent specificity and sensitivity on the serological and epidemiologic studies on schistosomiasis mansoni. Although the exact reason why our COPT had lower sensitivity remains unknown, such a discrepancy may be due to the difference of test subjects and condition.

Evaluation of the reliability of our ELISA using the sera isolated from the human subjects lived in the non-endemic area of the northeast Brazil also suggests that they has a sufficient specificity, and also that the ELISA-egg has much higher sensitivity than that of ELISA-adult. On the other hand, the sera from the primary school pupils in Cabo, an endemic area for schistosomiasis, showed a high positive rate by both types of ELISA, in particular by the ELISA-egg, as compared with the prevalence estimated by single stool examination. A high sensitivity of the ELISA-egg was confirmed by our present study as described above, which may be the reason why a high rate of positive reaction was detected in the sera of persons in the endemic area by the ELISA with egg antigen. If the serologic test were highly sensitive, it could, theoretically,

detect specific antibody in the sera of the subjects lived in the endemic area despite they are still negative after multiple stool examination. Therefore, a high incidence of positive reaction on the ELISA in this study may be considered to result from its high sensitivity to detect extremely low intensity infections, infection with unisexual schistosome, or persons no longer infected in whom low level of anti-egg antibodies persist.

Among the **S. mansoni** egg-positive subjects, a large number of false negatives were found by the intradermal skin test as well as three serological tests such as COPT, Ouchterlony test and ELISA-adult in this study, which confirms previous observations[24, 35]. Low sensitivity of these serologic tests seems to be primarily due to their inability to detect antibodies in the sera from the pupils, because the results of these immunological tests fairly correlated with those of stool examinations in the adult subjects (Table 2). Thus, it seems reasonable that low reactivity of the sera from young subjects is of considerable importance concerning the sensitivity and specificity of the various serologic tests for schistosomiasis in the northeast Brazil.

The low sensitivity of the serologic tests due to subject age has been observed on various immunological tests for schistosomiasis[12, 24, 25, 35]. In general, the low sensitivity of these immunological tests have been demonstrated principaly in the young subjects. As shown in Fig. 3 and 4, the present study demonstrated more distinctly the different reactivity of the sera isolated from different age populations to the ELISA using egg or adult antigen; the mean E / A absorbance ratio of the sera isolated from young groups (under 20 years old) was significantly higher than that of aged groups. This probably means that the sera from young groups could react more strongly with the antigen prepared from schistosome eggs than did those from aged groups. Conversely, the sera from adult groups (over 21 years old) could react more positively with the antigen prepared from adult worms. This finding probably indicates that the different reactivity of the sera from young subjects on the ELISA appears to affect its sensitivity, that is, a high reactivity of the sera from young subjects to undifined antigenic molecule(s) in the soluble egg antigen might supports the high sensitivity and specificity of ELISA with egg antigen.

Although the information provided by sero-epidemiologic study on schistosomiasis have been considered to have a limit to determining of the reliable prevalence of schistosome infection in endemic areas because of the insensitivity and nonspecificity of serological methods employed[17, 18], recent field studies suggested that improved serological tests have made significant contributions to the epidemiology of schistosomiasis according to the increase in their specificity and sensitivity[14, 22]. For example, the ELISA using some types of purified antigens from schistosome eggs or adult worms has been reported to have a high degree of species specificity as compared with those using crude antigens[31, 32, 33]. Our present investigation pointed out an applicability of soluble egg antigen as the antigen of the ELISA when applied for sero-epidemiological study on schistosomiasis in the northeast Brazil. It is, therefore, absolutely necessary to characterize and identify the antigenic molecules in the egg extract which can react with the sera from egg-positive young subjects. Detailed characterization of the purified antigens from **S. mansoni** eggs is under investigation.

RESUMO

Estudo sorológico na esquistosomíase mansônica no nordeste do Brasil (I)

Soros de pacientes com esquistosomíase mansônica do Hospital das Clínicas da Universidade Federal de Pernambuco (N = 10), de residentes da cidade do Cabo (PE) — 51 casos ovo-positivo e 452 casos ovo-negativo —, de pacientes do Hospital Instituto Materno Infantil de Pernambuco (IMIP) (N = 37), de imigrantes japoneses residentes na cidade de Petrolina (PE) (N = 127), assim como de japoneses supostamente saudáveis (N = 30) foram examinados sorologicamente através de testes como ELISA; os antígenos utilizados foram preparados a partir de ovos (ELISA-ovo) e de vermes adultos de **Schistosoma mansoni** (ELISA-adulto).

