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Schistosoma haematobium DNA and eggs in urine of patients from Sohag, Egypt

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Abstract

Background: Diagnosis of schistosomiasis depends mainly on stool or urine microscopy for *Schistosoma* egg detection as well as immunoassays. The low sensitivity of these conventional tests makes molecular detection the diagnostic method of choice. The study aimed to detect the molecular prevalence of urine schistosomiasis and evaluate microscopic examination vs. PCR technique for detection of *Schistosoma haematobium* (*S. haematobium*) in urine of patients with suggestive symptoms or previous history of urine schistosomiasis coming from endemic regions.

Results: This cross-sectional study was performed on eighty patients attending the urology clinic of Sohag University Teaching Hospital from August 2016 to July 2018. Socio-demographic data and clinical data were collected. Urine samples from all study individuals were collected and examined microscopically for *S. haematobium* eggs as well as detection of *S. haematobium* DNA of using PCR assay. Microscopic examination and PCR were positive among (68.8%) and (87.5%) of cases, respectively. There was 60% agreement between microscopy and molecular assay. Microscopy was a good test to rule in cases of urine schistosomiasis, with 100% specificity and 100% PPV, but was of limited sensitivity (NPV = 40%) and missed 12.5% of positive cases. Among studied patient variables, only hematuria showed association with urine schistosomiasis with statistical significance.

Conclusion: Urine schistosomiasis was highly prevalent in studied population. Considering the high sensitivity and specificity of PCR, it should be implemented as the test of choice, especially in chronic urinary schistosomiasis with low infection setting. In our study population, patients presenting hematuria were likely to have *S. haematobium*.

Keywords: *Schistosoma haematobium*, PCR, Hematuria, Dysuria, Urine microscopy

Background

Urinary schistosomiasis or bilharziasis caused by *Schistosoma haematobium* (*S. haematobium*) is a blood fluke infection that may cause fatal health sequelae, with significant socio-economic consequences in developing countries (King, Dickman & Tisch, 2005). It occurs in many parts of the world, particularly Middle East, Africa, a few countries in West Asia (Iraq, Yemen, KSA) and Corsica, France, where >600 million individuals live in endemic areas (Gryseels, Polman, Clerinx & Kestens, 2006; Berry et al., 2014). To effectively control the disease, reliable, accurate, precise, specific, and sensitive diagnostic methods are needed for proper detection of *Schistosoma* spp. infection and assessment of the efficacity of treatment in field situations (Wang et al., 2011). The epidemiological assessments of *S. haematobium* burden depend mainly on the microscopic examination of urine, the gold-standard diagnostic method. This method provides an economic and easy technique for determining the concentration of *S. haematobium* eggs in urine specimens (Lengeler, Utzinger & Tanner, 2002). Samples must, however, be collected at suitable or specific times for a good recovery of parasitic eggs and must be processed, with a thorough examination to increase the sensitivity,

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especially with low-intensity infections (Gryseels & De Vlas 1996). Consequently, direct parasitological techniques are often limited by low sensitivity, with cases missed in early or low-intensity infections and when used for the evaluation of treatment (Wang et al., 2011).

Immunodiagnostic techniques are easier to carry out and more sensitive than microscopy. They are a common epidemiological screening tool in low endemicity setting. *Schistosoma spp.* immunoassays are of reduced specificity, making them unreliable for diagnosis of new infections and the assessment of treatment (Gawish, Bayoumy, Abd El Raheem, Abo-hashim & El-Badry, 2021; Rabello, Pontes & Dias-Neto, 2002). Polymerase chain reaction (PCR) techniques, due to their high sensitivity and specificity, were successfully used to detect DNA from many parasites including *Schistosoma spp.* (Obeng et al., 2008).

