

Original Research Article
**Comparative Study between Molecular and Different Diagnostic
Methods of *Schistosoma haematobium* Gezira State Sudan**

Abstract

Over 650 million people globally are at risk of infection with Schistosomiasis, with more than 200 million people infected, and higher disease rates occur in children.

Objective: The aim of this study is to compare the Molecular diagnostic method with different diagnostic methods (filtration, haematuria ultrasound). **Methods:** Eighty three students of Quran School for boys in Radwan village, Gezira state were selected. The age range was between 6-20 years (mean 12.45 ± 3.2). Urine samples were collected and different diagnostic methods were used. DNA was extracted from the eggs and polymerase Chain Reaction was done for all samples. **Results** 45/83 (54.2%) were positive with filtration method. 23/83(28%) were positive by microhaematuria. 51/83(61.4%) were positive by ultrasound. 60/83 (73.3%) samples were positive by PCR. The comparison between the diagnostic method and gold standard syringe filtration techniques showed that haematuria sensitivity was 42.2% and specificity 89.4% with a significant association, ($P < 0.05$). No significant difference was found in ultrasound ($P > 0.05$) the sensitivity was 71% and specificity 50%, while PCR sensitivity was 100% and specificity 60.5%, with a significant association between PCR and filtration techniques. **Conclusion:** The study concludes that PCR was 100% sensitivity while microhaematuria method is highly specific 89% ultrasound lower specificity compared with other diagnostic test. the study recommends that different diagnostic techniques could be applied according to the situation of the disease.

Key words: *S. haematobium*, Molecular diagnosis, ultrasound, Gezira State, Sudan

1. Introduction

Schistosomiasis, or bilharziasis, is a parasitic disease affects man and mammals in tropical and sub-tropical area and it's the second disease after malaria in socio-economic and public health importance [1]. It is endemic in more than 70 low income countries where it occurs in rural areas and the fringes of cities [2]. Over 650 million people globally are at risk of infection, with more than 200 million people infected, higher disease rates occur in children [2] with infection high frequently found in those less than 14 years in many risk areas [3]. A number of studies [4] from Africa have shown that the estimated incidence of urinary bladder cancer is higher in areas with a high prevalence of infection with *S. haematobium* than in areas with a low prevalence [4]. Haematuria is a rapid indicator for assessing endemicity of urinary schistosomiasis in communities [5]. Lack of microhaematuria is a more valid indicator of the absence of urinary schistosomiasis, and showed moderate sensitivity and high specificity for the disease [6]. Microhaematuria was more prevalent among egg- positive. The degree of microhaematuria was significantly associated with the intensity of the infection [7]. A new and inexpensive filtration device was tested against the commonly used (Millipore device). The new device is effective as the millipore device [8] Urine filtration was long used in surveys to measure the distribution, prevalence and intensity of infection and is considered as the golden standard technique for detecting *S. haematobium* egg [9]. Urine filtration is one of methods recommended by the World Health Organization as gold standard Method, the egg count of *S. haematobium* by filtration technique reported by per 10 ml of urine [10]. Ultrasonography has proven its value as a safe, rapid and non-invasive technique for morbidity assessment in schistosomiasis. The technique has the invaluable advantage of directly visualizing the organ-specific schistosomiasis-associated changes seen in the liver and urinary bladder, as well as additional disease complications such as portal hypertension and hydronephrosis [11]. Studies from different *S. haematobium* endemic settings in Kenya, Tanzania, Ghana and Niger using the standardized protocol have shown that reversal of urinary tract morbidity occurs within six months of treatment, and that the rate of reappearance of

urinary tract morbidity depends on the level of re infection [12,13]. Several Studies had been conducted in detecting *S. haematobium* using polymerase chain reaction (PCR) [14, 15, 16, and 17]. Hamburger and his colleague cloned *S. haematobium* genome repeated sequence, using the DraI repeated sequence [14]. Ibironke et al Study the validation of a new test for *S. haematobium* parasite specific DNA Dra1 fragments, they found that Dra1 fragment is a definitive test for the presence of *S. haematobium* infection [15]. Lodh et al investigated the efficacy of detecting parasite specific DNA amplified from urine sediment obtained by filtration from people infected with both *S. mansoni* and *S. haematobium* either singly or together. They found that it was a practical advantage that avoids the need for specimens and was more effective than standard [16].