Em 100% dos soros de indivíduos não infectados residentes em áreas não endêmicas, tanto na ELISA-ovo como na ELISA-adulto os resultados foram negativos. No tocante a reações cruzadas, em 78 soros provenientes de indivíduos portadores de diferentes parasitas intestinais, apenas um apresentou reatividade frente aos antígenos de vermes adultos e ovos.

O ELISA-ovo realizado com o soro de 51 indivíduos portadores de ovos de **S. mansoni** revelou uma alta sensibilidade (98% de casos ovo-positivos); contudo grande número de resultados falso-negativos foi observado em reações como ELISA-adulto, Ouchterlony usando antígeno de verme adulto, reação periovular e intradermoreação. Em casos de jovens portadores de ovos, uma baixa sensibilidade destes testes, exceto para ELISA-ovo, parece ser, em princípio, devido a uma incapacidade de detecção de anticorpos do soro destes pacientes.

Parece não existir nenhuma correlação positiva entre os valores dos dois tipos de ELISA (ELISA-ovo e ELISA-adulto) dentre os soros isolados de indivíduos com ELISA-positivo. Não obstante, a reação destes soros com antígeno de ovos ou vermes adultos pode ser dividida em dois sub-grupos: em um a reação é mais positiva frente a antígenos de ovos do que frente a antígenos de vermes adultos.

Tudo indica que os soros de jovens com menos de 20 anos são mais reativos aos antígenos de ovos do que os soros provenientes de indivíduos de outras faixas etárias.

Estes dados sugerem que ELISA preparado com antígeno de ovos, e não de vermes adultos, pareça ser bastante útil em pesquisas sorológicas na esquistosomíase mansônica em áreas endêmicas tais como o nordeste do Brasil.

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REFERENCES

1. ASAMI, K.; MIURA, S.; YOKOGAWA, M.; OYA, H.; AOKI, T. & KOJIMA, S. — Prevalence of parasites in school children of São Lourenço Village, Pernambuco, Brazil in 1975 and 1979. **In:** REPORT OF OVERSEAS SCIENTIFIC EXPEDITION. Clinico-pathological and immunological investigations on schistosomiasis mansoni in northeast Brazil. Tokyo, Keio University, 1980. p. 17-28.

2. BARBOSA, F. S. — Cross-sectional studies on **Schistosoma mansoni** infection in northeastern Brazil. **Ann. trop. Med. Parasit., 69:** 207-216, 1975.

3. BOROS, D. L. & WARREN, K. S. — Delayed hypersensitivity-type granuloma formation and dermal reaction induced and elicited by a soluble factor isolated from **Schistosoma mansoni** eggs. **J. exp. Med., 132:** 488-507, 1970.

4. CAMARGO, S. — The impact of the country development in the expansion of schistosomiasis. **Rev. Inst. Med. trop. S. Paulo, 22** (suppl. 4): 2-4, 1980.

5. CERRI, G. G.; ALVES, V. A. F. & MAGALHÃES, A. — Hepatosplenic schistosomiasis mansoni: Ultrasound manifestations. **Radiology, 153:** 777-780, 1984.

6. CHAFFEE, E. F.; BAUMAN, P. M. & SHAPIRO, J. J. — Diagnosis of schistosomiasis by complement fixation. **Amer. J. trop. Med. Hyg., 3:** 905-913, 1954.

7. COKER, C. M. & LICHTENBERG, F. — A revised method for isolation of **Schistosoma mansoni** eggs for biological examination. **Proc. Soc. exp. biol. Med. (N. Y.), 92:** 780-782, 1956.

8. FARAG, H. F. & BARAKAT, R. M. R. — The enzyme-linked immunosorbent assay in the diagnosis of human bilharziasis. **Tropenmed. Parasit., 29:** 12-14, 1978.

9. HILLYER, G. V. & FRICK, L. P. — Immunoprecipitins in **Schistosoma mansoni** infections. I. Mouse Infections. **Exp. Parasit., 20:** 321-325, 1967.

