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A COMPARATIVE EPIDEMIOLOGIC STUDY OF SPECIFIC ANTIBODIES (IgM AND IgA) AND PARASITOLOGICAL FINDINGS IN AN ENDEMIC AREA OF LOW TRANSMISSION OF *Schistosoma mansoni*

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SUMMARY

The diagnostic potential of circulating IgM and IgA antibodies against *Schistosoma mansoni* gut-associated antigens detected by the immunofluorescence test (IFT) on adult worm paraffin sections was evaluated comparatively to the fecal parasitological method, for epidemiological purposes in low endemic areas for schistosomiasis. Blood samples were collected on filter paper from two groups of schoolchildren living in two different localities of the municipality of Itariri (São Paulo, Brazil) with different histories and prevalences of schistosomiasis. The parasitological and serological data were compared to those obtained for another group of schoolchildren from a non-endemic area for schistosomiasis. The results showed poor sensitivity of the parasitological method in detecting individuals with low worm burden and indicate the potential of the serological method as an important tool to be incorporated into schistosomiasis control and vigilance programs for determining the real situation of schistosomiasis in low endemic areas.

KEYWORDS: Schistosomiasis mansoni; Epidemiology; IgM; IgA; Immunodiagnosis.

INTRODUCTION

The diagnosis of schistosomiasis mansoni for epidemiological purposes usually relies on the demonstration of parasite eggs in feces by the Kato-Katz method[18]. This technique is not a sensitive diagnostic method when performed as an one-sample assay[4,11], as used in schistosomiasis control programs. Under these conditions, the true prevalence of active disease can be underestimated, especially in areas of low transmission, as is the case for some schistosomiasis endemic areas in São Paulo state, Brazil[5,7,8,19,22].

The detection of IgM antibodies against gut antigens by means of the immunofluorescence test (IFT) on worm paraffin sections proved to be valuable for the diagnosis of both acute and chronic schistosomiasis infection. The presence of gut-specific IgA antibodies, on the other hand, can indicate acute or recent infection[17,25].

The objective of the present study was to evaluate the diagnostic potential of circulating IgM and IgA antibodies detected against gut-associated antigens, as compared to the fecal parasitological method, in a low endemic area for schistosomiasis mansoni. The stability of the IgM antibodies from blood samples collected on filter paper was investigated by comparing the results obtained with blood samples stored at -20°C for one month or for 4 years prior to testing.

MATERIAL AND METHODS

1. Study population

The study was conducted on two groups of schoolchildren living in different villages in the municipality of Itariri, located in the Vale do Rio Ribeira de Iguape (São Paulo state, Brazil). *Biomphalaria tenagophila* is the only inter-

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mediate host present in this area where a focal distribution of schistosomiasis has been described [23]. According to the survey carried out in 1990, by SUCEN (Superintendência do Controle de Endemias), a division of the São Paulo State Health Department, the mean autochthonous schistosomiasis prevalence in the municipality was 3.1%, ranging from 0 % to 9.2 % in different villages. Selected treatment with anti-schistosome drugs only for individuals with active *S. mansoni* infection has been performed in the schistosomiasis endemic areas of the Vale do Rio Ribeira de Iguape with either Hycanthone[1] or Oxamniquine[27].

The first group studied in the present investigation ("endemic A") comprised 628 children from four schools (two from the village of Ana Dias, one from Raposo Tavares and one from Caixa D'Água), located in the rural area of the municipality of Itariri, where schistosomiasis prevalence indices were reported to be the highest in the municipality according to the above survey. The second endemic group ("endemic B") comprised 150 children from a school located in the village of Caraguava, located in the suburban area of Itariri. The first autochthonous cases of schistosomiasis were reported in this area in 1988, suggesting that the disease has been recently introduced[26]. A control group was also included in this study, and consisted of 115 schoolchildren living in the village of Areia Branca, located in the rural area of the municipality of Itariri. This village has been considered by SUCEN as a non-endemic area. Ten children from this area, although negative for *S. mansoni* eggs in feces, presented *S. mansoni*-specific IgM antibodies. Eight were excluded from the control group after an epidemiological investigation classifying them as imported cases from other endemic localities of Itariri or neighboring municipalities. Patterns of high mobility have been observed among the populations of the villages and towns located in this valley.

Intestinal parasitic diseases such as ascaridiasis, trichuriasis, and enterobiasis were equally common in the three groups studied (Table 1).

Only individuals found to be excreting *S. mansoni* eggs were treated with Oxamniquine (20-25 mg/kg body weight).