The risk of acquiring schistosome infection is wide-spread in Sudan but appears to be particularly high in the major irrigation systems that have been built such as Gezira area, the Gezira Irrigation Scheme, the largest irrigation scheme in Africa, lies in the central part of the Sudan, between the Blue and the White Nile Rivers. The prevalence of *S. mansoni* reaches up to 70% and that of *S. haematobium* reaches 15%. During 1990 [18]. Sudan has seen a serious increase in endemicity and prevalence of both *S. haematobium* and *S. mansoni* infections since 1990 as a result of expansion in water resource projects, population movements, and unsuccessful control measures and stop of the Blue Nile health project 1980-1990 [19]. One of the main challenges to schistosomiasis prevention and control in the country has been a lack of recognition of the schistosomiasis problem, coupled with a lack of political commitment. The aim of this study is to compare the Molecular Method Polymerase Chain Reaction (PCR) with the other diagnostic methods of *S. haematobium* in Gezira State Sudan.

2. Material and Methods

2.1. Study Area

This study was conducted in Roudwan village. It is located in the south west site of Wadmedeni city. This village is a part of Gezira irrigation scheme and surrounded by

canals from the south and east. Its population is about six thousand. Most of its population is working in agriculture as laborers or hand craft. There is only one small health center and no school most of the children learn in Khalwa (Quranic school). No safe water source, canals is the main source of water, safe water is payable. A total of 83 students in Quranic School of boys (Khalwa) were recruited for this study. All the students were at risk of infection of schistosomiasis due to the daily exposed to the infection by swimming and daily activities in canals water.

2.2. Urine Examinations

Ten ml of collected urine was collected the 83 students after informed consent and examined by macro, micro examination of each specimen. The urine was examined by filtration method [10] microscopically for *S. haematobium* eggs. All the positive cases were treated with praziquantel after obtaining the result.

2.3. Molecular Analysis

The urine was centrifuged and the deposit was kept in -80 °C as preparation for DNA extraction, DNA extraction was done using QIAamp DNA Mini kit (Germany).

Random Amplified PCR Reaction (RAPD) test the amplification conditions were based on the original method described by [20] with minor modifications.

In this study five primers were used to genotyping of *S. haematobium* strain (A01, 5'-CAGGCCCTTC-3'; A02, 5'-TGCCGAGCTG-3'; A12 , 5'-TCGGCGATAG-3';A13, 5'-CAGCACCCAC-3'; Y20 ,5'-AGCCGTGGAA-3';), primers was selected by [21]. The reaction was done using Perkin Elmer thermocycler and the program as followed, an initial at 95 °C for 5 min for 2 cycles through the following temperature profile: 30°C for 2 min for annealing, 72°C for 1 min for extension and 30 s at 95°C followed by 33cycles where the annealing step was altered to 40°C. In the final cycle the extension step was for 5 min., the product was run in 1.5% agarose gel with a 100-bp DNA ladder (Life Technologies).

2.4. Ultrasound Examination

Ultrasonographic examination was done for the 83 recruited students by expert radiologist for. Transabdominal US examination of the urinary tract was performed by Aloka (5100) with a 3.5 MHz convex probe and Shimadzu with convex probe 2-5 and 5-10 MHz ultrasonographic assessments were performed for all the study subjects according to WHO standards [22].

2.5. Ethical Approval

Ethical clearance was taken for this study from National Cancer Institute Research Ethical committee NCI-REC University of Gezira was conducted for this study. Written consent was obtained from the local leaders. All infected subjects were treated immediately with Praziquantel provided from the Ministry of Health Gezira State.