10. HILLYER, G. V. & GOMEZ DE RIOS, I. — The enzyme-linked immunosorbent assay (ELISA) for the immunodiagnosis of schistosomiasis. **Amer. J. trop. Med. Hyg., 28:** 237-241, 1979.

11. HILLYER, G. V.; RAMZY, R. M. R.; EL ALAMY, M. & CLINE, B. L. — The circumoval precipitin test for the serodiagnosis of human schistosomiasis mansoni and haematobia. **Amer. J. trop. Med. Hyg., 30:** 121-126, 1981.

12. HILLYER, G. V.; RUIZ-TIBEN, E.; KNIGHT, W. B.; GOMEZ DE RIOS, I. & PELLEY, R. P. — Immunodiagnosis of infection with **Schistosoma mansoni**: Comparison of ELISA, radio immunoassay, and precipitation tests performed with antigens from eggs. **Amer. J. trop. Med. Hyg., 28:** 661-669, 1979.

13. KAMIYA, H.; YAMASHITA, K.; ISHIGOOKA, S.; YOSHIMURA, K. & BLAS, B. L. — Enhancement of circumoval precipitin reactivity by sonication in the paraformaldehyde-fixed eggs of **Schistosoma mansoni** and **S. japonicum. Jap. J. Parasit., 34:** 21-26, 1985.

14. LEWERT, R. M.; YOGORE, M. G. & BLAS, B. L. — Seroepidemiology of schistosomiasis japonica by ELISA in the Philippines. II. Unreliability of stool examination in the assessment of incidence. **Amer. J. trop. Med. Hyg., 33:** 872-881, 1984.

15. LOWRY, O. H.; ROSEBROUGH, N. J.; FARR, A. L. & RANDALL, R. J. — Protein measurement with Folin phenol reagent. **J. biol. Chem., 193:** 265-275, 1951.

16. MACHADO, P. A. — The brazilian program for schistosomiasis control, 1975-1979. **Amer. J. trop. Med. Hyg., 31:** 76-86, 1982.

17. MADDISON, S. E. — Schistosomiasis. **In:** WALLS, K. W. & SCHANTZ, P. M. — **Immunodiagnosis of parasitic diseases. V. 1 — Helminthic diseases.** London, Academic Press, 1986. p. 1-37.

18. MADDISON, S. E. — The present status of serodiagnosis and seroepidemiology of schistosomiasis. **Diagn. Microbiol. infect. Dis., 7:** 93-105, 1987.

19. MATSUDA, H.; TANAKA, H.; BLAS, B. L.; NOSENAS, J. S.; TOKAWA, T. & OHSAWA, S. — Evaluation of ELISA with ABTS, 2,2'-azino-di-(3-ethylbenzthiazoline sulfonic acid), as the substrate of peroxidase and its application to the diagnosis of schistosomiasis. **Jap. J. exp. Med., 54:** 131-138, 1984.

20. McLAREN, M.; DRAPER, C. C.; ROBERTS, J. M.; MINTER-GOEDBLOED, E.; LIGTHART, G. S.; TEESDALE, C. H.; AMIN, M. A.; OMER, A. H. S.; BARTLETT, A. & VOLLER, A. — Studies on the enzyme linked immunosorbent assay (ELISA) test for **Schistosoma mansoni** infections. **Ann. trop. Med. Parasit., 72:** 243-253, 1978.

21. McLAREN, M. L.; LONG, E. G.; GOODGAME, R. W. & LILLYWHITE, J. E. — Application of the enzyme-linked immunosorbent assay (ELISA) for the serodiagnosis of **Schistosoma mansoni** infections in St. Lucia. **Trans. roy. Soc. trop. Med. Hyg., 73:** 636-639, 1979.

22. MOTT, K. E. — Diagnostic tools for **Schistosoma japonicum** in control programs. **Arzneimittel-Forsch, 34:** 1217-1220, 1984.

23. OKAZARI, M.; OKAZAKI, M.; MIRANDA, P.; NETO, J.; DIEGUES, V.; ALVES, J.; CAUAS, M.; TANABE, M.; KOBAYASHI, S.; KANEKO, N.; NAGAKURA, K.; KOBAYASHI, M.; MOTTA, S.; TATENO, S. & TAKEUCHI, T. — Parasitological and serological studies on amoebiasis and other intestinal parasitic infections in Recife and its suburban area, northeast Brazil. **Rev. Inst. Med. trop. S. Paulo, 30:** 313-321, 1988.