2. Fecal examination

In the "endemic A" group, from the total of 628 individuals enrolled in the project, 115 did not submit fecal samples to the parasitological method. The Kato-Katz technique[18] was initially performed on one fecal specimen from each of the 513 children (three slides per sample). *S. mansoni* eggs were quantified and other helminth eggs were identified, but not counted. In some selected cases, negative for *S. mansoni* eggs, but with high levels of IgM antibodies by IFT, the parasitological examination was repeated in the same and/or in other stool samples. Some of the children had 2 to 3 fecal samples examined, also employing the sedimentation technique[21]. In the "endemic B" group, where schistosomia-

TABLE 1
Prevalence indices (%) of intestinal helminthiasis in the studied populations.

Parasite species	Endemic Population		Control
	A[a]	B[b]	Population[a]
Ascaris lumbricoides	54.9% (50.4-59.2) [c]	67.9% (59.2-75.6)	49.5% (40.4-58.8)
Trichuris trichiura	44.5% (40.2-49.0)	54.5% (45.7-63.0)	33.0% (24.9-42.3)
Hookworm	–	8.9% (4.9-15.4)	–
Enterobius vermicularis	3.1% (1.9-5.1)	5.2% (2.3-10.7)	1.6% (0.3-6.4)
Strongyloides stercoralis	–	1.5% (0.3-5.8)	–
Schistosoma mansoni	8.6% (6.4-11.4)	0.7% (0.04-4.7)	–

a = Prevalence data obtained for one fecal sample by the Kato-Katz method only.
b = Prevalence data obtained for three fecal samples by three parasitological methods (Kato-Katz, Lutz, and Rugai).
c = (95% confidence intervals)

sis had recently been introduced, the search for helminth eggs was done using three different parasitological techniques: KATO, modified by KATZ et al.[18], LUTZ[21], and RUGAI[24]. In the "control group", from the area where autochthonous schistosomiasis had never been reported, intestinal helminthiasis was diagnosed by Kato-Katz technique[18] on one fecal sample. Sixteen and 11 children from the "endemic B" and "control group", respectively, did not submit fecal samples to the parasitological investigation (Table 2).

3. Blood samples

Several drops of capillary blood from a fingertip onto Whatman #3 filter paper were collected from each individual. Filter paper with the blood was dried at room temperature and stored in a plastic bag at -20°C. For the IFT, the dried blood spot discs were cut from the Whatman filter paper using a punch 8 mm in diameter. The paper discs were eluted in 120 µl of 0.01 M PBS (Phosphate buffer solution, pH7.2) and incubated overnight at 4° C. The serum dilution after elution and removal of the paper disc was estimated to be 1:16[12].

The blood samples on the filter paper were stored at -20°C for 4 years, and 242 of them were randomly selected and resubmitted to IFT in order to test the stability of the IgM antibodies on filter paper.

4. Immunofluorescence test (IFT)

For the detection of IgM and IgA antibodies against gut antigens, paraffin sections of *S. mansoni* worms treated with Rossman's solution were utilized in the IFT as described[17]. The eluates from the blood samples were diluted to 1:16, and commercial anti-human IgM and IgA fluorescent conjugates (Biolab, R.Janeiro, Brazil) were used according to the respective titers determined by block titration against known positive and negative standard serum samples. From the total of 893 schoolchildren enrolled in the project, six , one of the "endemic A" and five of the "control group", did not submit blood samples to the serological method (Table 2). IgA-IFT was carried out in only 354 IgM-IFT positive samples, from the total of 627 samples submitted to the serological method (Table 3).

5. Data analysis

Data from the three groups were analyzed using the Epi-Info database package[3]. The same program was used to analyze possible differences between diagnostic methods, by determining the 95% confidence intervals (CI) and the Kappa indices (k). Prevalences and mean ages were compared using the Chi-square test and Student's t-test, respectively. The ordinary least squares (OLS) regression analysis[2] was used to analyze the correlation between prevalence indices and age groups.

RESULTS

The data presented in Table 2 show a significantly ($p < 0.0001$) higher prevalence of schistosomiasis by the serological (IgM-IFT) method in comparison to the parasitological (Kato-Katz) method. The number of collected blood specimens exceed the number of stool specimens for all groups, which reflect the difficulties for recovering feces in field studies.

Table 3 presents the parasitological and serological prevalence rates according to the age group in the "endemic A" group. The positive slope by age group in the OLS regression analysis indicated an increasing trend for the IgM-IFT prevalences in the older age groups ($p < 0.01$). This was

TABLE 2
Prevalence indices for schistosomiasis mansoni obtained by parasitological (Kato-Katz) and serological (IgM-IFT) methods for the three studied groups.

