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**ORIGINAL RESEARCH ARTICLE**

# Prevalence of urinary schistosomiasis in Egypt: result of a study in high versus low endemic governorates

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**Abstract.** Schistosomiasis is one of the most prevalent parasitic infections worldwide. Establishment of sensitive assays to detect schistosomiasis in situations with low transmission is crucial. The present study aimed to estimate the current status of *S. haematobium* infection in different Egyptian localities. Urine samples were collected from 960 asymptomatic men from Great Cairo (low-endemic) and Fayoum (endemic) governorates. Samples were examined microscopically for *S. hematobium* eggs followed by Dip stick test and one step test to detect *Schistosoma* circulating cathodic antigen (CCA). *Schistosoma hematobium* eggs were detected in eight cases from Fayoum and one case from Great Cairo. The overall occurrence of *S. haematobium* infection was 0.93% (0.2% in Great Cairo & 1.66% in Fayoum). CCA antigen was positive in 11 cases and haematuria by dip stick test was detected in the 9 microscopically positive cases. Microscopy remains the most appropriate technique for detection of *S. haematobium* infection and haematuria by dipstick test is helpful for initial screening of urinary schistosomiasis cases.

**Keywords:** *Schistosoma haematobium;* Circulating cathodic antigen; Dip stick; Haematuria.

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## Introduction

*Schistosoma haematobium* was discovered by Theodore Bilharz in 1851 during autopsy at Kasr El Ainy hospital (Bilharz, 1853). In 1915, the life cycle of the *Schistosome* parasite was first described by Leiper (1915). Schistosomiasis comes after malaria among parasitic diseases as regards the number of people infected and those at risk of infection (Chitsulo et al., 2000). Schistosomiasis is a major public health problem, with 200 million people infected worldwide and 700 million people residing in areas of infection risk. Although five species of waterborne trematodes in the genus *Schistosoma* cause human infection, the most important are *Schistosoma mansoni* and *Schistosoma haematobium*, and the majority of cases occur in sub-Saharan Africa (Gryseels et al., 2006). The pathology of schistosomiasis results from egg-mediated immune response and

granuloma formation followed by fibrosis leading to obstructive manifestations in the gastrointestinal tract in case of intestinal schistosomiasis and in the urinary tract in the case of *S. haematobium*. However, eggs can be disseminated to other organs, e.g., the brain, the spinal cord, genital organs, and the lungs leading to severe morbidity (Gryseels et al., 2006). Squamous cell carcinoma is one of the serious complications of urinary schistosomiasis in Egypt and North Africa (Fedewa et al., 2009).

In 50% of newly acquired *Schistosoma* infections, patients remain asymptomatic. However, asymptomatic carriers can potentially transmit schistosomiasis in areas that lack adequate sanitation as in many areas of our country highlighting great importance of early detection of infection (Whitty et al., 2000).

Current estimates of the prevalence of schistosomiasis depend on the use of well-established diagnostic tests. The accepted diagnostic standard of schistosomiasis is evidence of viable eggs in urine *(S.haematobium),* faeces *(S. japonicum & S.mansoni)* or tissue biopsies. Microscopic examination can quantify the intensity of the *S.haematobium* infection, however it is relatively insensitive especially in situations involving low level infections (De Vlas et al., 1997; Colley et al., 2013). In endemic areas, where past *Schistosoma* infections and polyparasitism are frequent, serological testing requires high specificity to avoid false-positive results (Tsang and Yung, 1991).

Assessment of *Schistosoma* circulating cathodic antigen (CCA) derived from adult worms in urine of infected individuals using ELISA has been proposed as an alternative technique to overcome those problems inherent in serological testing (Van Etten et al., 1994; Van Lieshout et al., 2000). A newly developed one-step reagent strip test for the detection of *Schistosoma* CCA in urine was evaluated by Van Dam and other researchers who reported high sensitivity and specificity of *Schistosoma* CCA for the epidemiological study of schistosomiasis (Van Dam et al., 2004).

The one step *Schistosoma* circulating cathodic antigen (CCA) test was reconsidered in this work aiming to discover *S. haematobium* asymptomatic infection among men seeking pre-employment routine investigation, taking the direct parasitological examination as the gold standard.