The current study aimed to assess the true (molecular) prevalence of *S. haematobium* in Sohag University Hospital, Governorate of Sohag, using the PCR assay to detect DNA of *S. haematobium* in urine vs. urine microscopy and to evaluate the diagnostic performance of microscopy and also to determine the associated variable that can be a predicator for the occurrence of urinary schistosomiasis.

Methods

This cross-sectional study was performed for detection of *S. haematobium* for patients attending the Outpatient Clinic of Sohag University Hospital from August 2016 to July 2018, who had suggestive symptoms, previous history of urine schistosomiasis, or history of contact with water canals, or received *Schistosoma* treatment and were coming from endemic regions.

Related patient socio-demographic and clinical data were collected from each patient. Single urine sample (at least 60 ml of mid-morning urine) was collected from each patient in labeled clean dry containers and transported to the laboratory of the Faculty of Medicine (Assiut), Al-Azhar University. The samples were subjected to complete urine analysis and detection of *Schistosoma spp.* eggs through direct microscopic examination of a centrifuged sedimented sample. Another part of the specimens was filtered on Whatman filter paper No.3 and used for the molecular assay.

All molecular work was done in Lab of Molecular Medical Parasitology (LMMP), Medical Parasitology Department, Kasr Al-Ainy Faculty of Medicine, Cairo University, Cairo, Egypt. After filtration of urine sample, all collected paper discs were opened and dried in fly-proof boxes. Dry paper discs were packed individually in plastic sleeves with desiccant and maintained at – 20 °C. A 2-cm in diameter circular central portion of each filter paper was excised and divided by sterile scissors into four quadrants (Ibironke, Phillips, Garba, Lamine & Shiff, 2011) and then placed into Eppendorf tube; then, genomic DNA was extracted using the Qiagen QIAamp mini-kit (Qiagen Sciences, MD) following the manufacturer's instructions. DNA was tested for purity and concentration spectrophotometrically using NanoDrop.

A multiplex PCR targets the *Schistosoma*-specific COX-1 gene sequences of both *S. haematobium* and *S. mansoni* which were done for identification of specific *Schistosoma spp.* DNA in urine specimens (Ten Hove et al., 2008). Each PCR run was done in duplicate and included negative control and positive *S. haematobium* and *S. mansoni* control samples (supplied by Prof. Ayman A. El-Badry Lab., LMMP). The PCR condition was done following Ten Hove et al (2008). The multiplex PCR products were examined on agarose gel and were visualized under ultraviolet light (Fig. 1; Table 1).

Primers' titration to calculate the optimum primer concentrations was done using increased stepwise concentrations 50, 100, 200 and 400 nM each. Calculation of the melting temperature (Tm) of the primers was done using oligonucleotide properties calculator (OligoCalc) (http://basic.northwestern.edu/biotools/) according to Kibbe (2007). Finally, gradient annealing temperatures in a gradient thermocycler (from 58 to 70 °C) were tested.

The collected data of the study were tabulated and analyzed statistically using version 26 of Software Statistical Computer Package (SPSS) (SPSS Inc, USA). The mean