Group	Number of Schoolchildren Enrolled	Kato-Katz			IgM-IFT		
		Total[a]	Positives		Total[b]	Positives	
			No.	%		No.	%
Endemic A	628	513	44	8.6	627	354	56.5
Endemic B	150	134	1	0.7	150	15	10.0
Control	115	104	0	–	110	2	1.8
Total	893	751	45	6.0 (4.5 - 8.0)[c]	887	371	41.8 (38.6 - 45.2)

a = Total number of fecal samples submitted to Kato-Katz, in each group
b = Total number of blood samples submitted to IgM-IFT, in each group
c = (95% confidence intervals)

TABLE 3

Prevalence indices for schistosomiasis by parasitological (Kato-Katz) and serological (IgM and IgA-IFT) methods according to age group in the "endemic A" group.

Age group	Total	Kato-Katz			IgM-IFT			IgA-IFT		
		Total[a]	Positives		Total[b]	Positives			Positives[d]	
			No.	%		No.	%	Total[c]	No.	%
5 - 6	54	52	1	1.9	53	18	34.0	18	1	5.6
7 - 8	146	128	13	10.2	146	64	43.8	64	4	6.2
9 - 10	162	137	10	7.3	162	92	56.8	92	9	9.8
11 - 12	126	107	12	11.2	126	85	67.5	85	5	5.9
13 - 14	95	63	6	9.5	95	62	65.3	62	5	8.1
15 - 18	45	26	2	7.7	45	33	73.3	33	3	9.1
Total	628	513	44	8.6	627	354	56.5	354	27	7.6

a = Total number of fecal samples submitted to Kato-Katz, in each category
b = Total number of blood samples submitted to IgM-IFT, in each category
c = Total number of IgM-IFT-positive samples submitted to IgA-IFT
d = Number and percentage of IgA-IFT-positive samples among IgM-IFT positive cases.

TABLE 4

S. mansoni prevalence indices determined by parasitological (Kato-Katz) and serological (IgM-IFT) methods, according to the condition: "Previous treatment for *S. mansoni* infection".

Previous Treatment	Total	Kato-Katz			IgM-IFT		
		Total[a]	Positives		Total[b]	Positives	
			No.	%		No.	%
Yes	122	112	10	8.9 (4.6-16.2) [c]	122	104	85.2 (77.4-90.8)
No	506	401	34	8.5 (6.0-11.8)	505	250	49.5 (45.1-53.9)

a = Total number of fecal samples submitted to Kato-Katz, in each category
b = Total number of blood samples submitted to IgM-IFT, in each category
c = (95% confidence intervals)

not seen for the parasitological or the IgA positivity rates ($p > 0.8$ and $p > 0.15$, respectively), suggesting no correlation between these variables and age group. The presence of IgA antibodies, indicating possible recent infection, was similar in all age groups.

In the "endemic A" group, *S. mansoni* egg counts in Kato-Katz-positive individuals ranged from 8 to 1,792 eggs per gram of feces (epg), with a geometrical mean of 57.8 epg.

The data in Table 4 suggest that previous treatment for *S. mansoni* did not interfere with the parasitological prevalence indices, since no significant difference ($p > 0.8$) was detected between the two groups, i.e., "Yes" or "No" for this condition. But the serological prevalence was significantly higher ($p < 0.0001$) in the previously treated group. Comparing the mean ages of the "Yes" and "No" groups, respectively 11.3 (sd = 2.6) and 9.9 (sd = 2.8), the difference was significantly higher ($p < 0.001$) in the "Yes" group. Also, the proportions of boys and girls in the two groups were not the same; in the "Yes" group there were more boys (78) than girls (44), while in the "No" group, the number of boys (250) was practically the same as the number of girls (256).

The serological prevalence was significantly higher ($p < 0.0001$) in the male than in the female group, respectively 64.3% (95% confidence interval = CI: 58.8-69.5) and 47.8% (CI: 42.1-53.6) , but no statistical difference ($p > 0.6$) was detected between the male and female groups in relation to the parasitological prevalence indices, respectively 8.1% (CI: 5.3-12.2) and 9.1% (CI: 5.9-13.6). The mean ages and standard deviations (sd) of the male and female groups were 10.1 (sd = 2.8) and 10.2 (sd = 2.8), respectively, with no statistical difference ($p > 0.6$).

