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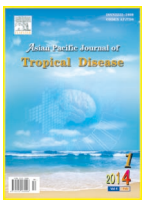

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Evaluation of microscopical and serological techniques in the diagnosis of *Schistosoma mansoni* infection at Sennar State, Central Sudan

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PEER REVIEW

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Comments

In this study, authors focused on how to diagnose an important neglected disease using different laboratory methods, selecting in principle the cost-effective ones. These applied diagnostic methods have been compared to a reference method and the sensitivity and specificity of these methods were determined which aim to reach a feasible conclusion. These findings are useful to improve the diagnosis and assist in the control of schistosomiasis.
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ABSTRACT

Objective: To determine the prevalence of *Schistosoma mansoni* (*S. mansoni*) infection among schoolchildren and to evaluate the sero-diagnostic techniques of indirect-haemagglutination (IHA) and enzyme-linked immunosorbent assay (ELISA) in comparison with Kato-Katz smear examination.

Methods: A descriptive cross-sectional study was conducted from August, 2011 to January, 2012 at Sennar State, central of Sudan. Stool and blood samples were collected from schoolchildren (n=214) those with age groups from 6 to 16 years old. Kato-Katz smear was used for the detection of *S. mansoni* eggs and then the intensity of infection was determined as per standard procedure. IHA and ELISA assays were applied to detect *S. mansoni* antibodies. Considering the Kato-Katz as a reference method, the sensitivity, specificity, positive predictive values (PPV) and negative predictive values of serological methods were evaluated.

Results: Out of 214 schoolchildren enrolled, 45 (21%) were found to be positive for infections using Kato-Katz technique. Of these, 84.4% were having light infections, 6.7% with moderate infections and 8.9%with heavy infections. Shistosomiasis was significantly higher (P=0.007) among boys (33/124; 26.6%) than girls (12/90; 13.3%). In comparison between the applied methods, the majority of the positive cases were detected by ELISA (56.1%; 120/214) followed by IHA (33.2%; 71/214) and Kato-Katz (21%; 45/214). The sensitivity of the ELISA was 93.3% compared to 84.4% given by IHA. Furthermore, the specificity was reduced to 53.8% in ELISA compared to the 80% detected by IHA. The PPV was increased in IHA (53.3%) than that of in ELISA (35%). The combination use of the ELISA and IHA were yielded good sensitivity (93.3%), increased the rates of specificity to 85.8% and PPV to 55.1%.

Conclusions: In the settings where the prevalence of *S. mansoni* infection was high with a low infection intensity, performing of serodiagnostic methods together with a microscopical examination are required to detect more positive cases.

KEYWORDS

Schistosoma mansoni, Detection methods, Schoolchildren, Sudan

1. Introduction

Schistosomiasis is a tropical parasitic disease endemic in many developing countries. It affects over 200 million people worldwide and about 90% of them are found in sub-Saharan Africa[1,2]. Different species of the genus *Schistosoma* known to cause the disease in the humans such as *Schistosoma mansoni* (*S. mansoni*), *Schistosoma haematobium*, *Schistosoma japonicum* and *Schistosoma intercalatum*[1]. *S. mansoni* is a causative agent of intestinal schistosomiasis, and it is endemic in over 70 countries and widely distributed in Africa, South America, the eastern Mediterranean regions and the Caribbean Sea[2,3]. People are at risk of infection due to agricultural, domestic and recreational activities which expose them to infested water[2]. The highest rates of schistosomal infections are

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commonly found among children and young adults and in recognition of these schoolchildren are the main target of schistosomiasis control programmes[4].