2.6. Statistical Analysis

Data were entered into Microsoft Access 2007 and analyzed relevant statistical methods, by using SPSS version 16 statistical package. Descriptive, specificity, sensitivity and positive and negative predictive value were calculated.

3. Results

3.1 Study Area and Population:

The study was conducted in Roudwan a village near Wadmedni city. The eighty three recruited students were males; the mean age was 12.45 ± 3.2 with age range between 8 - 20 years. The majority of age groups were between 9-11 years 36.1% (30/83) as shown in table (1).

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Table (1) Shows Distribution of the study subjects by age range, N=83

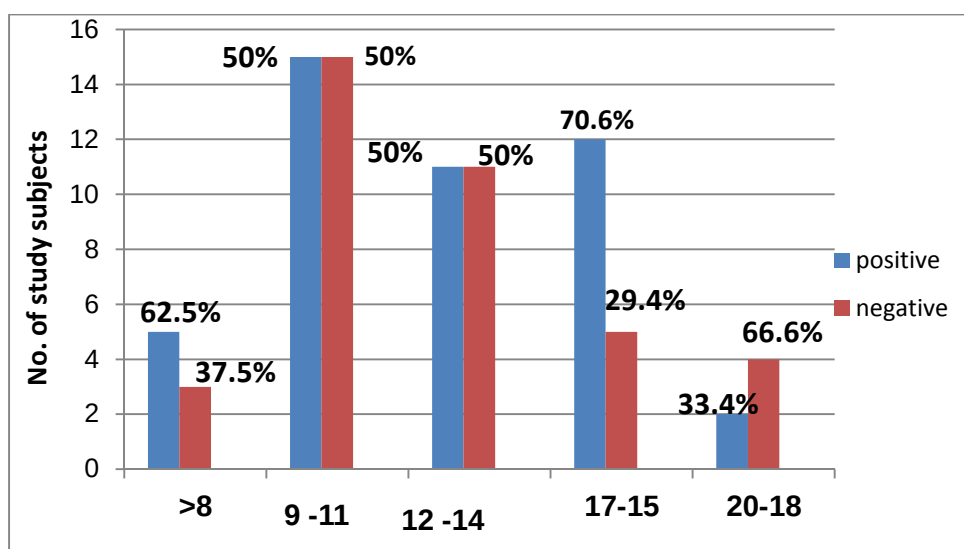
age range	No. of Subjects (%)
>8	08 (09.6)
9-11	30 (36.1)
12-14	22 (26.5)
15-17	17 (20.5)
18-20	06 (07.2)
Total	83 (100)

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139 3.2 Parasitological Examination of *S. haematobium*

140 The prevalence of *S. haematobium* among the study participants was 54.2% (45/83) by
 141 filtration techniques as shown in figure1. Diagnosis by microhaematuria method found
 142 that 28% (23/83) was positive for *S. haematobium*.

143 **Figure (1) shows the results of filtration method age groups by filtration technique, N=83**



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145 All the khalwas' students were diagnosed by ultrasound, 85% (51/83) of study subjects
 146 had pathological changes. The changes included 85.9% (49/51) abnormal wall thickness,

3.9% (2/51) one polyp and 56.8 % (29/51) multiple nodularities and 1.9% (1/51) wall calcifications of urinary bladder. Regarding kidneys and ureters, the pathological changes were 11.73% (22/51) abnormal ureteric thickness and 3.9% (2/51) echogenic kidneys. The age range 9-11 year had a high frequency of abnormality in bladder thickness and bladder masses compared with the other age groups, while 12-14 year age group had higher frequency regarding abnormality of kidneys as shown in table 2.

