

Original Research Article

Comparative study between Direct Microscopy and Indirect Haemagglutination Methods Used in Diagnosis of Urinary Schistosomiasis

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Abstract: **Background:** Schistosomiasis is one of the world's most prevalent parasitic infections, with at least 200 million people infected and about 700 million at risk in Africa, Asia, and South America. Although five species of waterborne trematodes in the genus *Schistosoma* are capable of causing human infection, the most important are *S. haematobium* & *Schistosoma mansoni* and the majority of cases occur in sub-Saharan Africa. **Methods:** A comparative study aimed to compare between indirect-hemagglutination assay and Golden Stander Method which used for diagnosis of *Schistosoma haematobium*. Total of 73.0 samples were collected from people located in two villages in the areas of Sudan endemic with *Schistosoma haematobium*. The samples were analyzed by the centrifuged parasitological examination and indirect hemagglutination assay (IHA). **Result:** Statistical analysis of the results showed that the sensitivity value of the IHA test was 95.8% and specificity was 4.1%. **Conclusion:** A search for a good diagnostic test that can be applied in field situations in Sudan should be given high priority.

Keywords: Urinary schistosomiasis, direct microscopy, indirect haemagglutination, Patients, Khartoum, Sudan.

INTRODUCTION

Schistosomiasis is one of the world's most prevalent parasitic infections, with at least 200 million people infected and about 700 million at risk in Africa, Asia, and South America [1]. Although five species of waterborne trematodes in the genus *Schistosoma* are capable of causing human infection, the most important are *Schistosoma mansoni* and *S. haematobium*, and the majority of cases occur in sub-Saharan Africa [2]. Schistosomiasis is a parasitic trematodiasis caused by several species of the genus *Schistosoma*, of which *S. mansoni*, *S. japonicum*, *S. mekongi*, and *S. haematobium* are of public health importance. These worms live in the veins around the intestine or urinary bladder. Eggs are released in the stool or urine of the host and hatch in water [1]. Humans are usually infected when they come into contact with contaminated fresh water such as collecting water, washing, bathing, playing, fishing, or cultivating crops. In general, children, women, fishermen, and farmers are the high risk groups in schistosomiasis, also other people can infect in the irrigation channels or rivers and suffer from hematuria and anemia, enlargement of the liver and spleen, and growth retardation [3, 4]. Due to the geographical distribution of schistosomiasis and the affected populations, schistosomiasis is listed as a neglected tropical disease and a neglected infection of poverty. Apart from this, imported schistosomiasis has been recognized as an emerging clinical problem in countries where the disease is not endemic [5-7, 18]. The infection affects expatriates and immigrants but also travelers, especially in association with adventure and ecotourism [5-7, 18].

During early stages, schistosoma infections might cause severe manifestations, such as Katayama fever, schistosomal myeloradiculopathy, and pneumonitis [9, 10, 8, 12]. However, up to 50% of newly infected patients remain asymptomatic [13-15]. Independent of the initial presentation, untreated schistosomiasis might lead to complications such as obstructive uropathies, hepatic fibrosis, or granulomatous cerebral lesions [7, 17, 18]. To prevent those late manifestations, any case of schistosomiasis should be detected and treated [18].

Like the case for other parasitic infections, the diagnostic approach to schistosomiasis depends on the epidemiological situation. In endemic settings, parasitological examinations are the mainstay of diagnosis. Serological examinations, such as screening for antischistosomal antibodies, are of limited use for the diagnosis of active infection, as large parts of the population may carry antibodies due to past infections [2].

The diagnosis of imported schistosomiasis in individuals from countries where the disease is not endemic bears other challenges. First, those patients seem to be more prone to acute manifestations, which occur during early stages of infection and sometimes during the pre-patent period [2]. Furthermore, exposure to cercarial larvae is usually limited, resulting in infections with low parasite loads [19, 8, 11]. Therefore, direct parasitological methods often fail [19]. For this patient group, serological tests detecting antischistosomal antibodies are an important diagnostic tool [20-22]. Although the “seronegative window” has to be considered in very early infections [5, 8, 23].

Over the last decades, various serological methods have been developed to detect antibodies against *Schistosoma* antigens. Different techniques have been applied, including indirect immunofluorescent-antibody tests (IFATs), indirect hemagglutination assays (IHAs), and enzyme-linked immunosorbent assays (ELISAs) using different antigens, such as crude or purified adult worm antigen (AWA), soluble egg antigen (SEA), and cercarial antigen (CA) preparations [24-36, 21, 37, 16, 22].

Nevertheless, very few studies have addressed the value of serological assays for diagnosis of schistosomiasis in individuals from areas where the disease is not endemic and who are carrying light and/or recently acquired infections; most of those describe single in-house assays and are limited by small sample numbers [39, 40]. Only one study analyzed a commercial test together with an in-house assay [16].

MATERIAL AND METHOD

This is a comparative experimental study carried out to compare between two methods used for diagnosis of schistosomiasis. The samples were collected from 73 patients confirmed having schistosomiasis by urine samples which were analyzed using Microscope method from area of Om usher (jebelaulia) governorate southern Khartoum state.

Inclusion Criteria: (Resident in Um usher village, Jebel Aulia governorate, Khartoum state - Known patient with urinary schistosomiasis).

Exclusion Criteria: (Resident outside Om usher village, Jebel Aulia governorate, Khartoum state- People Free from urinary schistosomiasis). Principle of method called.

Shistosomiasisfumouze: Is based on indirect heamagltuination sensitized red blood cells are composed of sheep red cell coated with *shistosoma mansoni* antigen.