## Materials and methods

*Study design*

A cross-sectional study was conducted from May 2014 to July 2015. A total of 960 participants from different age groups were included in the present study. The participants were selected from asymptomatic men attending Kasr Al Ainy outpatient clinic and Fayoum University Hospital outpatient clinic for pre-employment assessment. The participants were selected from those living in 2 governorates in Egypt; Great Cairo as low endemic area and Fayoum as an endemic area for *S. haematobium* infection. Informed consent was obtained from each participant. All data were confidential for the research use only. Initial assessment included collection of demographic information by questionnaire, followed by a standard medical history and examination. Individuals were divided by age range (18–25, 26–35, 36–45, +45), previous to the sample collection.

*Collection of samples*

Clean plastic cups were distributed to each individual to provide (10–15 ml) of fresh midstream urine samples (Weber et al., 1967). Each container was labeled and transferred to the Research Unit in the Medical Parasitology Department, Kasr Al-Ainy. Collected urine samples were kept in the dark till examined to avoid hatching of miracidia.

*Dip stick test*

Each urine sample was examined for protein and blood (an indirect indicator of *S.haematobium* infection) by the dip stick method (Combur 10 Test®, Roche Diagnostics Gmbh, D-68298 Mannheim, Germany). After dipping the dipstick in the urine sample (for one second), it was left for a minute on a bench.

Then, it was read as negative, 1+, 2+,3+, 4+ according to the manufacturer's instructions by comparing the color reaction in the test area to the matching standard labeled on the urinalysis dipstick bottle.

*Parasitological examination*

Urine samples were microscopically examined for the presence of *S. hematobium* eggs. Detection and counting of eggs in urine samples were performed using the urine filtration method. Concisely, urine samples were vigorously shaken, and 10 ml of well mixed urine of each sample were pushed *via* a 13-mm diameter small-meshed filter (20 µm) (Sefar AG; Heiden, Switzerland). Each filter was placed on a microscope slide and examined using a light compound microscope at 10X magnification. The number of *S. haematobium* eggs was recorded and expressed as number of eggs/10 ml of urine (Cheesbrough, 2006).

*Immunological test*

Urine samples were tested for the presence of circulating cathodic antigen using a commercially available lateral flow cassette assay (Rapid Medical Diagnostics, Egypt). The assay was performed according to manufacturer instructions. Briefly, all reagents were brought to room temperature before performing the test. After opening the one-step pouch and placing the CCA strip test on a flat surface, one drop of fresh urine was transferred to the circular well of the test cassette till completely absorbed into the specimen pad. Then, one drop of buffer was added to the circular well. After 20 minutes result was read from the test zone band, compared to the control zone pink band and recorded as negative or positive.

*Statistical analysis*

Results were collected, tabulated and statistically analyzed using the statistical package SPSS version 12. Data were tabulated as mean and standard deviation (SD) for quantitative variables and percent for qualitative variables. *P* values <0.05 were considered as statistically significant.

## Results

The present study was carried out on 960 asymptomatic men of different age groups attending outpatient clinics in Kasr Al-Ainy Hospital and Fayoun University Hospital. Eggs of *S. haematobium* were observed by microscopy in 9 urine samples out of 960 collected samples in the present study. Eight cases out of 480 collected samples from Fayoum governorate representing 0.83% of the total collected urine samples and one case in Great Cairo (0.1%) out of 480 collected samples were positive for *S. haematobium* eggs. Microscopy was considered the gold standard method for the diagnosis of *S. haematobium* infection in the present study. Therefore, the overall occurrence of *S. haematobium* infection in collected samples from both governorates was 0.93%. The infection ranged from 0.2% in Great Cairo to 1.66% in Fayoum with statistically significant difference (P value <0.05) (table 1).

**Table 1.** *S. haematobium* eggs in urine samples from 2 governorates in Egypt

| Governorate | Number of *S. haematobium* positive samples | Percent |
|---|---|---|
| **Great Cairo (480 urine samples)** | 1 | 0.2 % |
| **Fayoum (480 urine samples)** | 8 | 1.66% |
| **Total (960 urine samples)** | 9 | 0.93% |

Statistically significant higher occurrence of infection was reported among the first and second age groups (18-25 and 26-35 years old) (P value <0.05), where 5 cases (55.6%) and 4 cases (44.4%) were positive respectively. No infection was reported in the other age groups. All positive cases gave history of living in ezbas (ezbas are small villages that lack safe water supply, people use canal water for drinking and in summer they swim in canal water as no swimming pools are available ) and no cases were from large villages. The average intensity of infection was below 10 eggs per 10 ml of urine and ranged from 4 to 10 eggs per 10 ml of urine. All detected eggs were light in colour, viable and three of the observed eggs showed

flickering of the excretory flame cells. Fully developed miracidia were seen in 2 urine samples followed by hatching. Concerning macroscopic appearance, urine samples proved positive for *S. haematobium* eggs were cloudy and 5 urine samples were reddish brown in colour.