The Kato-Katz microscopic smear is a standard laboratory method recommended for diagnosing of intestinal schistosomiasis in the field study because it is quantitative, relatively inexpensive, simple and fast[3]. In addition, the semi-quantitative egg counts can be performed by this method to determine the intensity of the infection[5]. Despite the specificity is very high, the sensitivity of Kato-Katz in single stool sample examination is becoming not satisfied, especially when the number of worms is low or the test is done after the eggs were eliminated from the body[1,6], which leads to measurement error in estimating the presence of infection[1]. Nevertheless, there is a possibility to increase the sensitivity of Kato-Katz smear through examination of multiple samples[5], but this is a limiting procedure for field study[7]. Moreover, this microscopic method is un-sufficient in the areas of low endemicity, in post-treatment situations, and in the control of transmission[8,9]. Therefore, other diagnostic assays like detection of parasite-specific antibodies have been shown to be more sensitive than the parasitological examination and are needed to plan and monitor control measure[3,9].

Various serodiagnostic methods have been developed to detect anti-schistosomal antibodies, such as indirect hemagglutination assays (IHA), and enzyme-linked immunosorbent assays (ELISA) using different type of antigens[10,11]. In Sudan, the high endemicity of *S. mansoni* infections have been reported in many parts of the country[12-14]. Therefore, the first approach for prevention and control is to diagnose the disease through applying different laboratory methods. The present study aimed to determine the prevalence of *S. mansoni* infection among schoolchildren in Sennar State, central of Sudan, to evaluate the sero-diagnostic techniques of the IHA and ELISA in comparison with Kato-Katz smear examinations, in order to be applied and adopted as to improve the diagnosis and assist in the control of schistosomiasis.

2. Materials and methods

2.1. Study design and setting

This was a descriptive cross-sectional study conducted at the Sennar State (300 km south of Khartoum capital), central of Sudan, during the period from August, 2011 to January, 2012. In this state, the Sennar Dam distributes water through canals for irrigation purpose. Although most villages have a chlorinated water supply, the water contact takes place along the untreated canals for recreation purposes or for domestic uses (washing utensils, bathing and watering animals)[15].

2.2. Study population

The study population was comprised of schoolchildren of both genders those were selected from three basic schools of Huzaifa Ibn Alyaman School, Al khansaa Basic School and Alkeila Basic Co-educated School at Sennar State. The age groups of the subjects were ranged from 6 to 16 years with the mean age of 11 years old.

2.3. Sample size ethical considerations

The sample size was obtained as recommended by the World Health Organization[16]. For this purpose, a total of 214 stool and blood samples were collected from the study subjects. Before the onset of the sample collections, informative meetings were held and the aim of the study was discussed with the headmasters and teachers of the selected schools and then a lecture about the disease was introduced to the students in each school. A written consent was obtained from each student or his/her parents after informing them about the importance of the study. The study was approved by a Committee of Research Council of Faculty of Medical Laboratory Science, University of Khartoum.

2.4. Collection of samples

To obtain the stool samples, each student was given a wide dry and clean container and informed him or her to provide at least 10 g of a stool sample. Whereas about 5 mL of venous blood sample was extracted from each subject using sterile disposable syringe. Only data from individuals who provided the recommended samples were included in the final analysis.

2.5. Microscopic examination of S. mansoni egg's

Upon receiving the stool sample, it was immediately processed in the study field using Kato-Katz technique for the detection of *S. mansoni* eggs[17]. Each sample was pressed through a sieve and the amount of 41.7 mg sieved stool measured by a standard template was transferred to a microscope slide where it was pressed by another slide. The slides were then examined microscopically within 15 min. Intensity of infection was categorized according to the eggs count per gram of stool (epg): light (1-99 epg), moderate (100-399 epg) and heavy ($\geqslant$400 epg)[18].