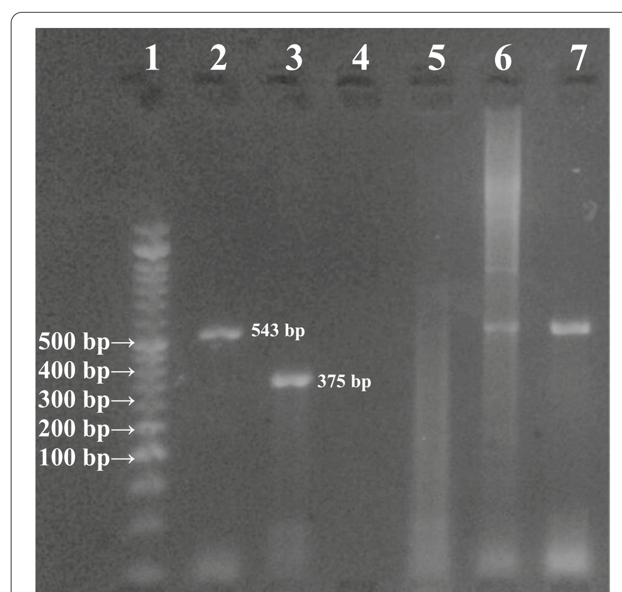

Fig. 1 Agarose gel stained with ethidium bromide for *Schistosoma spp.* PCR product: Lane 1; molecular weight marker (50 bp), Lane 2; positive control *S. haematobium* (543 bp), Lane 3; positive control *S. mansoni* (375 bp), Lane 4; negative control, Lane 5; negative sample, Lanes 6 and 7; positive samples (543 bp)

Table 1 Used primers and their sequences

Primers	Sequence	Expected product size (bp)	Annealing temp. (°C)
Common *Schistosoma spp* forward primer	5′-TTT TTT GGT CAT CCT GAG GTG TAT-3′		
S. haematobium reverse primer	5′-TGA TAA TCA ATG ACC CTG CAA TAA-3′	543	58
S. mansoni reverse primer	5′-TGC AGA TAA AGC CAC CCC TGT G-3′	375	

Table 2 Diagnostic performance of microscopic examination and PCR assay for detection of urine schistosomiasis

		PCR		
		Positive	Negative	Total
Microscopic examination	Positive	55 (68.8%)	0 (0%)	55 (68.8%)
	Negative	15 (18.7%)	10 (12.5%)	25 (31.2%)
	Total	70 (87.5%)	10 (12.5%)	80 (100%)

and standard deviation were calculated for quantitative data. The Student's (t) test was used to compare between two means; the one-way analysis of variance (ANOVA test) was used to compare between more than two means. The number and percent distribution were calculated for qualitative data. Independent t test was used for mean comparison. Fisher's exact test was used to compare the frequency between groups. Chi (χ^2) square was the test of significance. Significance was when P value is <0.05 for interpretation of results of tests of significance.

Oral and written consent was taken from all patients. The study aim was explained to all study patients before the specimens and data collection, and the privacy of all collected data was assured. The work was approved by Al-Azhar Assiut University ethical committee.

Table 3 Diagnostic yield and accuracy of microscopy using PCR as standard reference test

Measure	Value (%)
Sensitivity	78.6
Specificity	100
Positive predictive value (PPV)	100
Negative predictive value (NPV)	40
Accuracy	81.3
Kappa agreement	57.8

Results

Among the 80 patients, *S. haematobium* DNA was found in the urine specimens for 70 patients (87.5%), while microscopy failed to detect *Schistosoma* eggs for 15 patients (21.4%), as presented in Table 2. There was 60% agreement between microscopy and molecular assay (Table 3). Microscopy was a good test to rule in cases of urine schistosomiasis with 100% specificity and 100% PPV, but was of limited sensitivity (NPV = 40%) and missed 12.5% of positive cases (Table 3).

The mean age of study individuals was 7.3 (±2 years). Out of the examined patients, 70% were males and 30% were females. Rural inhabitants represented 83.8%, while 16.2% were urban. The most common symptoms were hematuria (58.8%), dysuria (55%) and suprapubic pain (12.5%), respectively (Tables 4 and 5). Results of distribution and association between urine schistosomiasis and age, socio-demographic and symptoms of study population using PCR are presented in Tables 4 and 5. Among studied variables, only hematuria showed association with urine schistosomiasis with statistical significance.

