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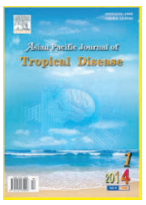

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Evaluation of microscopical and serological techniques in the diagnosis of *Schistosoma mansoni* infection at Sennar State, Central Sudan

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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