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## **Comparative Study between Molecular Diagnosis and Different Diagnostic Methods of *Schistosoma haematobium* in Gezira State Sudan**

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### **Authors' contributions**

*This work was carried out in collaboration between all authors. Author NG designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author NEK did the practical work and managed the analyses of the study. Authors AOH and AEE did ultrasonography revised and edited the manuscript. All authors read and approved the final manuscript.*

**Original Research Article**

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### **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.

**Objectives:** The aim of this study was to compare the Molecular diagnostic method with different diagnostic methods (urine filtration, haematuria ultrasound).

**Methods:** Eighty three students of Quran School for boys in Radwan village, Gezira state were recruited. The mean age was 12.45±3.2 with a range between 6-20 years. Urine samples were collected. Different diagnostic methods were used. DNA was extracted from the eggs. Polymerase Chain Reaction was done for all samples.

**Results:** Fifty-four percent of the cases were positive with urine filtration method, of whom microhaematuria was seen in 28%, while 61.4% were positive by ultrasound. Using polymerase chain reaction 73.3% of the samples were positive for *Schistosoma haematobium*. The comparison between the different diagnostic methods and gold

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standard urine filtration techniques showed that haematuria had a sensitivity of 42.2% and specificity of 89.4% with a significant association, ( $P < 0.05$ ). No, significant difference was found with ultrasound ( $P > 0.05$ ) which had a sensitivity of 71% and specificity of 50%, while PCR showed a sensitivity of 100% and specificity of 60.5%, showing a significant association between PCR and filtration techniques.

**Conclusion:** The study concluded that PCR was 100% sensitive, while microhaematuria method was highly specific 89%. Ultrasound had lower specificity as compared with other diagnostic tests. It is recommended that different diagnostic techniques should be applied according to the situation of the disease.

**Keywords:** *S. haematobium*; molecular diagnosis; ultrasound; Gezira State; Sudan.

## 1. INTRODUCTION

Schistosomiasis, or bilharziasis, is a parasitic disease that affects man and mammals, in tropical and sub-tropical area and it is 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 cases. 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]. 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 [10]. 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 reinfection [11,12]. Several Studies had been conducted in detecting *S. haematobium* using polymerase chain reaction (PCR) [13-16]. Hamburger and his colleague cloned *S. haematobium* genome repeated sequence, using the DraI repeated sequence [13]. Ibironke et al. [14]. Studied 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. Lodh et al. [15] 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.

The risk of acquiring schistosoma 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* reached up to 70% and that of *S. haematobium* reached 15%. During 1990 [17]. Sudan has seen a serious increase in endemicity and prevalence of both *S. haematobium* and *S. mansoni* infections as a result of expansion in water resource projects, population movements, and unsuccessful control measures and the stop of the Blue Nile Health Project 1980-1990 [18]. 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 was to compare the Molecular Method Polymerase Chain Reaction (PCR) with the other diagnostic methods for the detection of *S. haematobium* in Gezira State Sudan.

## 2. MATERIALS 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 them are 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 supply source, canals is the main source of water, safe water is payable. A total of 83 students from Quranic School of boys (Khalwa) were recruited for this study. All the them were at risk of infection with schistosomiasis due to the daily exposure to infection by swimming and by daily activities in canals water.

### 2.2 Urine Examinations

Ten ml of collected urine were collected the 83 students after informed consent and examined by macro, micro examination technique of each specimen. The urine was then examined by filtration method [19] 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, utilizing the amplification conditions were based on the original method described by [20] with minor modifications.

In this study five primers were used to genotype *S. haematobium* strain (A01, 5'-CAGGCCCTTC-3'; A02, 5'-TGCCGAGCTG-3'; A12, 5'-TCGGCGATAG-3'; A13, 5'-CAGCACCCAC-3'; Y20 ,5'-AGCCGTGGAA-3';) [21]. The reaction was done by using Perkin Elmer thermocycler follows: an initial at 95°C for 5 minutes for 2 cycles, then 30°C for 2 minutes 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

minutes. Controls were utilized as positive and negative to validate the PCR test. The product was then 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 an expert radiologist. 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 obtain for this study from National Cancer Institute Research Ethical committee NCI-REC, University of Gezira. Written consent was obtained from the local leaders. All infected subjects were treated immediately with Praziquantel provided by the Ministry of Health Gezira State.

## 2.6 Statistical Analysis

Data were entered into Microsoft Access 2007 and analyzed the relevant statistical methods, by using SPSS version 16 statistical package. Descriptive, specificity, sensitivity and positive and negative predictive value were calculated. Chi-square cross tabulation was applied using SPSS version16.

