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Detection of schistosomiasis antibodies in urine patients as a promising diagnostic maker

Salma Medani Elhag[1*], Eshtiyag Abdalla Abdelkareem[2], Ashraf Siddig Yousif[2], Ehab Ahmed Frah[1], Ahmed Babiker Mohamed[3]

[1]*Department of Vector and Biomedical Studies, Tropical Medicine Research Institute, National Centre for Research, Khartoum, Sudan*
[2]*Department of Immunology and Biotechnology, Tropical Medicine Research Institute, National Centre for Research, Khartoum, Sudan*
[3]*National Centre for Research, Ministry of Science and Technology, Khartoum, Sudan*

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ABSTRACT

Objective: To determine secreted *antischistosoma* antibodies in urine and to discern the epidemiological situation of schistosomiasis in the agricultural field labourers' camps city in the Gezira State-central Sudan. **Methods:** Total of 66 urine and 66 serum paired samples were collected from those who confirmed parasitologically positive and negative with schistosomiasis from the two camps. Samples were tested using ELISA technique to measure and compare the immunoglobulin G (IgG) levels in serum and urine samples of schistosomiasis patients. **Results:** The overall prevalence of *S. mansoni* and *S. haematobium* was 53.8% and 15.4%, while the intensity were (2.04 GMEC) and (0.9 GMEC) respectively. The relative percentage of positive IgG individulas in urine was 92.40% where as 96.97% in serum. Statistically no significant difference between the IgG levels in serum and urine samples was observed. **Conclusions:** This study shows that the detection of secreted IgG antibodies in urine can substitute serum for diagnosis of schistosomiasis.

1. Introduction

Schistosomiasis is one of the most widespread of all parasitic infections of man. The World Health Organization (WHO) estimated that schistosomiasis and soil transmitted helminths represent more than 40% of the global disease burden caused by all tropical diseases, excluding malaria[1]. In the Sudan, especially in Gezira Agricultural Irrigation Scheme (GAIS) between the Blue and White Nile Rivers, *S. haematobium* and *S. mansoni* are widespread[2]. Diagnosis of schistosomiasis, one of the major parasitic diseases in tropical areas, is usually performed by parasitological (microscopic detection of eggs), and/or immunological methods (antibody and antigen detection)[3]. The demonstration of parasite eggs in urine or feces directly indicates the presence of the worms, but the disadvantages of this approach include a high fluctuation in egg counts, easily missed low infections, and a relatively time-consuming methodology. Immunological methods such as enzyme-linked immunosorbent assays (ELISAs) usually require more advanced laboratory settings may yield a higher sensitivity (particularly for antibody detection).

For the past decade, praziquantel has been the main drug of choice for treatment of all species of schistosomes because of its efficacy, ease of administration, safety, and cost[4]. A single dose of 40 mg/kg has been widely accepted as the standard dosage, resulting in cure rates of 60%-95%[5]. Although the efficacy of treatment remains difficult to determine because of specific antibodies continue to be present long after the worms have disappeared.

The experience from seroepidemiological surveys, and apart from test procedures, indicates the sample collection of the blood and its processing are sometimes cumbersome and require trained personnel for venopuncture and

*Corresponding author: Salma Medani Elhag, Department of Vector and Biomedical Studies, Tropical Medicine Research Institute, National Centre for Research, Khartoum, Sudan.
Tel: + 249 9 124 644 13; + 249 9 124 644 13
Fax: +249 83 781845
E-mail: selmamedani@hotmail.com

separation of serum samples under field conditions. In addition, venopuncture is painful and not widely accepted by children and certain ethnic and religious groups[6]. ELISA has shown a high sensitivity and specificity for antibody detection in schistosomiasis[7].

A drawback of the method is that it cannot distinguish between previous and current infections. However, the ELISA method is still a useful sensitive diagnostic tool for surveillance, especially in finding new endemic foci. It is also be useful for evaluating the control programmes. Recently, to overcome the difficulties of convincing the people to provide blood, urine was successfully substituted for serum as a sample to diagnose lymphatic filariasis and visceral leishmaniasis with ELISA[8,9].

Compared with serum samples, urine can be easily and safely collect, especially from children. This facilitates and increases the compliance of people, in the field, to provide samples for the different activities[10]. To answer the question of simplicity in immunodiagnosis of schistosome infection, it was suggested to use urine sample in which the appropriate markers for schistosomiasis infection, either antibody or antigen, can be detected is expressed[11]. The present study was carried out to determine the schistosomiasis epidemiological situation and to investigate the reliability of using IgG in the urine for schistosomiasis diagnosis.