2.6. Immunological diagnosis of S. mansoni

2.6.1. IHA method

IHA assay was performed for the detection of *S. mansoni* antibodies using erythrocytes coated with specific adult worm antigen as described by Gool *et al*[10]. The IHA test kit (Fumouze Diagnostics Company, Paris, France) was prepared following the manufacturer's instructions. An exactly 50 μL of phosphate buffer solution was delivered to all eight wells of the microplate, then 50 μL of serum stock dilution was added to the first well, mixing it well with the buffer solution, and then 50 μL from the first well was transferred to the second well. Then similar action was repeated for all wells up to the well number six. Then the last 50 μL from the well number six was discarded as to obtain serial dilutions of 1:80, 1:160, 1:320, 1:640, 1:1280 and 1:2560. Then 50 μL of the stock serum dilution was added to the well number seven, mixed and 50 μL was aspirated and discarded, to get 1:80 dilutions constituting the serum control. The well number eight was left only with the buffer solution to serve as reagent control. Then carefully, one drop of the sensitized red blood cells was delivered into the first six wells and to the well number eight. One drop of un-sensitized red blood cells was added to the seventh well number seven (serum control). Very carefully, the wells content was homogenized

and allowed to remain motionless protected from vibration, for 2 h. The plate was examined for any agglutination. Each sample yielded agglutination reaction equal to/or more than 1:160 was considered as a significant reaction.

2.6.2. ELISA method

The test method of ELISA was carried out for detection of *S. mansoni* IgG-class antibodies as previously described[10]. ELISA reagent kits (Nova Tec Immundiagnostica GmbH Technologic & Waldpark Company, Dietzenbach, Germany) were prepared according to the manufacturer's instructions. The microtitre strip was pre-coated with *S. mansoni* specific antigen. The strip was designed as follows: first well (A1) for substrate buffer, second well (B1) for the negative control, third and fourth wells (C1 and D1) for the cutoff control and the well number five for the positive control. An exactly 100 μL of the positive and negative controls and the diluted samples were dispensed on their corresponding wells, then the strip was covered with a foil and then incubated in an ELISA incubator at 37 °C for 1 h. The foil was removed, aspirated the content of all wells and washed five times with a 300 μL diluted washing solution and the remaining solution was removed carefully by tapping the plate on a tissue paper. Then 100 μL of protein a conjugate was added to all wells except the blank well and then the plate was covered with a foil and incubated at room temperature for 30 min. By the end of the second incubation, the plate again was washed five times with the diluted washing buffer, and then 100 μL of the solution was dispensed to all wells and then incubated for 15 min at room temperature in dark. Then a 100 μL of stop solution was added into all wells. The plate was examined on ELISA reader at a wavelength of 620 nm. The result was considered to be positive when the absorbance value was higher than 10% over the cutoff titre.

2.7. Quality control

A total of 20 blood samples which were collected from healthy non-infected subjects and free of schistosomiasis from non-endemic area were applied as a quality control measure. Control samples were examined for the presence of disease by ELISA and IHA methods when every batch of patient samples was carried out.

2.8. Data analysis

Data were analyzed using the Statistical Package for Social Sciences (IBM SPSS for windows, version 16 software). Considering the Kato-Katz methods as a reference method, the results of serological tests of IHA and ELISA were tabulated and calculated their sensitivity, specificity, positive predictive values (PPV) and negative predictive values (NPV). Proportions of the prevalence of schistosomiasis between boys and girls were compared using *Chi*-square test with *P*-value less than 0.05 was considered as statistically significant.

3. Results

3.1. Prevalence and intensity of infection

A total of 214 schoolchildren (124 boys and 90 girls) were recruited from three basic schools at Sennar State, central of Sudan. Forty five (21%) were found to be positive for *S. mansoni* infections using Kato-Katz as a reference method. Of these 45 positive cases, 33 were boys while 12 were girls. The frequency of *S. mansoni* infection was significantly higher (*P*=0.007) among boys (33/124; 26.6%) than girls (12/90; 13.3%) (Figure 1).