24. OYA, H.; AOKI, T.; ASAMI, K.; MIURA, S.; YOKOGAWA, M.; KOBAYASHI, M. & KOJIMA, S. — Hemoglobin-specific protease from **Schistosoma mansoni** and **Schistosoma japonicum;** Antigenic properties for radio-allergosorbent test and applications to field trial. **In:** REPORT OF OVERSEAS SCIENTIFIC EXPEDITION. Clinico-pathological and immunological investigations on schistosomiasis mansoni in north-east Brazil. Tokyo, Keio University, 1980. p. 49-67.

25. PELLEY, R. P.; WARREN, K. S. & JORDAN, P. — Purified antigen radioimmunoassay in serological diagnosis of schistosomiasis mansoni. **Lancet, 2:** 781-785, 1979.

26. PESSOA, S. B. — Helmintologia. **In:** PESSOA, S. B. — **Parasitologia médica.** Rio de Janeiro, Guanabara Koogan, 1972. p. 419-735.

27. RUIZ-TIBEN, E.; COX, P. M. & GREENBERG, E. R. — Simplified criteria for interpretation of the schistosomiasis skin test. **Ann. trop. Med. Parasit., 67:** 341-348, 1973.

28. RUIZ-TIBEN, E.; HILLYER, G. V.; KNIGHT, W. B.; GOMEZ DE RIOS, I. & WOODALL, J. P. — Intensity of infection with **Schistosoma mansoni:** its relationship to the sensitivity and specificity of serologic tests. **Amer. J. trop. Med. Hyg., 28:** 230-236, 1979.

29. SCHINSKI, V. D.; CLUTTER, W. C. & MURRELL, K. D. — Enzyme and ^{125}I-labelled anti-immunoglobulin assays in the immunodiagnosis of schistosomiasis. **Amer. J. trop. Med. Hyg., 25:** 824-831, 1976.

30. SPEISER, F. & WEISS, N. — Comparative evaluation of 7 helminth antigens the enzyme-linked immunosorbent assay (ELISA). **Experientia, 35:** 1512-1513, 1979.

31. TRACY, J. W.; DOMINGO, E. O. & MAHMOUD, A. A. F. — Evaluation of purified **Schistosoma japonicum** glycoprotein egg antigen for the immunodiagnosis of infection in man. **Amer. J. trop. Med. Hyg., 34:** 92-95, 1985.

32. TSANG, V. C. W.; HANCOCK, K.; MADDISON, S. E.; BEATTY, A. L. & MOSS, D. M. — Demonstration of species-specific and cross-reactive components of the adult microsomal antigens from **Schistosoma mansoni** and **S. japonicum** (MAMA and JAMA). **J. Immunol., 132:** 2607-2613, 1984.

33. TSANG, V. C. W.; TSANG, K. R.; HANCOCK, K.; KELLEY, M. A.; WILSON, B. C. & MADDISON, S. E. — **Schistosoma mansoni** adult microsomal antigens, a serologic reagent. I. Systematic fractionation, quantitation, and characterization of antigenic components. **J. Immunol., 130:** 1359-1365, 1983.

34. WARREN, K. S.; KELLERMEYER, R. W.; JORDAN, P.; LITTELL, A. S.; COOK, J. A. & KAGAN, I. G. — Immunologic diagnosis of schistosomiasis. I. A control study of intradermal (immediate and delayed) and serologic tests in St. Lucians infected with **Schistosoma mansoni** and in uninfected St. Vincentians. **Amer. J. trop. Med. Hyg., 22:** 189-198, 1973.

35. YOKOGAWA, M.; KOBAYASHI, M.; KOJIMA, S.; OYA, H.; AOKI, T.; ASAMI, K. & MIURA, S. — Intradermal tests with antigens of proteolytic enzyme and veronal buffered saline extract of **Schistosoma mansoni. In:** REPORT OF OVERSEAS SCIENTIFIC EXPEDITION. Clinico-pathological and immunological investigations on schistosomiasis mansoni in north-east Brazil. Tokyo, Keio University, 1980. p. 29-48.

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