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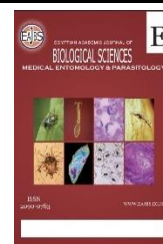
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## Role of Clinical and Parasitological data in Diagnosis of Chronic Urinary Schistosomiasis in comparison with other techniques

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### ABSTRACT

The gold standard for diagnosis of *schistosomiasis haematobium* is a microscopic examination of urine for parasite eggs. However, direct detection of eggs is difficult among people who have chronic infections. The aim of the current study was to detect the role of clinical and parasitological examination in the diagnosis of *S. haematobium* in comparison with PCR in chronic patients. Materials and methods: This study was conducted on 115 urine samples; 60 samples were collected from patients with cancer bladder, 35 samples from cases of urinary schistosomiasis, and 20 samples from a healthy control. Samples enrolled in the study were subjected to patient socio-demographic history, clinical data, and the collection of histopathological reports (in the case of cancer bladder patients). All urine samples were subjected to parasitological examination for the detection of *S. haematobium* eggs. Multiplex PCR was used for the detection of DNA fragments of *S. haematobium* in urine. Results: By multiplex PCR, it was revealed that 5 cases (8.3%) were positive for *S. haematobium*, while 55 cases (91.7%) were negative. PCR-positive cases were in the age of 44- 67 years. Diagnosis of *S. haematobium* infection in symptomatic (dysuria & haematuria) suspected bilharziasis patients by using the traditional parasitological examination for detection of eggs in urine revealed absolute diagnostic efficacy (100%). *S. haematobium* eggs were detected in the examined patients of age group from 15 to over 40 years. Conclusion: Using clinical and direct parasitological methods in association with history taking could be enough for the diagnosis of *S. haematobium*, especially in aged patients.

### INTRODUCTION

Schistosomiasis is the second most common complicating parasitic disease after malaria, affecting almost 240 million people in developing countries. In Africa, *Schistosoma haematobium* (*S. haematobium*) predominants and manifests as a urogenital disease (Bamgbola, 2014).

Urinary schistosomiasis is a disease that dates to Egyptian pharaohs who were among the first to detect the disease. Haematuria was the main symptom as pointed out in the Egyptian papyri (El-Aal *et al.*, 2015).

Schistosomiasis complications are due to immunologic reactions to eggs of *Schistosoma* trapped in tissues and the formation of granuloma. *S. haematobium* infection has a strong connection with cancer bladder, leading to severe and chronic morbidity (Brindley and Hotez, 2013).

The gold standard for diagnosis of *schistosomiasis haematobium* is a microscopic examination of urine for parasite eggs. However, direct detection of eggs is difficult among people who have a chronic infection due to inflammation and fibrosis (Ibironke *et al.*, 2011). Pathologists may proceed to tissue biopsy where *Schistosoma* eggs can be seen. Inadequate diagnosis is a serious problem, especially in cases of chronic infection with *S. haematobium* especially when it has been associated with damage to the urinary tract and the development of bladder squamous cell carcinoma (Shiff *et al.*, 2010).

An alternative to the direct detection of eggs by microscopy is the detection of *Schistosoma* DNA fragments (Ibironke *et al.*, 2011).

The aim of the current study was to detect the role of clinical and parasitological examination in the diagnosis of urinary Schistosomiasis in comparison with PCR in chronically infected patients.

#### MATERIALS AND METHODS

A cross-sectional study was performed on cancer bladder patients as well as patients suffering from symptoms of urinary schistosomiasis as well as control healthy people. The study was carried out in the period from May 2015 to July 2019 as a part of a Master thesis entitled "Detection of *Schistosoma haematobium* DNA in the urine of patients with cancer bladder in Egypt"

(Ali *et al.* 2016).

#### Inspected Samples:

1- Fresh urine samples from 60 cancer bladder patients (admitted to the inpatient of the Urology department, Theodor Bilharz institute) were

collected. The samples were from both sexes and age groups from 35 – over 65 years old with previous old symptoms suggestive of urinary schistosomiasis (dysuria or haematuria), some of them received anti-bilharzial treatment and most of them gave a history of contact with water canal.

2- Thirty-five samples from patients attending outpatient Urology clinic at Kasr alainy of both sexes, ages ranging from 15 to 45 years. Patients included were complaining of hematuria and dysuria (with a previous history suggestive of bilharziasis whether received anti-bilharzial treatment or gave a history of contact with a water canal).