2. Materials and methods

2.1. Study area and study population

This study was carried out in the permanent agricultural camps (Hababna and Elshajara) of Tayeba village area in the GAIS. GAIS lies to the south of the junction of the Blue and White Niles with total area 2.2 million feddans approximately. The population of the two camps is working mostly in agriculture and they belong to different ethnic groups. In Hababna camp all the inhabitants are from Nuba mountains area in Kordofan State in the mid west of the Sudan, while the residents of Elshajara camp are from Darfur State in the far west of the Sudan. The estimated population of each camp was about 500 individuals. The canal is the main source of water for the inhabitants of the camps; although each of them has a low-yield well with a hand pump.

2.2. Study design and data collection

Following elucidation the aim of the study and obtaining informed consent from the head of each camp and the residents, a cross-sectional immuno-epidemilogical study was done. It involved 208 individuals 97(46.6%) female and 111(53.4%) male ranging 4-80 years were chosen randomly from the camps. Disposable containers were labeled by specific ID number and distributed to each participant (close relative in case of children) to provide stool and urine samples for schistosomiasis diagnosis.

2.3. Parasitological examination

Developed modified Kato technique was used to check stool samples by means, which described before[12] and sedimentation technique was used for urine samples as described by Braun-Munzinger RA[13]. Egg count was detected for all samples to state the index of infection (intensity) egg count was performed for *S. mansoni* and the intensity of infection was expressed as egg count per gram (EPG) of stool for each individual[14].

2.4. Serum and urine samples preparation

Five ml of blood in non-heparinized tube was collected from all volunteers of two forms of schistosomiasis infections and co-infection plus control from endemic area at the same time. 15 mL tubes were used to collect urine samples from the same individuals. Blood was allowed to clot at room temperature, and then sera were separated by centrifugation at 2 000 rpm for 15 min and stored at −70 ℃ utill further use. Urine samples were centrifuged at 3 000 rpm for 10 min and then supernatant discarded and about 20 μL of the residue and the pellet of urine mixed well with 100 μL of the phosphate buffer saline (PBS) and stored at −20 ℃ until used.

2.5. Determination of IgG antibody in serum and urine using ELISA technique

Blood and urine samples (from the same individuals) who confirmed positive were collected in addition to healthy control from endemic and non-endemic area. Indirect ELISA (Enzyme linked-immunosorbent Assay) technique was used to detect IgG antibody adjacent to Soluble Worm Antigen (SWA) in the serum and urine samples as described by Itoh M[8]. Optical density (OD) was measured at 492 nm and 450 nm by ELISA reader laboratory system Multiskan plus (Serial 3140) for serum and urine respectively. An (OD) less than 2.21 was considered the cut off point for serum and (OD) less than 0.386 was considered the cut off point for urine IgG antibodies.

2.6. Statistical analysis

The Statistical Package for Social Science (SPSS 13) was used for data analysis. The immunological factors, the quantitative variables were subjected to analysis using t-test and One-way Anova ($P<0.05$).

3. Results

3.1. Prevalence and Intensity of infection

The overall prevalence of *S. mansoni* and *S. haematobium* in the two camps was 53.8% and 15.4%, while the intensity (2.04 GMEC) and (0.90 GMEC) respectively. The prevalence of *S. mansoni* in Hababna camp was 48.4% and the intensity of infection was 1.92 (GMEC). Whereas in Elshajara camp, the prevalence of *S. mansoni* infection was 58.4% and the intensity of infection was 2.12 (GMEC) (Figure 1). There was no significant difference ($P>0.05$) between the prevalence of *S. mansoni* infection or in the intensity of infection ($P>0.05$) in the two camps.

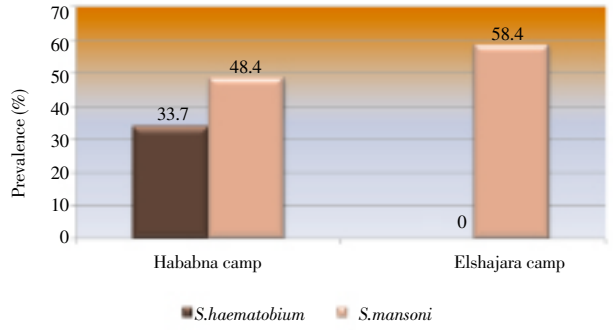

Figure 1. Prevalence of *S. mansoni* and *S. haematobium* infections in each camp.