In the present study haematuria was observed in 25 urine samples including the 9 cases which were positive for *S. haematobium* by microscopy. Proteinuria and bacteriuria were checked by observing colour changes in nitrite and leukocyte testing areas on urine strip used in addition to detection of motile bacteria while examining the urine samples by the light microscope. Accordingly, proteinuria and bacteriuria were detected in 5 samples out of 9 positive samples for *S. haematobium*.

As regards the immunological method, the antigen test was positive in 7 cases out of the 9 positive cases for *S. haematobium* diagnosed by microscopy. The total positive cases detected by the antigen test were 11, of which only seven were also diagnosed by microscopy.

All positive cases for *S. haematobium* infection diagnosed by microscopy were recorded and reported to the corresponding clinic for further treatment while cases that were positive for CCA or dipstick method were informed by the results for further investigation and interpretation of results to achieve the proper diagnosis.

## Discussion

During the past 20 years, much progress in combating schistosomiasis has been achieved based on World Health Organization (WHO) recommendation strategies. Remarkable decline in the prevalence and morbidity of the disease was achieved in many endemic countries (Lammie et al., 2006; King, 2009). Many countries of the EMR (Eastern Mediterranean Region) specifically Egypt, Iraq, Syria, Libya, Oman and Saudi Arabia have now reached low schistosomiasis endemicity. For instance, the overall prevalence of schistosomiasis in Egypt was about 40% in 1967 before the national control program initiated by WHO. Urinary schistosomiasis was clustered in Upper Egypt with prevalence of infection ranging from 4.8 % in Qena to 13.7 % in Fayoum (Abdel-Wahab et al., 2000; El-Khoby et al., 2000). In 2006, due to different control measures, the overall prevalence fell down to <3%. However, there are still hot spot transmission foci with prevalence rate about 10%. Therefore, lack of well-structured prevention, control and elimination programs could lead to emergence or resurgence of the controlled disease. Schistosomiasis has been eliminated in Iran, Lebanon, Morocco and Tunisia with absence of new recorded cases in the past few years. Moreover, WHO adopted a resolution calling on countries in low transmission areas, to sustain successful control activities to eradicate schistosomiasis (WHO, 2007). In these conditions, where eradication of schistosomiasis is aimed for, detection of cases may pose a problem because the frequently used diagnostic methods may lack the necessary sensitivity to accurately determine the prevalence of schistosomiasis or parasite burden (Utzinger et al., 2005; WHO, 2005). Therefore, WHO asked for sensitive assays for active surveillance, particularly in situations with no or very low transmission (WHO, 2007).

In the present work, prevalence of schistosomiasis was assessed in 2 localities; Fayoum governorate as endemic area and Great Cairo region as low endemic area. The diagnostic strategies of urinary schistosomiasis traditionally rely on the detection of eggs in urine and immunological techniques based on antigen and/or antibody detection. In our study, eggs of *S. haematobium* were detected in 9 urine samples (0.93%) out of 960 collected urine samples.

The prevalence of *S. haematobium* in the present study is less than the prevalence reported by King et al. (1982) who studied schistosomiasis *haematobium* in six rural villages of Qena governorate and reported a prevalence of 37.1%. Similarly, El Khoby et al. (2000) found that the prevalences of *S.mansoni* and *S. haematobium* in Ismailia governorate were 42.9% and 3.5% respectively, Kafr-El-Sheikh; 39.1% and 2.5%, Gharbia: 37.7% and 2.06%, Monofia: 28.49% and 5.5%, Qalubia: 17.47% and 1.53%, Fayoum: 4.3% and 9.95%,

Minya: 1.04% and 8.47%, Assiut: 0.42% and 6.63% and Qena: 0.44% and 7.04%.

More recently, Zaher et al. (2011) studied schistosomiasis over 5 years (2005-2010) and reported the presence of *S. haematobium* eggs in urine samples of 6 cases (0.02%) and *S.mansoni* eggs in stool samples of 99 cases (0.33%) out of the 30,000 outpatients.