3- Twenty urine samples from healthy patients of ages ranging from 25 to 50 years were also enrolled in the study as a control.

Urine samples included in the present study were examined microscopically and those with parasitic infections other than *S. haematobium* were excluded.

Samples enrolled in the study were subjected to:

- Related patient socio-demographic, clinical data and collection of histopathological reports (cancer bladder patients).
- Parasitological examination of urine samples for detection of *S. haematobium* eggs.
- Detection of *S. haematobium* DNA fragments in urine using PCR.

#### Parasitological Examination:

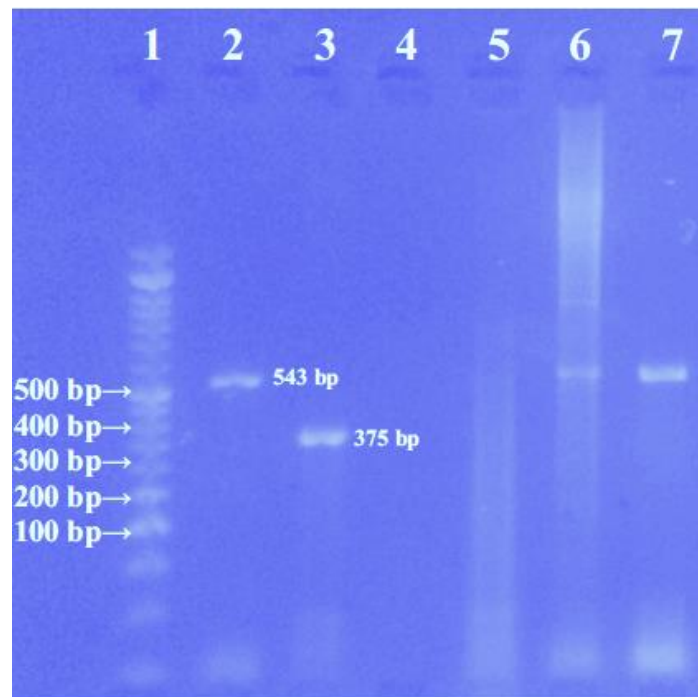
A urine sample was collected from each patient in a clean dry labeled container and transported to the laboratory of the Faculty of Medicine, Cairo University. Complete urine analysis was done on all urine samples. All samples were centrifuged and the sediment was examined microscopically for eggs of *Schistosoma spp.* Another part of the urine samples was filtered using Whatman filter paper No.3 and was used for the molecular assay.

**PCR:**

The molecular work was carried out in the Lab of Molecular Medical Parasitology (LMMP), Medical Parasitology Department, Faculty of Medicine, Cairo University. At first urine samples were filtered, paper discs were collected then opened and left to dry in closed boxes. The paper discs were put in plastic sleeves with desiccant and stored at  $-20^{\circ}\text{C}$ . 2 cm circular portion from the center of each filter paper was cut and divided into 4 quadrants by sterile scissors (Ibironke *et al.*, 2011) and put into an Eppendorf tube. Genomic DNA was extracted using the Qiagen QIAamp mini-kit (Qiagen Sciences, MD) following the

instructions of the manufacturer. DNA was examined spectrophotometrically for concentration and purity by the use of NanoDrop.

A multiplex PCR directed to the *Schistosoma*-specific COX-1 gene sequences of *S. haematobium* for detection of specific DNA of *Schistosoma spp.* in urine samples (Ten Hove *et al.*, 2021). Each PCR run was performed in duplicate and negative control and positive *S. haematobium* control samples were used. The PCR condition was carried out according to Ten Hove *et al.* (2008). The multiplex PCR products were examined by the use of agarose gel. Ultraviolet light was used to be visualized (Fig.1).



**Fig. 1:** Ethidium bromide-stained agarose gel contained PCR products of the tested samples.; Molecular weight marker (L 1), L 2; *S. haematobium* +ve control, *S. Mansoni* +ve control (L.3), (L.4) -ve control, (L.5) -ve sample, (L.6&L. 7) +ve samples.

Common *Schistosoma spp* forward primer sequence was 5'-TTT TTT GGT CAT CCT GAG GTG TAT-3'. While *S. haematobium* reverse primer used was 5'-TGA TAA TCA ATG ACC CTG CAA TAA-3' and the expected product size was 543 bp and the annealing temperature was  $58^{\circ}\text{C}$ . *S.*

*mansoni* reverses primer sequence was 5'-TGC AGA TAA AGC CAC CCC TGT G-3' and the expected product size was 375bp.