The prevalence of *S. mansoni* infection was significantly higher in males than females ($P<0.05$). The intensity of *S. mansoni* infection in males was 2.05 GMEC, and in females was 2.01 GMEC ($P>0.05$). On the other hand, there was no significant difference between the prevalence of *S. haematobium* infection and gender ($P>0.05$); or between the intensity of infection in males (0.85 GMEC) and in females (0.84 GMEC) ($P>0.05$) (Figure 2).

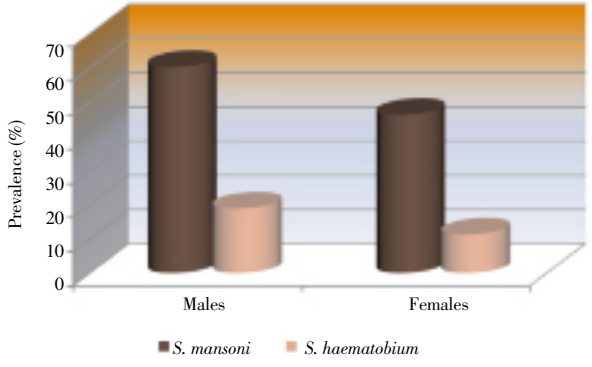

Figure 2. Prevalence of schistosomiasis infections in males and females within the two camps.

The prevalence, of both species, was higher in the age group 4–15 years old, 60.7% and 78.1% for *S. mansoni* and *S. haematobium* respectively and then dropped sharply to 18.8%, for both species, in the age group 16–25 years old and decreased gradually in the following age groups (Figure 3). There was a highly significant difference in the overall prevalence of *S. mansoni* infection ($P<0.05$) as well as in the overall prevalence of *S. haematobium* infection ($P<0.05$) among the different age groups in the two camps.

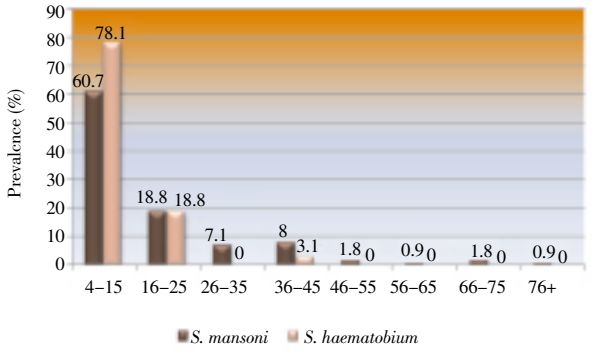

Figure 3. Overall prevalence of *S. mansoni* and *S. haematobium* infections by age groups in the two camps.

3.2. Anti–Schistosoma IgG levels in serum and urine

The total of paired samples of urine and serum in Hababna and Elshajara camps was 66. The relative percentage of positive IgG individulas in urine and serum were 92.40% and 96.97% respectively, compare to negative IgG individuals which were 7.60% and 3.03% in urine and serum, accordingly the variation between the negative urine and serum individuals is not immense as well as positive urine and serum individuals.

Figure 4 shows the optical density of anti–*Schistosoma* IgG in urine and serum samples of the study group according to the schistosome infection. Generally, the IgG levels in the serum were higher than the IgG levels in the urine. The optical density of the serum samples of those infected with intestinal schistosomiasis were much higher in comparison with those infected with urinary schistosomiasis. There were no significant differences between IgG levels in urine and serum samples ($P>0.05$).

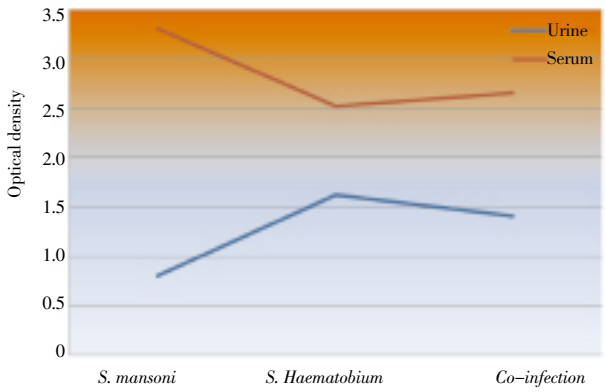

Figure 4. Optical density of anti–*schistosoma* IgG in urine and serum samples study groups according to their infection.