In the present study, the intensity of infection ranged from 4 to 10 eggs per 10 ml of urine and according to Cheesbrough (2006), this count is considered mild urinary schistosomiasis while urine samples with egg count more than 50 eggs per 10 ml were categorized as heavy infection. All infected cases gave history of contact with canal water with lack of pure water supply or good health services. In the same context, Abdel-Wahab et al. (2000) and El-Khoby et al. (2000) reported that extreme poverty, unawareness of the risks, inadequacy or total lack of public health facilities plus the unsanitary conditions, are all factors contributing to the risk of infection (Fenwick et al., 2003).

In the present study, haematuria dipstick test was helpful in the initial screening of the collected urine samples for *S. haematobium*, as haematuria was confirmed by the test in 25 urine samples including the 9 samples positive for *S. haematobium* by microscopy. Recent studies showed that semi-quantitative reading of dipstick test correlated well with intensity of *S. haematobium* infection and ultrasound pathology (Koukounari et al. 2006; Koukounari et al. 2007). Proteinuria and bacteruria were detected in 5 samples out of 9 positive for *S.haematobium*. Proteinuria is frequently present in schistosomiasis *haematobium* infection and bacteriuria may accompany it also (Cheesbrough, 2006).

Testing micro-haematuria and proteinuria as an alternative to urine examination for initial screening in the field for *S. haematobium* infection are cheap, rapid and easy to apply (Mott et al., 1985; Brooker et al., 2009). They also have no technical requirements and are less influenced by the circadian production of *Schistosome* eggs (Murare et al., 1987). Moreover, some studies have shown that the sensitivity of these strips is higher than that of urine filtration (French et al., 2007; Robinson et al., 2009). Such features make these strips suitable for initial mapping and screening of urogenital schistosomiasis in the field. However, microhaematuria and proteinuria are non-specific signs that could also result from other ailments such as urogenital infection, malignancy, immune system disorders, metabolic disorders and trauma (Cheesbrough, 2006). Therefore, results should be interpreted against the background of risk for schistosomiasis, as well as any other signs and symptoms that could be indicative of other diseases.

Concerning the immunological method, CCA antigen test was positive in 7 cases out of the 9 positive cases for *S. haematobium* infection diagnosed by microscopy. The total positive cases detected by the antigen test were 11, out of these positive cases, only seven were also diagnosed by microscopy. El-Morshedy et al. (1996) and Van Dam et al. (2004) suggested that antigen based assays, as circulating cathodic antigen (CCA) detection in urine and circulating anodic antigen (CAA) in serum, have proven to be a helpful and field applicable method. In *S. mansoni* infection, a positive association between urine CCA assay and microscopic examination of stool samples for *S.mansoni* eggs to indicate the intensity of *S.mansoni* infections was reported (Stothard et al., 2006; Legesse and Erko, 2007). As CCA antigens are genus cross-specific, the test does not discriminate between urinary and/or intestinal schistosomiasis which, from a control perspective, has the advantage of capturing, but not discriminating, both forms of disease in a single test (Stothard et al., 2006).

Ayele et al. (2008) studied *S. haematobium* infection in a total of 206 urine samples collected from Hassoba Elementary school children, Afar, Ethiopia, a low *S. haematobium* endemic setting. They detected *S. haematobium* eggs in 47 urine samples which were negative by CCA strip while 38 egg-negative samples were found positive by CCA strip. They concluded that urine CCA could not be used as an alternative to microscopic examination of urine samples to diagnose urinary schistosomiasis.

The total positive cases detected by the antigen test in the present study were 11, out of these positive cases, only seven were diagnosed by microscopy. Missed infections may be due to the fact that only one urine sample was examined for each participant in our study. According to Cheesbrough (2006), more than one urine sample should be examined for each individual to to increase the sensitivity of microscopy and avoid false negative results by microscopy.

## Conclusion

The present findings revealed a decrease in the prevalence of schistosomiasis that may be explained by the present policy of schistosomiasis control in Egypt. Microscopy is still the most appropriate technique for detection of *S. haematobium* infection. Further studies in different governorates in Egypt are needed and examination of multiple samples to increase the sensitivity of microscopy is recommended to reflect the real prevalence of schistosomiasis. Inclusion of haematuria dipstick test in the initial screening for *S.haematobium* and monitoring process of human mass chemotherapy programs would be of great help. Further studies for antigen detection tests are also needed and should be compared with the microscopic examination for both *S. haematobium* and *S. mansoni*.

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