Table 2 Ultrasound finding of the Study Subjects by age range

Age range	Total (%)	Thickness (%)	Mass		Wall	Ureter thickness (%)	Echogenicity (%)
			One Polyp (%)	Multiple (%)	Calcification (%)		
>8	5 (9.8)	5 (10.2)	1 (50)	3 (10.3)	0	0	0
9-11	21 (41.2)	20 (40.8)	0	14 (48.3)	0	7 (31.8)	0
12-14	16 (31.4)	15 (30.6)	1 (50)	9 (31.0)	1 (100)	8 (36.4)	0
15-17	7 (13.7)	7 (14.3)	0	2 (6.9)	0	5 (22.7)	1 (50)
18-20	2 (3.9)	2 (4.1)	0	1 (3.4)	0	2 (9.1)	1 (50)
Total (%)	51 (100%)	49 (100)	2 (100)	29 (100)	1 (100)	22 (100)	2 (100)

3.3 Comparison between different diagnostic techniques:

The gold standard method for *S. haematobium* diagnosis is the filtration technique. This method was compared by microhaematuria method the result showed that 19 (22.9%) were positive for both methods with 42.2% sensitivity, 89.4% specificity and the positive predictive (PPV) value was 82.6 % as shown in table 3 and 4.

The comparison between gold standard ultrasound showed that 23 (38.5%) students were positive for both methods as shown in table 3. The sensitivity was 71.1%, specificity was 50% with 62.7% PPV as table 4.

All the positive subjects with filtration technique were positive by PCR while only 15 (18.1%) of subjects were negative to filtration method and positive by PCR shown in table 3. The sensitivity was 100% and specificity was 60.5% with PPV 75.0% table 3.

Table 3. Comparison between different diagnostic techniques

Techniques	True		False		Total (%)
	+ ve (%)	- ve (%)	+ ve (%)	-ve (%)	
Micro haematuria	19 (22.9)	34 (50.0)	4 (4.8)	26 (31.3)	83 (100)
Ultrasound	32 (38.5)	19 (22.9)	19 (22.9)	13 (15.7)	83 (100)
PCR	45 (54.2)	23 (27.7)	15 (18.1)	0 (00)	83 (100)

Table 4. Sensitivity and specificity and

Positive predictive value of different diagnostic techniques

Techniques	Sensitivity% 95%(Confidence Interval)	Specificity% 95% (Confidence Interval)	PPV % 95% (Confidence Interval)	NPV 95% (Confidence Interval)	P value Chi square
Micro haematuria	42.2 (27.7 - 57.8)	89.4 (75.2 - 97.0)	82.6 (61.20 - 95.1)	56.7 (43.2 - 69.4)	0.001
Ultrasound	71.1 (55.7 -83.6)	50.0 (33.4- 66.6)	62.7 (48.1 - 75.9)	59.4 (40.6 - 76.3)	0.05
PCR	100 (92.0 - 100.0)	60.5 (43.39 - 75.95).	75.5 (62.14 - 85.27)	100 (85.0- 100.0)	0.00

4. Discussion

Schistosoma haematobium start increasing in the last decade after control of *S. mansoni* in Gezira area [23]. This schistosomiasis is affecting males more than females' unpublished report 2011. It may be due to that males are more exposed to the water supply than females. Eighty three study subjects were recruited; age range between 8 - 20 years. The prevalence of infection with *S. haematobium* was 54.2%. There was little increasing of prevalence than this was found in a study done in the same area in 2004, showed that the prevalence of *S. haematobium* was (34%) [23], the increasing of prevalence may be due to change environmental and ecological factor of snail in addition to increase of activities of water contact in addition to the irregular control measures. Comparing The prevalence of *S. haematobium* in this study (54.2%), with a study in southern Darfur showed high prevalence 81.1% [24], other study done by Mahgoub et al in eastern Sudan mentioned that 18.0% of the school children were infected with *S. haematobium* [25]. The decreasing of the prevalence in compared with southern Darfur may be due to the use of control measures in Gezira State and Eastern Sudan. Micro-hematuria had 4.3% false positive result compare with other methods, this may be due to those had urinary tract infection or bacterial infection. In case of ultrasound 22.9% were false positive; this may be due to the chronicity of the infection by continuous exposure to source of infection canals. All the students were exposed more than three years minimum. In addition to chronic urinary tract infection which may affect urinary tract abnormality. Laboratory technician sometime cannot see the schistosomiasis eggs in the microscopic examination because for very low intensity, this may be the main reason for the false positive result of PCR method (18.1%). In this study 85% of study subjects revealed abnormal pathological conditions, while in Mali a study done in children found 3.4% irregular bladder wall abnormality [26]. The increasing of the abnormality of urinary tract this study may be our study subjects were more exposed to *Schistosoma* infection with lack of irregular mass chemotherapy which may affect the chronicity of the disease.