Discussion

Schistosoma haematobium infection continues to be a clinically important challenge (Kabiru et al., 2007). In our study, urine schistosomiasis was highly prevalent. Out of 80 patients suspected to be infected with *S. haematobium*, 55 cases were microscopically positive (which represents about 68.8%) and 25 cases were microscopically negative (which represents about 31.2%). On the other hand, when using the PCR technique, 70 cases were positive and 10 cases were negative. We used filter paper to

Table 4 The age distribution among study population

Variable	Category	PCR positive	PCR negative	Total $n = 80$	P value*
Age (year)	Mean ± SD	7.30 ± 2.1	7.00 ± 1.5	7.26 ± 2.0	0.583

* P value less than 0.5 is statistically significant

Table 5 The frequency of socio-demographic and symptoms of study population

Variable	Category	PCR positive ($n=70$)	PCR negative ($n=10$)	Total ($n=80$)	*P* value*
Sex	Male	20 (28.6%)	4 (40%)	24 (30%)	0.345
	Female	50 (71.4%)	6 (60%)	56 (70%)	
Residence	Urban	12 (17.1%)	1 (10%)	13 (16.2%)	0.488
	Rural	58 (82.9%)	9 (90%)	67 (83.8%)	
Symptoms					
Hematuria	Yes	36 (51.4%)	0 (0%)	36 (45%)	0.002*
	No	34 (48.6%)	10 (100%)	44 (55%)	
Dysuria	Yes	28 (40%)	5 (50%)	33 (41.3%)	0.393
	No	42 (60%)	5 (50%)	47 (58.8%)	
Suprapubic pain	Yes	62 (88.6%)	8 (80%)	70 (87.5%)	0.364
	No	8 (11.4%)	2 (20%)	10 (12.5%)	

P value less than 0.5 is statistically significant

collect urine for DNA extraction and amplification; Ibironke et al. (2011) concluded that using urine filter paper to collect and transfer urine from field was sufficiently sensitive to identify cryptic and low infections, which is missed by egg microscopy.

In our study, school children were the most affected age-group. A study was done by Mahgoub et al. (2010) in Eastern Sudan, which reported that 18.0% of school children had urinary schistosomiasis. Similar high prevalence of urinary schistosomiasis in school children was reported from in Ethiopia (Degarege et al., 2015) and Senegal (Senghor et al., 2014). In contrast, a cross-sectional study was done by Ndassi, Anchang-Kimbi, Sumbele, Wepnje and Kimbi (2019) reporting that the 5–15 years age group was less associated with excretion of *Schistosoma* ova regardless of continuous contact with the water streams. The decrease in prevalence of schistosomiasis in school children may be due to mass treatment for this age group (Ghazy, Tahoun, Abdo, El-Badry, & Hamdy, 2021).

Regarding sex, males represented about two-thirds (70%), while females represented about one third (30%) of the total samples. This can be due to more frequent exposure of males to cercaria-infected water canals because of the various outdoor activities of males, such as the social habits of bathing in infected water streams. Our finding agrees with Ibironke, Koukounari, Asaolu, Moustaki and Shiff (2012) and Afifi, Ahmed, Sulieman and Pengsakul (2016) who reported that the prevalence of *S. haematobium* infection in males was 48.83% and in females was 26.66%. In contrast, other studies indicate that the prevalence of infection in females was higher than in males (Ekpo, Laja-Deile, Oluwole, Sam-Wobo & Mafiana, 2010), while yet other studies indicate that there were no differences in the prevalence of infection between males and females (Degarege et al., 2015; Kavana, 2018). The difference in the prevalence rates of schistosomiasis between males and females may be affected by peculiar ecology, degree of contact with contaminated water canals and the extent of exposure to cercariae (Ndassi et al., 2019).

Regarding residence, 83.8% of the cases were inhabitants from rural areas while about 16.2% were residents from urban areas. Our finding is in agreement with studies performed by Kavana (2018) and Ndassi et al. (2019). This can be explained by the low socio-economic level and behavior of people living in the rural areas and their continuous water-contact activities, inadequate sanitation, and shortage of safe water supply. Ndassi et al. (2019) reported that increased water contact activities such as bathing, farming and fishing were correlated with increased risk of infection.

The most common symptoms among the studied cases were hematuria, presented in 47 cases (58.8%), followed by dysuria in 44 cases (55%) and suprapubic pain in 10 cases (12.5%).