Specific serum antibodies are revealed by an agglutination of the sensitized red blood cells a reddish-brown film can be observed in the absence of specific antibodies these red blood cells deposit forming a ring in well bottom. The un-sensitized red blood cells ensure the reaction specificity and the elimination of interference due to natural anti sheep antibodies (For ssmahetero antibodies, infectious mononucleosis antibodies). The reaction was performed in U-micro plate. The test procedure is easy and rapid. The result is obtained in 2 hours. Data was analyzed using SPSS version 25. The results expressed as frequency and percentage. Chi-square test was used to compare the difference between IHA and Golden standard methods. Sensitivity was defined as the proportion of patients with a positive test result among those with proven infection. Specificity was calculated as the proportion of patients with a negative test result among samples of the control group.

RESULT

A total of 73 patients male (10-40 years) from Om ushar in the areas endemic with *Schistosoma hematobium* were analyzed by the parasitological examination and hemagglutination assay (IHA).

Table-1: Frequency and percentage of indirect-hemagglutination assay and Golden Standard Method for diagnosis of Schistosoma (n=73)

Methods	+Ve F (%)	-Ve F (%)
IHA	70 (96%)	3 (4%)
GSM	73 (100%)	0 (0.0%)

F= frequency

The result showed in Table-1 determine that the frequency of IHA was (4%) and specificity was (4.1) while the frequency of golden standard method (0%)

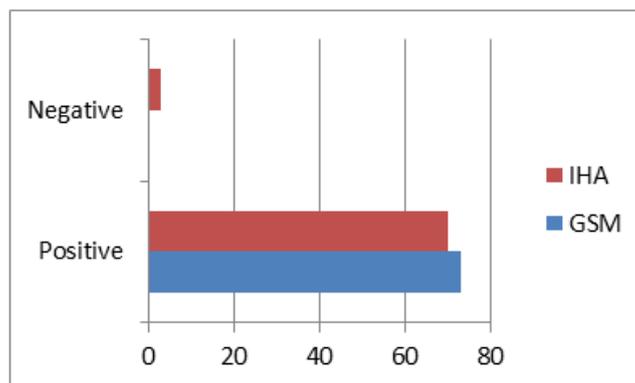


Fig-1: Compare positivity of IHA against GSM for diagnosis of *Schistosoma haematobium* (n = 73)

Table-2: Shows Sensitivity and positive predictive value (PPV) of IHA assay in compare to GSM for diagnosis of *Schistosoma haematobium*

Method	Sensitivity%	PPV
IHA	94.87	95.89%

The result showed in Table-2 determine that the sensitivity of IHA was (94.87%) and have a PPV of (95.89%).

DISCUSSION

In areas of endemicity, where past *Schistosoma* infections, high *Schistosoma* loads, and polyparasitism are frequent, serological testing that have high specificity to avoid false-positive results. In such settings, and combination of serological tests (screening followed by confirmation) has been widely used in endemic areas because it is more sensitive than parasitological diagnosis and antibodies are easier to detect than antigens [42]. In this study, we used three circulating antibody detection methods (IHA) tests which are the most widely used assays in People's Republic of china [42]. Our study highlights the usefulness within this patient group, serology has two aims: (i) to diagnose symptomatic infections and (ii) to screen asymptomatic individuals with reported freshwater exposure in areas where schistosomiasis is endemic. A known obstacle to serodiagnosis of acute or recent schistosome infection is the prolonged seronegative window period. Antibody production in newly infected individuals usually starts 4 to 7 weeks after infection, and although the majority of patients exhibit seroconversion within 3 months [10, 13, 14], prolonged seronegative window periods of up to 6 months have been described [9, 10].

This study showed that commercially available IHA test is sensitive and tests for the serodiagnosis of schistosomiasis in endemic tropic area. Among our serum samples, IHA method revealed sensitivity similar to that detected by Tom van Gool *et al.*, [16] in the Netherlands when they got 94% sensitivity when they investigated patient sera infected by *Schistosoma mansoni* and *Schistosoma haematobium*. This suggests that almost all patients had seroconvert at the time that the samples were taken. [12] In contrast our result showed higher sensitivity to detect *Schistosoma* antibodies when it's compared to similar study done by Annie Sulahian *et al.*, [43] in France when they evaluated sensitivity of IHA method in a total of 48 sera sample from patients with parasitological confirmed cases of schistosomiasis out of them 35 were positive giving sensitivity equal to 72.9%. Also ours study result is higher than the result obtained by Hans-Friedemann Kinkel *et al.*, [44] in Germany that showed 73% sensitivity when they investigated 37 patient with confirmed, this Cleary state that our study is high and these differences might have been caused by differences in the serum panel that was used in these studies however this highlights the variability of test sensitivity in different clinical settings and the difficulty in comparing diagnostic test performances obtained in different studies.

Some limitations of the present study are that it included sera only from patients with egg-proven schistosomiasis. Also since the prevalent period of schistosomiasis is 4 to 6 weeks, our gold standard had a negative bias for very early infections and a probable positive bias toward larger parasite burdens, since parasitological methods are more sensitive to larger worm loads [8]. Still, parasitological proof is the accepted diagnostic gold standard [9]. A multicenter study including large sample size and comparing other serological and advanced molecular technique is needed to overcome most of the above-mentioned limitations.

CONCLUSION

This study concluded that the IHA test was useful tool for the diagnosis schistosomiasis in endemics area and had good sensitivity values to be used as screening and diagnostic tool.

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Ethical Consideration

Informed consent was written and signed by each participant following explanation of the study and sample collection procedure. The participants' information was kept confidential.

Strength

The study focusing in important public health problems located in agricultural areas causing social and economic problems.

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