## 3. RESULTS

### 3.1 Study Area and Population

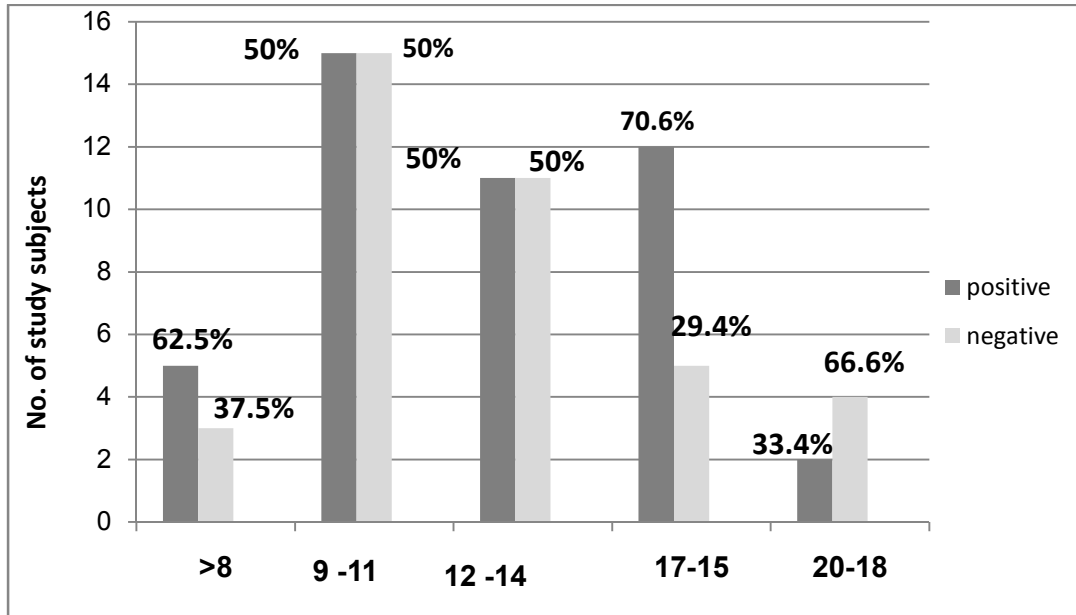
The study was conducted in Roudwan, a village near to Wadmedni city. The eighty three recruited students were males; the mean age was  $12.45 \pm 3.2$  with an age range between 8 - 20 years. The majority of age groups were between 9-11years 36.1% (30/83) as shown in (Table 1).

**Table 1. Shows distribution of the study subjects by age, N=83**

<b>Age range</b>	<b>No. of subjects (%)</b>
>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)

### 3.2 Parasitological Examination for *S. haematobium*

The prevalence of *S. haematobium* among the study participants was 54.2% (45/83) by filtration techniques as shown in (Fig. 1). Diagnosis by microhaematuria method has found that 28% (23/83) were positive for *S. haematobium*.



**Fig. 1. Shows the results of filtration method age groups by filtration technique, N=83**

All the khalwas' students were diagnosed by ultrasound, 85% (51/83) of study subjects had pathological changes. The changes included: 85.9% (49/51) had an abnormal bladder wall thickness, 3.9% (2/51) had one polyp and 56.8% (29/51) had multiple nodularities and 1.9% (1/51) showed wall calcifications of the urinary bladder. Regarding kidneys and ureters, the pathological changes were: 11.73% (22/51) showed an abnormal ureteric thickness and 3.9% (2/51) had echogenic kidneys. The age range between 9-11 year had a high frequency of abnormality in bladder thickness and bladder masses compared with the other age groups, while those between 12-14 years had a higher frequency regarding abnormalities of the kidneys as shown in (Table 2).

### 3.3 Comparison between Different Diagnostic Techniques

The gold standard method for *S. haematobium* diagnosis is the filtration technique. When this method was compared with the 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%. Pearson Chi-square=10.3 and p-value=0.001 as shown in (Tables 3 and 4).

The comparison between the gold standard technique and ultrasound showed that 23 (38.5%) of students were positive for both methods as shown in (Table 3). The sensitivity was 71.1%, specificity was 50% with a positive predictive value of 62.7%. Pearson Chi-square=3.8 and p-value=0.05 (Table 4).

All the positive subjects with filtration technique were found to be positive by PCR, while only 15 (18.1%) of the subjects were negative for filtration method and were positive for PCR as shown in (Table 3). The sensitivity was 100% and specificity was 60.5% with PPV of 75.0%. Pearson Chi-square=37.7 and p-value=0.000 (Table 4).