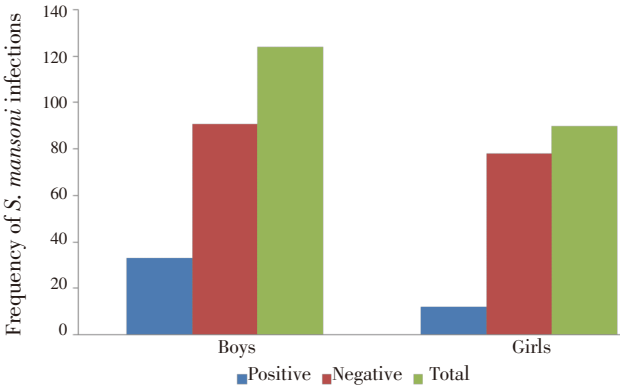

Figure 1. Frequency of *S. mansoni* infections according to the schoolchildren gender's at Sennar State, central Sudan.

The intensity of infection was expressed according to the number of egg's count by Kato-Katz method. Among the 45 infected individuals, 38 (84.4%) were having light infections, 3 (6.7%) with moderate infections and 4 (8.9%) with heavy infections.

3.2. Screening of infection using Kato-Katz, IHA and ELISA methods

Table 1 summarizes the distribution of positive results determined by all the three applied test methods of Kato-Katz, IHA and ELISA. The majority of the positive cases were detected by ELISA (56.1%; 120/214) followed by IHA (33.2%; 71/214) and Kato-Katz (21%; 45/214). Out of the 45 positive cases by Kato-Katz smear, 42 and 38 cases were found to be positive by ELISA and IHA, respectively. Of the total 214 cases, 78 (36.4%) were recorded as false positive results by ELISA, whereas 33 (15.5%) false positive were detected IHA.

Table 1
Screening of *S. mansoni* infections using Kato-Katz, IHA and ELISA methods.

Test method			Number of positive
Kato-Katz	IHAT	ELISA	
+	+	+	38
+	–	+	4
+	–	–	3
–	+	+	31
–	+	–	2
–	–	+	47
45 (21%)	71 (33.2%)	120 (56.1%)	125

3.3. Comparative analysis of the three applied methods

Considering Kato-Katz results as a true positive, the sensitivity, specificity, positive predictive value and negative predictive value of the ELISA and IHA were evaluated (Table 2). Of the 45 true positive cases by Kato-Katz, 42 and 38 were positive by ELISA and IHA, respectively. On the other hand, out of 169 negative cases by Kato-Katz method, 78

were positive and 91 were negative by ELISA, while 33 were positive and 136 were negative by IHA method (Table 2). On the basis of these results, the sensitivity of the ELISA was 93.3% compared to 84.4% given by IHA. Furthermore, the specificity was reduced to 53.8% in ELISA compared to the 95.1% detected by IHA. Moreover, the PPV was increased in IHA than ELISA (53.5% *vs.* 35%, respectively), while both tests were given a similar percentage of NPV (95.1%; 96.8%, respectively).

When we evaluated the combination of positive results of both ELISA and IHA, the sensitivity was high 93.3%; the specificity and PPV were increased to 85.8% and 55.1%, respectively compared to the ELISA method (Table 2).

Table 2

Comparison between IHA and ELISA assays in diagnosis of *S. mansoni* in term of sensitivity, specificity, PPV and NPV.

Methods	Sensitivity	Specificity	PPV	NPV
ELISA	42/45 (93.3%)	91/169 (53.8%)	42/120 (35.0%)	91/94 (96.8%)
IHA	38/45 (84.4%)	136/169 (80.0%)	38/71 (53.5%)	136/143 (95.1%)
IHA & ELISA	38/45 (93.3%)	145/169 (85.8%)	38/69 (55.1%)	145/152 (95.4%)