The sensitivity of the IgM-IFT was excellent with only one false negative result in 44 parasitologically confirmed cases, giving an index of 97.7 % (CI: 86.5-99.9). Because of the large number of IgM-IFT-positive and Kato-Katz-negative cases, it was observed a poor agreement between the two diagnostic methods. The Kappa indices (k) for the agreement between the parasitological and serological methods were low for both groups, "Yes" and "No" in relation to previous treatment. However, it was significantly higher in the "No" group (k = 0.182; CI: 0.125 - 0.240) when compared to the "Yes" group (k = 0.01; CI: -0.031 - +0.05).

The comparative results of the IgM-IFT carried out on 242 blood samples collected on filter paper, and tested after one month (IFT_1) and 4 years (IFT_2) of storage at -20° C, showed a high degree of agreement, giving a Kappa index of 0.933 ($p < 0.0001$). The results agreed in 234 samples, 129 gave positive and 105 negative result in both situations; seven samples were IFT_1 negative but IFT_2 positive, and one sample IFT_1 positive but IFT_2 negative.

DISCUSSION

The IgM-IFT has proved to be a very sensitive technique capable of detecting *S. mansoni* infection in a very early phase, even before the adult worms begin to lay eggs[28]. In previous papers[17,25], the IgM-IFT on worm paraffin sections showed good specificity when tested with serum samples from normal individuals and high sensitivity when applied to known cases of schistosomiasis, also in those with a low worm burden[20]. In this study, we evaluated the potential of this technique for field studies using blood samples collected on filter paper in an area with a high prevalence of other parasites. Thus, the "control group" for this study was composed of schoolchildren living in an area with climatic and geographic conditions similar to those of endemic areas, and also presenting a high prevalence of other helminth infections, but where autochthonous *S. mansoni* infections had never been reported (Table 1).

The good specificity of the IgM-IFT obtained for our "control group" (98.2%), with only two false positive results (Table 2), permitted us to better interpret the serological data obtained with the schoolchildren from the schistosomiasis endemic areas. The higher prevalence rates obtained by the serological methods in comparison to the parasitological methods have been commonly observed in other epidemiological studies by different authors, especially in endemically low areas for schistosomiasis[5,6,9,10,13,14,19]. In these areas, when only fecal examination is used, the poor sensitivity of the parasitological methods in detecting individuals with low egg counts has led to underestimated prevalence indices[4,11]. Actually, relatively small number of individuals could be diagnosed by the Kato-Katz method in the "endemic A" group, but the serological data demonstrated high serological prevalence in our study area. This must be interpreted as indicative of possible high exposure of the children to the risk of *S. mansoni* infection. Also, in the "endemic B" area, where the parasitological data showed a very low prevalence rate of less than 1% (Table 2), the serological results indicate that some vigilance program must be introduced to control the expansion of schistosoma infection.

It is well known that prevalence rates by age group in an endemic area for schistosomiasis follow a predictable pattern, peaking around the age of 10 to 20 years[7]. In our study, to evaluate the potential of the IgM-IFT for epidemiological purposes, we chose groups of schoolchildren aged 5 to 18 years living in a low endemic area for schistosomiasis. We included in this study young children from villages with relatively high parasitological prevalence rates in the municipality, where we assumed to detect individuals with recent exposure to *S. mansoni* cercariae. This was important to evaluate the feasibility of the IgA-IFT for the detection of recent infection in the field in children with no acute clinical symptoms. In previous studies, the IgA-IFT proved to be useful for differential diagnosis of acute from chronic schistosomiasis, with IgA antibodies against gut antigens being detected only in the acute phase of the disease[16,17,25]. In the present study, IgA antibodies were detected in all age groups (Table 3), suggesting the possible applicability of this method as an indicator of recent and active transmission of schistosomiasis in this kind of low endemic area.

Our results also showed a higher percentage of IgM-IFT-positive results among older children (Table 3). This probably occurs due to the presence of IgM antibodies in some individuals with chronic infection, when the detection of eggs in the feces becomes in general very difficult, and also due to the persistence of IgM antibodies in some individuals cured after drug treatment.

The significantly higher serological prevalence observed in the male group when compared to the female group could also be explained by the higher chance boys have to be exposed to *S. mansoni* cercariae. MARÇAL Jr. et al.[22], studying the risk factors for *S. mansoni* infection in a municipality close to Itariri, in the Vale do Rio Ribeira do Iguape, concluded that recreational activities like swimming, playing and fishing in the river were the main reasons for schistosomiasis transmission in the area, and these activities are usually performed more frequently by boys.