Microscopic examination revealed that 68.8% of the studied cases were positive and 31.2% were negative. The number of positive cases increased to 87.5% using the PCR technique. This can be explained by the ability of PCR assay to detect very low levels of *Schistosoma spp.* DNA. The low sensitivity of microscopy is attributable to its inability to detect the intermittent excretion of eggs and very low infection intensity levels (Cavalcanti, Silva, Peralta, Barreto & Peralta, 2013). In addition, following treatment, *Schistosoma* re-infections are less severe and microscopy become less effective and miss the detection of *Schistosoma* eggs in asymptomatic carriers. These asymptomatic carriers become the source of persistent transmission (King, 2010). The validity criteria of microscopic examination versus PCR of the studied cohort for diagnosis of *S. haematobium*

were of limited sensitivity (79%) and perfect specificity (100%). Many studies confirm that the sensitivity of PCR is higher than microscopic examination for the detection of *S. haematobium* eggs (Hessler et al., 2017; Lodh, Mwansa, Mutengo & Shiff, 2013), with high positive predictive values and negative predictive values (100%). Further studies found that using PCR to amplify species-specific *Schistosoma*-DNA gave higher sensitivity (99%–100%) and perfect specificity (100%) for both schistosome species (Lodh et al., 2013; Lodh, Naples, Bosompem, Quartey & Shiff, 2014).

Mohammed, Ismail and Omar (2017) performed PCR on 50 urine specimens, with 100% for both sensitivity and specificity. Another cross-sectional study by Hessler et al. (2017) in Chongwe and Siavonga Districts in Zambia, carried out PCR on filtered urine specimens of 111 school children (7–15 years) with 100% for both sensitivity and specificity, compared to poor sensitivity (7%) in microscopy of urine filtration.

Effiong et al. (2017) in Wamakko, Nigeria, carried out another cross-sectional study on urine specimens of 50 primary school children. Eggs of *S. haematobium* were identified only in the urine of five children, while *S. haematobium Dra*1 DNA fragments were identified in the urine of three children. The PCR sensitivity was 60.0%, and specificity was 95.92%. These results do not mean that the performed PCR assay is not sensitive; it can be because the sensitivity, specificity, as well as PPV and NPV, are affected by the low disease prevalence in the population and small sample size.

Conclusion

Urine schistosomiasis was highly prevalent in our cohort of individuals studied. Considering the high sensitivity and specificity of PCR, it should be the test of choice, especially in cases of chronic urinary schistosomiasis in low infection settings. In our study population, patients presenting hematuria are likely to have *S. haematobium*.

Abbreviations

NPV: Negative predictive value; PCR: Polymerase chain reaction; PPV: Positive predictive value; *Schistosoma haematobium*: *S. haematobium*; *Schistosoma mansoni*: *S. mansoni*.

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Not applicable.

Authors' contributions

BAA has contributed to work design, analysis, drafted the work, and approved the submitted version. ASB has contributed to the conception, interpretation of data, drafted the work, revised the work, and approved the submitted version. MSE has contributed to the work design, data analysis, revised the work and approved the submitted version. NMA has contributed to the work design, data analysis, revised the work and approved the submitted version. AAE has contributed to the conception, work design, supervised the work, data analysis, data interpretation, drafted the work, revised the work and approved the submitted version. All authors read and approved the final manuscript.

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Availability of data and materials

All data that support the study are included in the manuscript.

Declarations

Ethics approval and consent to participate

The study was approved by the Ethical Board of the Faculty of Medicine, Al- Azhar University, Cairo, Egypt. Oral and written consent was taken from all patients. The study aim was explained to all study patients before the specimens and data collection, and the privacy of all collected data was assured.

Consent for publication

All authors have read and approved the final version of the report and the manuscript.

Competing of interests

The authors declared that they neither have competing interests nor received fund.

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