4. Discussion

In consider of microscopic examination using Kato-Katz smear as the gold standard method for the diagnosis of intestinal schistosomiasis, the prevalence of *S. mansoni* infection among schoolchildren in Sennar State, central of Sudan was 21%. In recent studies carried out in many parts of Sudan, different prevalences of *S. mansoni* infection have been recorded, *e.g.* in Wadi Halfa (ancient Nubia) was 26.1%[12], in central Sudan was 13% prevalence among pregnant women[13] and was 36.7% among schoolchildren[19]. Furthermore, worldwide studies have been determined the prevalence of *S. mansoni* infection. For example, the prevalence of *S. mansoni* infection was up to 62.2% among the schoolchildren in Tanzania[20], 60.5% in Kenya[21], 24.5% in Southern Sudan[22], 20.3% in Côte d'Ivoire among pre-school-aged children[7], 23.7% in Brazil[23] and 26.3% in Ethiopia[24]. These results with our finding reflecting that there were various geographical distributions of *S. mansoni* infections, indicating the needs to implement eradication programs in many endemic areas.

In this study, the important causes of this high prevalence of schistosomiasis in Sennar area were might be due to the habit of playing, swimming and using canal water for domestic uses. Moreover, we noticed that the water canals are surrounding the villages and most of the population daily cross these canals to reach either their school or their farms. However, they can easily access to this full infected water. In a previous study carried out in Kenya, the specific water-related activities such as swimming and fishing have been documented as risk factors for *S. mansoni* infections[25].

In out settings, despite of a relatively high prevalence of infection, we found 84.4% of infected children with light intensity infection, indicating of low endimicity of *S. mansoni* among the schoolchildren. Similar findings have been reported by other researchers[22,25]. In this study, the prevalence of *S. mansoni* infection was found to be significantly higher among the boys (33%) than that of in the girls (12%). This might be due to difference in exposure status. In our setting, the variations in the prevalence of infections between both sexes could be attributed to social and traditional habits which give the boys freedom to find more chances to play outside than girls. Similar findings have been reported among schoolchildren in the Al Gunaid area in Central Sudan[19]. In contrast, higher prevalence in females than males has been reported by others[25]. In a study carried out in Tanzania, no significant differences of infections were detected between boys and girls[20]. Likewise, in ancient Nubia, Northern Sudan, Hibbs *et al.* have reported that no statistically significant differences were in prevalence between males and females[12].

In comparison between microscopic and serological methods, we found higher positive results detected by serological tests of ELISA (56.1%) and IHA (33.2%) than Kato-Katz microscopic smear (21%). These findings could be compared with a survey conducted in Brazil, where the stool samples were examined using the Kato-Katz and serum samples were tested by IgG-ELISA. Of the total screened individuals, 49% showed positive serological test results. Of these, 16 (6%) had positive results in stool examination in the first sample batch[26]. Burlandy *et al.* reported that the high difference between serologic and parasitologic prevalence data is undoubtedly due to the low diagnostic sensitivity of the parasitologic methods[27]. Therefore, microscopical method is not satisfied, especially when the number of worms is low or the test is done after the eggs were eliminated from the body[6]. In such cases, we should increase the number Kato-Katz smear per sample or run multiple Kato-Katz tests in interval days[5,26].

In this study, we found high proportions of positive results that detected by the serological methods of the IHA and ELISA in spite of negative Kato-Katz examinations. In a study carried out by da Forta *et al.*, a single Kato-Katz smear detected only 12% of the 25 infections[9]; this increased to 44% (three smears, one stool sample), 84% (five smears, three stool samples) and 96% (six smears, four stool samples). In our study, since we examined a single Kato-Katz slide from each patient sample, the false positive results which detected by the serological tests should be followed up and confirmed by increasing the number of Kato-Katz smears per sample or repeating the stool sample in interval of days. However, these cases could be due to previous infections[11], or due to the cross reactions with other parasites[5]. Also it could be useful to determine whether there is cross-reactivity with antibodies against other helminthes infections[7].