Filtration is the gold standard technique and compared with other diagnostic technique for *S. haematobium*; in this study. Our results demonstrate that hematuria sensitivity was 42.2% and specificity 89.4% with significant difference $P=0.001$. In Southern Sudan Robinson et al found that hematuria was highly sensitive (97.8%) but moderately specific (58.8%) [27]. Our study was contrary to study done Ibironke et al in Nigeria, they found that sensitivity 87.6% and the specificity 34.7% [15], this may refer to hematuria was mostly caused by *S. haematobium* egg in our study. Obeng et al in Ghana assessed micro-hematuria with the microscopic results, they recorded sensitivities of >77% and specificity of 90% [28]. A study from Ethiopia done by Birrie et al found that 80% the sensitivity and 96% specificity of hematuria [29]. The sensitivity of hematuria in this study was least when compared by studies done in Ghana and Ethiopia it may be due to the infection cause by *S. haematobium* in study area in early stage and density of parasite was decrease. In this study 19/51 (37%) were positive by ultrasound but negative for *S. haematobium* by filtration technique. The sensitivity is 71.1% and specificity 50% with PPV (62%) no significant difference $P= 0.05$ compared with other method. Hamburger cloned *S. haematobium* from free free-swimming cercaria, DraI-based PCR assay was done for genotyping of *S. haematobium* they found that repeated sequence in the genome of *S. haematobium* developed a highly sensitive PCR assay for the detection of this parasite [14]. Many studies confirmed the sensitivity of PCR to detect egg of *S. haematobium* in urine is more sensitive than filtration technique. Lodh et al. in Zambia found that, PCR was the strongest indicator of positive cases with sensitivity and specificity of 100% in comparison to circulating cathodic antigen (CCA) (67% and 60%) and Kato-Katz (KK) (50% and 100%), with high positive and negative predictive values (100%) [16] DNA was extracted from the filtration of urine, in this study PCR results showed 100% sensitivity while the specificity was low (60%.5) with PPV 75.5 %. Lodh et al. found the amplification of species-specific DNA by PCR showed much higher sensitivity (99%–100%) and specificity (100%) compared to KK and haematuria (sensitivity: 76% and 30% respectively) for both schistosome species.[17]. In Western Nigeria the Presence of parasite specific DNA Dra1 fragments both males and

females Sensitivity was 100%; Specificity 100% [14]. Study was performed in Ghana PCR was found sensitivity 100% and specificity was 89% [28]. In our study the specificity and PPV of PCR is lower compared with the studies mentioned above this may be due to the use of the random primers and the cross reaction of the parasite due to less specificity of the primers (RAPD).

5- Conclusion

This study concludes that haematuria is more specific 89.4%, while PCR were 100% sensitive, i contrast ultrasound had lower specificity 50% .PCR is a definitive test for the presence of *S. haematobium* infection. It is well-known that the sensitivity of PCR to diagnosis *S. haematobium* is higher compare with other diagnostic test. The study recommended that PCR was effective test in children as it is difficult to find the eggs in spite of suspicion of infection. Thus different diagnostic techniques could be applied according to the situation of the disease.

6- Consent

Written informed consent was taken from the teacher after explaining the study to the students

7- Ethical approval

Ethical clearance was taken from National Cancer Ethical committee before the study was conducted

8- Acknowledgement

I would like to express my thanks to the staff members of the Quranic school (Khalwa) for facilitating our work. Great thanks to Ministry of Health Gezira State for providing drugs for treatment of infected study subjects.

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