4. Discussion

The epidemiology of schistosomiasis is very complicated. It involves the parasite, the intermediate and the final hosts, among the factors related to the final hosts are the indices of infection (prevalance and intensity). This study showed that School age children comes to knwon the group with the highest rate of infection and agree with study by Biu AA[15], that the disease was found to exist at high prevalence in the endemic areas all over the world, which based on focus individual discussions with adult men and women revealed and that infection in children was primarily due to their swimming activities in earth dam reservoirs particularly during the active rains and that infection in pre-school and school children was primarily due to exposure occasioned by washing, bathing, dry season farming, and fishing activities[15-17]. The indices of infection decrease with age. As people, grow up they become wiser. The indices of infection according to the gender are incompatible to some extend. This suggests a different pattern of exposure to the infection between the gender, which could be due to the fact that males are field workers and are exposed continuously to the infection in the fields. Or for behaviour of males swimming freely in the canal so they exposed to the cercariae, where as the females rarely swim naked. The conventional ways of diagnosing the parasite eggs in the faeces and urine to confirm an active infection by the schistosome worms are the parasitological techniques. These are either qualitative or quantitative techniques. All of them have drawback in sensitivity and specificity, especially in light infections. They are also time-consuming methods. They are not standardized when they report the results. Therefore, they are not reliable to be used to compare and/or to assess the epidemiology or the success of the control programmes and diagnosis. The aim of the control is different among the areas of high or low transmission. Decrease of morbidity control is the goal of control in high endemic transmission areas and transmission control should be the goal in the low endemic ones[18]. As already has been achieved by WHO[19].

Therefore, diagnosis of human schistosomiasis is very central to make a decision on individual case management and to achieve control programs. Several immunological and serological tests have been developed, tested and evaluated for diagnose schistosomiasis. Such as, detection of antibodies IgG against antigens of *S. mansoni* eggs in saliva, CAA (circulating anodic antigen), CCA (circulating cathodic antigen). Each method has its merits and drawbacks. The experience from seroepidemiological surveys, and apart from test procedures, shows that the sample collection of the blood and its processing are sometimes cumbersome and require trained personnel for venopuncture and separation of serum samples under field conditions. In addition, venopuncture is painful and not widely accepted by children and certain ethnic and religious groups[6,20]. A drawback of the method is that it cannot distinguish between previous and current infections. However, the ELISA method is still a useful sensitive diagnostic tool for surveillance, especially in finding new endemic foci. It is also be useful for evaluating the control programmes.

As we know the occurrence and development of schistosomiasis is strongly depended on human immunity against schistosomes[20]. Recently, to overcome the difficulties of convincing the people to provide blood, urine was successfully substituted for serum as a sample to diagnose lymphatic filariasis and visceral leishmaniasis with ELISA[8,9]. Compared with serum samples, urine can be easily and safely collected, especially from children. This facilitates and increases the compliance of people, in the field, to provide samples for the different activities[10] Because of the difficulties in obtaining blood samples in the Sudan, this study was carried out to determine the feasibility of using urine instead of blood or serum to determine those infected with schistosomiasis, both, the intestinal and the urinary forms. Also, to investigate the reliability of using (IgG) in the urine for schistosomiasis diagnosis instead of serum. The present study revealed that urine samples, from the schistosomiasis patients, contain IgG against *Schistosoma* Soluble Worm Antigen (anti-SWA), and IgG were significantly correlated with those of the serum samples. The IgG antibodies were detected in more than 85.0% of the urine samples from serum positive patients whereas all urine samples from a schistosomiasis non-endemic area were negative.

The differences in the IgG anti-bodies level, in urine and serum of the infected individuals, were insignificant. However, these levels are higher in the serum than urine of *S. mansoni* patients. However, they are higher in urine than in serum of those infected with *S. haematobium*. This is post probably due to the assembling of the antibodies near to the site of antigens. In conclusion, the schistosomiasis epidemiological situation in the agricultural labourers' camps compare with studies during the last twenty-five years has changed, due to several factors. *S. haematobium* re-appeared in the scheme and several socio-economical and epidemiological factors have changed. This requires, urgently, the initiation of new epidemiological studies in GAIS to determine the pattern of transmission, and, may be, to suggest integrated control. These study revaled that one of the tests that held promise is to use urine in ELISA which

had been used for the diagnosis of schistosomiasis and should take in consideration the adoption of the advanced techniques that have been developed recently to improve the diagnostic capabilities of the health units and to use urine sample in which the appropriate marker, either antibody or antigen, can be detected. It is, also, advisable.

Conflict of interest statement

We declare that we have no conflict of interest.

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