For a diagnostic tool, it should be both sensitivity and specificity of the applied technique should be evaluated, especially when used for human diagnosis[28]. In this study, we assessed the sensitivity and specificity of the IHA and IgG-ELISA techniques were by comparing them to the Kato-Katz reference method. We found a good sensitivity of IgG-ELISA (93.3%) comparable to the 84.4% given by IHA method, while the specificity of ELISA was relatively lower (53.3%) than IHA (80%). Earlier in a study in Ethiopia, the sensitivity and specificity of the ELISA test were 97.6% and 30.3%, respectively[29]. Despite the serological tests like ELISA are not able to discriminate between previous contact with the parasite and active infections[30], the high sensitivity ELISA has indicated this technique as one of the most successful serological tests for epidemiological studies to detect parasite burden[30,31]. In areas of endemicity, other researchers have suggested to carry out serological tests

that require high specificity to avoid false-positive results. In such settings, a conjunctive combination of serological techniques has been required. For the diagnosis of imported schistosomiasis. However, a high sensitivity is important, and a disjunctive combination of two or more serological tests seems more useful[11]. This has become obvious in our finding, when we combined the both results of IHA and ELISA the specificity was increased to 85.8%. Gool *et al.* reported that the combined use of these two tests enabled the serological diagnosis of schistosomiasis to be achieved with very high degrees of both sensitivity and specificity[10].

In a clinical diagnostic method, PPV is a useful measure of the proportion of patients with positive test results that are correctly diagnosed[28,32]. In our setting, where 45 out 214 individuals were diagnosed microscopically with schistosomiasis, serological assays of ELISA and IHA have good predictive value for the absence of the infection but positive results have a low predictive value. Thus it will be useful to apply a valuable alternative reference method like polymerase chain reaction which may obtain a more accurate assessment whether the infection is present[3,33].

The present study concluded that the prevalence of *S. mansoni* infection in Sennar State, central of Sudan was found to be relatively high (21%) with low infection intensities. Therefore, in such setting screening of anti-schistosomal antibodies followed by a multiple stool sample examinations for each suspected individuals are required to detect more positive cases. Although IHA and ELISA tests yielded high sensitivity, the specificity rates of each assay alone were found to be low. However, it could be useful to perform a combination of such methods or to use more reliable molecular assay.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

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Comments

Background

Intestinal schistosomiasis is a tropical parasitic disease endemic in many developing countries. The Kato-Katz microscopic smear is a standard laboratory method recommended for the diagnosis of *S. mansoni* infections. It is becoming less satisfactory due to measurement errors in estimating the presence of infection. Other diagnostic methods such as the detection of parasite-specific antibodies have been shown to be more sensitive than the parasitological examination such as IHA and ELISA.

Research frontiers

This research determined the prevalence of *S. mansoni* infection among schoolchildren. In addition, it evaluated the sero-diagnostic techniques of the IHA and ELISA in comparison with Kato-Katz smear examinations, in order to be applied and adopted as to improve the diagnosis and assist in the control of schistosomiasis.

Related reports

Similar studies have been done by others in many part of the world. The performance of the serological tests in the diagnosis, using the Kato-Katz method as a diagnostic reference has been established (Carneiro *et al*., 2012). Gool *et al.* (2002) reported the combined use of these two tests which enabled the serological diagnosis of schistosomiasis with very high degrees of both sensitivity and specificity. This in agreement with the finding in present study.

Innovations & breakthroughs

In developing countries, there are limitations in diagnostic tools that investigate schistosomiasis. This paper focused on different diagnostic methods that could help and confirm the presence of the disease with a high accuracy.

Applications

Standard parasitological method as well as sero-immunological methods such as IHA and ELISA assays can be used for the diagnosis of schistosomiasis.

Peer review

In this study, authors focused on how to diagnose an important neglected disease using different laboratory methods, selecting in principle the cost-effective ones. These applied diagnostic methods have been compared to a reference method and the sensitivity and specificity of these methods were determined which aim to reach a feasible conclusion. These findings are useful to improve the diagnosis and assist in the control of schistosomiasis.

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