Titration of Primers to measure the optimum primer concentrations was done using increased concentrations of 50, 100,

200 and 400 nM each. Measurement of the melting temperature (T<sub>m</sub>) of the primers was carried out using an oligonucleotide properties calculator (OligoCalc) (Kibbe, 2007). At last, gradient annealing temperatures in a gradient thermocycler (from 58 to 70 °C) were tried.

#### Ethical Considerations:

Approval of the study design was obtained from the research committee unit, Faculty of Medicine, Cairo university. The patients were informed verbally about the purpose of the study and consent was taken from them before the collection of samples.

#### RESULTS

The present study analyzed urine samples collected from 60 cancer bladder patients; some of them had a previous history of *S.haematobium* infection, had

egg granuloma as illustrated by histopathological reports, or previously received anti-bilharziasis treatment and some of them gave a history of contact with water canals. Parasitological examination of this group revealed no *S. haematobium* eggs in their urine. On the other hand, screening them using PCR succeeded in the detection of specific *S. haematobium* DNA in only 5 patients (8.33%). Five cases of SCC and 9 cases of TCC had egg granuloma by histopathological reports. Detection of DNA in urine samples by PCR revealed only 2 cases in SCC and 3 cases in TCC. Moreover, 3 cases who were PCR positive for *S. haematobium* received anti-bilharzial treatment and 2 PCR positive cases gave a history of contact with a water canal (Table 1).

**Table 1:** Rate of Infection by *S.haematobium* in Cancer bladder patients in relation to some data collected by history.

Patients History	Tested samples	Diagnosis of infection by				Histopathology			
		<i>S. haematobium</i> in Urine		PCR		SCC with egg granuloma		TCC with egg granuloma	
		No +Ve	%	No +Ve	%	No +Ve	%	No +Ve	%
Cancer bladder	60	0	0	5.0	8.33%	5	20%	9	25.7%
SCC with or without egg granuloma	25	0	0	2	8%				
TCC with or without egg granuloma	35	0	0	3	8.5%				
Previously treated	17	0	0	3	17.6%	0	0%	0	0%
Contact with water	38	0	0	2	5.26%	0	0%	2	2.22%

In the present study, all cancer patients who were PCR positive for *S. haematobium* were males. Of the PCR-positive cancer patients, one case was at the age of 44 years. 4 cases in the age group

55-67 years. By using histopathological reports, those with egg granuloma were 14 patients among SCC and TCC in the age group 55-67 years (Table 2)

**Table 2:** Relation between patient age and presence of *S. haematobium* DNA in urine and its Pathological changes in Cancer bladder patients.

Age of patients	Age of PCR & Histopathology Positive cases	
	No. of positive cases by PCR	No. of Patients with SCC or TCC showing egg granuloma
44 years	1	1(TCC)
55 years	1	2 (SCC)
62 years	1	4 (TCC)
65years	1	3 (SCC)
67 years	1	4(TCC)

On the contrary, diagnosis of *S. haematobium* infection in symptomatic (dysuria & haematuria) suspected bilharziasis patients (received previous anti-bilharzial treatment or gave a history of contact with water canals) by using the

traditional parasitological examination for detection of eggs in urine revealed absolute diagnostic efficacy (100%).

*S. haematobium* eggs were diagnosed in the examined patients of age groups from 15 to over 40 years old (Table: 3).

**Table 3:** Rate of Infection by *S. haematobium* in symptomatic patients (dysuria & haematuria) in relation to age.

Patients age groups	Tested samples	Diagnosis of infection by			
		<i>S. haematobium</i> egg in Urine		PCR positive	
		No +Ve	%	No +Ve	%
15-20 Years	13	13	100%	13	100%
20 - 30years	12	12	100%	12	100%
30- 40 years	10	10	100%	10	100%
Healthy control	20	0	0	0	0

None of these patients needed to perform histopathological investigations. All of these patients were farmers or gave a history of contact with water canals or received anti- bilharzial treatment.

**DISCUSSION**

Schistosomiasis is a chronic infection with significant residual morbidity and is of considerable public health value, with socioeconomic impacts (Weerakoon *et al.*, 2015)

Diagnosis of Parasites by detection of their specific DNA using PCR technology is considered one of the best specific sensitive and accurate diagnostic techniques (Siqueira *et al.*, 2021). PCR cannot be considered a field applicable test being non-economic, and time-consuming in comparison with the easy diagnostic parasitological methods, especially in the case of schistosomiasis (Ajibola *et al.*, 2018). PCR can be used to detect DNA of *S. haematobium* in old chronic infections

with no eggs in urine, however, this infection can be suspected simply from the history of patients.