In the "endemic A" group, the sensitivity of the IgM-IFT was excellent (97.7%) but its specificity was apparently low (47.6%), because of the high number of serologically positive and parasitologically negative cases. This result can be partially explained by the difficulties in finding eggs in individuals with a low worm burden. Actually, in nine nontreated children, positive for IgM-IFT but negative for *S. mansoni* eggs in the first fecal sample, *S. mansoni* infection

was parasitologically confirmed in one case by the finding of one egg on one of three Kato-Katz slides of a second fecal sample; in six children, among the nine above mentioned, negative for all three Kato-Katz slides in the second fecal sample, the confirmation was possible by the spontaneous sedimentation technique[21], when one to 60 slides had to be examined to find at least one *S. mansoni* egg; in the last two children, *S. mansoni* eggs were detected only in a third fecal sample. Other authors have shown that the use of parasitological methods for epidemiological purposes can be responsible for underestimated prevalence rates of schistosomiasis in low endemic areas[4,6,10,11].

Also, as expected, a higher serological prevalence was observed in the group of children previously submitted to treatment with schistosoma-specific drugs (Table 4), probably because of the persistence of IgM antibodies after cure in some children. No significant difference was detected between the two groups in relation to the parasitological prevalence rates, but the high serological positivity observed also in the group of children not previously submitted to treatment (49.5%) suggest that the current situation of schistosomiasis in the area is more serious than shown by the parasitological data. The repetition of the fecal examination, in some serologically positive cases, as already commented, have demonstrated how difficult is to find eggs in these individuals with low parasite burden. So, there is no doubt that the difference observed between the serological and parasitological prevalence indices is mainly because of lack of sensitivity of the parasitological method to diagnose *S. mansoni* infection in individuals with low worm burden. The high specificity (98.2%) obtained for the IgM-IFT, when applied in the "control group" , with only two false positive results (Table 2), can also corroborate this assumption.

The previously treated children ("Yes" group) showed the lowest level of specificity for the serological test (14.7%), and also the lowest agreement of results (k = 0.01) between the two diagnostic methods, indicating that the persistence of the IgM antibodies long after treatment may represent a limitation of the IgM-IFT. Thus, we can recommend this serological technique for endemic areas whose population was never submitted to anti-schistosomal chemotherapy , or even for areas previously submitted to selected treatment if it should be possible to analyze the data classifying the population according to this condition.

The powerful Kappa index of 0.933 showed a high degree of agreement between the IgM-IFT data obtained one month and 4 years after blood collection. This result indicated a good stability of the IgM antibodies on filter paper at -20°C, and the suitability of this process for seroepidemiological studies. Others investigators[15] obtained comparable results for the detection of egg-specific IgM and IgG-antibodies by ELISA when evaluating paired samples of venous blood sera and buffer-eluates of blood dried on filter paper.

Our results suggest that the IgM-IFT on worm paraffin sections, because of its high sensitivity and the high stability of the antigen at room temperature, constitutes a practical and useful test for epidemiological purposes, differentiating areas with and without active transmission better than parasitological methods. This method can be introduced as a valuable diagnostic tool to complement the parasitological data in this low endemic area for schistosomiasis.

RESUMO

Estudo comparativo entre detecção de anticorpos (IgM e IgA) específicos ao *Schistosoma mansoni* e métodos parasitológicos, para fins epidemiológicos em áreas de baixa transmissão

O potencial diagnóstico dos anticorpos IgM e IgA contra antígenos do tubo digestivo do *Schistosoma mansoni*, detectados através da reação de imunofluorescência indireta utilizando-se cortes parafinados de vermes adultos, foi avaliado, comparativamente aos resultados do exame parasitológico de fezes, para fins epidemiológicos em áreas de baixa endemicidade para a esquistossomose. Amostras de sangue em papel de filtro foram coletadas de escolares, residindo em duas localidades diferentes dentro do município de Itariri (São Paulo, Brasil), com características epidemiológicas distintas em relação à esquistossomose. Os dados parasitológicos e sorológicos foram comparados aos obtidos com um outro grupo de escolares, residentes em uma área não endêmica para esquistossomose. Os resultados demonstraram a falta de sensibilidade do método parasitológico para detecção de indivíduos com baixa carga parasitária e indicam a potencialidade do método sorológico como importante instrumento a ser incorporado aos programas de controle e de vigilância da esquistossomose, para verificação da real situação da esquistossomose em áreas de baixa endemicidade.

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