**Table 2. Ultrasound findings of the study subjects by age group**

Age range	Total (%)	Thickness (%)	Mass		Wall calcification (%)	Ureter thickness (%)	Echogenicity (%)
			One polyp (%)	Multiple (%)			
>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)

**Table 3. Showing the 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. Showing the 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* started to increase in the last decade after the control of *S. mansoni* in Gezira area [23]. Schistosomiasis is affecting males more than females in this area (unpublished report 2011). It may be due to the fact that males are more exposed to the water supply resources than females. Eighty three study subjects were recruited; age range was between 6-20 years. The prevalence of infection with *S. haematobium* was 54.2%. There was an increase of prevalence as compared with a study done in the same area in 2004, where the prevalence of *S. haematobium* was (34%) [23]. This increase of the prevalence rate may be due to environmental changes and ecological factors of snail, in addition to increase of activities of water contact and the irregular control measures. Comparing the prevalence of *S. haematobium* in this study (54.2%), was far less than a study done in Southren Darfur that showed a high prevalence of 81.1% [24]. Another study was done by Mahgoub et al. [25] in Eastern Sudan, showed that 18.0% of the school children were infected with *S. haematobium* [25]. The rapid increase of the prevalence rate in Southern Darfur may be due to the use of control measures in Gezira State and Eastern Sudan. Micro-hematuria had 4.3% false positive results as compared with other methods. This may be due to the fact that some of those had urinary tract 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 for a minimum more than three years. In addition to chronic urinary tract infection which may affect urinary tract abnormality, laboratory technicians sometime cannot see the schistosomiasis eggs during microscopic examination. Because for the very low intensity of infection, this may be the main reason for the false positive results of the PCR method (18.1%). In this study 85% of the study subjects showed abnormal pathological conditions, while in Mali, in a study done in children, has found that 3.4% had irregular bladder wall abnormality [26]. The increase of the abnormality of urinary tract this study, may be due to the fact that our study subjects were more exposed to *Schistosoma* infection, accompanied with the lack of irregular mass chemotherapy which may affect the chronicity of the disease.

Filtration is the gold standard technique and was compared with other diagnostic techniques for the diagnosis of *S. haematobium*; in this study. Our results demonstrated that hematuria had a sensitivity of 42.2% and specificity of 89.4%, with a 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 a study done by Ibironke et al in Nigeria, where they showed a sensitivity of 87.6% and the specificity of 34.7% [14]. This may signify hat hematuria was mostly caused by *S haematobium* egg in our study. Obeng et al in Ghana has assessed micro-hematuria with microscopic examination, they recorded sensitivities of >77% and specificity of 90% [28]. A study from Ethiopia done by Birrie et al found a sensitivity of 80% and a specificity of 96% for hematuria [29]. The low sensitivity of hematuria in this study, when compared with studies from Ghana and Ethiopia, may be due to the fact that infection cause by *S. haematobium* in study area, was in its early stage with a low density of the parasite. In this study 19/51 (37%) were positive by ultrasound but were negative for *S. haematobium* by filtration technique. The sensitivity was 71.1% and specificity was 50% with PPV of (62%). No, significant difference  $P=0.05$  was found as compared with other methods. Hamburger et al has cloned *S. haematobium* from free-swimming cercaria, Dral-based PCR assay was done for genotyping of *S. haematobium*, they found that the repeated sequence in the genome of *S. haematobium* has developed a highly sensitive PCR assay for the detection of this parasite [13]. Many studies confirmed that the sensitivity of PCR is higher than microhaemtuir and ultrasound for *S. haematobium* detection. Lodh et al. [15] 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% respectively) with high positive and negative predictive values (100%) [16]. In this study PCR results showed a sensitivity of 100% a specificity of (60%.5) and PPV of 75.5 %. Lodh et al. [16] found that the amplification of species-specific DNA by PCR has shown much higher sensitivity (99%–100%) and specificity (100%) when compared to KK and haematuria (sensitivity 76% and 30% respectively) for both schistosome species [16]. In Western Nigeria 100 % sensitivity and Specificity was reported for the presence of parasite specific DNA Dra1 fragments in both males and females [13]. Study was performed in Ghana that showed PCR was found to have a sensitivity of 100% and specificity of 89% [28]. In our study the specificity and PPV of PCR was lower as compared with the studies mentioned above. This may be due to the use of random primers and the cross reaction of the parasite due to less the specificity of the primers (RAPD).

## 5. CONCLUSION

This study concluded that haematuria was more specific 89.4%, while PCR was 100% sensitive, in contrast to ultrasound it carried a low specificity 50% .PCR is a definitive test for the presence of *S. haematobium* infection. The study recommended that PCR showed be considered as a gold standard 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.

## CONSENT

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

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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