In the present study regarding 60 cancer bladder patients, PCR succeeded in the diagnosis of *S. hematobium* in old aged cancer patients by a percentage that didn't exceed 8.33% (5 out of 60 patients). This was in contrast to Méabed *et al.* (2014) who illustrated that PCR was a significant diagnostic tool for the diagnosis of urinary schistosomiasis. Regarding the sensitivity of the molecular methods in schistosomiasis diagnosis, it has been documented by previous studies. Eighty-nine urine samples from school-age children in Niger were analysed by PCR by Ibrinke *et al.* (2011). The PCR method showed higher sensitivity

(57.3 %) compared to that observed by microscopic examination for eggs (49.4 %).

Cnops *et al.* (2013) analysed 110 urine samples with the Real-Time PCR directed toward *Schistosoma haematobium*-specific DraI sequence. A positive PCR signal was taken in 14 urine samples of which 7 were positive for *S. haematobium* eggs by using the microscope.

The low percentage of schistosomiasis detected in the current study might be due to a lack of value for determination of the cause of cancer especially if these patients had a previous history of bilharziasis. The present study noted that clinical and parasitological inspection appeared more fast, easy and more economic.

In the present study, out of the 17 patients with a history of anti-bilharzial treatment, 3 were found positive by PCR for *S. haematobium* DNA in urine. This means that not all the cases who received treatment had eliminated the parasite DNA and higher drug doses might be required. Similar results were obtained by Downs *et al.* (2013) who conducted a six-month cohort study to assess the efficacy of treatment with single dose praziquantel. 33 women with *S. haematobium* infection were included. After treatment, eggs disappeared from women's urine and cervical samples, however, the PCR method detected DNA of *Schistosoma* in 8 of the recruited women. In addition, He *et al.* (2016) found that a single dose of praziquantel didn't eliminate *S. haematobium* DNA in all the recruited patients.

Histopathological data (biopsy reports) of the study population revealed that 5 cases had SCC with egg granuloma (20%), and 9 cases had TCC with egg granuloma (25.7%).

One of the most important observations in the present study was that the cancer bladder patients that had *S. haematobium* DNA by PCR were all of the old age (44-67 years). This agreed with El-Aal *et al.* (2015) who conducted a study on 46 schistosomiasis *haematobium* patients, 24 were chronic cases with bladder cancer

and the mean age among patients was 62.5 years. On the other hand, Rambau *et al.* (2013) found that the mean age of patients with bladder cancer was 54.3 years.

By using histopathological data of these patients, 14 of these cancer patients showed bilharzial egg granuloma. All of these patients were old and complained of cancer symptoms rather than complaining of schistosomiasis and most of them gave history suggestive of a previous infection with schistosomiasis.

The authors of the present study noted that proper management of cancer bladder patients regardless of the cause might be of more important value than spending money in seeking the causative agents, especially with a previous history of schistosomiasis.

On the other hand, Zaghoul *et al.*, 2020 highlighted the association between cancer bladder and urinary schistosomiasis.

Similarly, an ultrasound examination study on schistosomiasis patients were done by Shiff *et al.* (2006) showed that numerous individuals had severe bladder damage without *S. haematobium* eggs in the urine. Shiff (2012) conducted community-based surveys and concluded that a strong inflammatory response from the host occurs in response to *Schistosoma* egg, metaplasia and finally the development of cancer. Regarding the 35 urine samples of symptomatic patients suspected previously infected with schistosomiasis all young patients who complained of haematuria and dysuria were harbouring *S. haematobium* eggs in urine with absolute diagnostic efficacy by using the traditional economic easy applicable parasitological techniques. The same efficacy was revealed by using PCR.

#### Conclusion

The current study concluded that identification of the cause of cancer bladder in *S. haematobium* infected patients could be expected from the history of the patients without the need for expensive time-consuming PCR. In this respect, using clinical and direct parasitological methods

in association with history taking could be enough for the diagnosis of *S. haematobium*, especially in aged patients. Surgical intervention would be required regardless of the cause in most cancer-infected patients. The study recommended using clinical and repeated parasitological examination for the diagnosis of Schistosomiasis being an easier and more economic diagnostic technique than PCR.

#### Conflict of Interests:

The authors declare that there is no conflict of interests regarding the publication of this paper.

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