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CIRCULATING CATHODIC ANTIGEN VERSUS MICROSCOPY FOR DIAGNOSING URINARY SCHISTOSOMIASIS AMONG CHILDREN, IN SOHAG UNIVERSITY HOSPITALS

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Abstract

Urinary schistosomiasis caused by *Schistosoma haematobium* constitutes a major public health problem in many tropical and sub-tropical countries. Rapid diagnostic tests are needed for the implementation and monitoring of national schistosomiasis control programs.

The study estimated prevalence and risk factors of *S. haematobium* by the circulating cathodic antigen test (POC-CCA) versus microscopic urine examinations. A cross-sectional study was conducted on 100 outpatient children aged 3 to 15 years attended Sohag University Hospitals. Demographic data and risk factors were collected using a structured questionnaire. Urine samples were examined by microscopic examination techniques (sedimentation centrifugation and Nucleopore filtration methods) for detection of *S. haematobium* eggs and by a commercially available cassette test POC-CCA, for detection of *S. haematobium* circulating cathodic antigens.

The results showed that *S. haematobium* infected children as indicated by microscopy was 23%. The study reported increasing age (OR=6.9-8.3), male (OR= 3.5), living in rural areas (OR=4.1), exposures to canal water (OR=26.4), history of schistosomiasis (OR= 3.3) and history of burning micturition (OR= 7.4) or hematuria (OR= 10.1) as significant risk factors**.** Using microscopy as the gold standard for *S. haematobium* detection, sensitivity, specificity, PPV & NPV of POC-CCA tests, were 56.5%, 92.2%, 68.4% & 87.7% respectively with an accuracy 84% and area under curve (AUC) was 0.744. In light cases, the POC CCA detected 52.6%, but in heavy cases it increased to 75% without significant difference ($P < 0.412$).

Keywords: Schistosomiasis *haematobium*, Microscope, Circulating cathodic antigen test.

Introduction

Schistosomiasis is a risky disease caused by *Schistosoma* species which severely affects the health and socio-economic well-being of more than 230 million people in 78 of the worldwide **(**McManus *et al*, 2018). This was mainly true in Sub-Saharan with highest burden (90%) of schistosomiasis by *S. mansoni* & *S. haematobium* (WHO, 2010). Schistosomiasis control focused on mass drug administration (MDA), with the effective oral praziquantel as the cornerstone (Olveda *et al*, 2013).

Before the implementation of MDA campaigns, infection prevalence should be assessed to guide program decision making. This is usually achieved through surveys based on the use of traditional parasitological methods (urine filtration for *S. haematobium* and Kato-Katz thick smears for *S. mansoni* infections) (Nausch *et al*, 2014). These methods are useful for detecting active infection, exhibit a high level of specificity however, these are insensitive and often miss light-intensity infections (Cavalcanti *et al,* 2013). This increased efforts to develop more sensitive tests as point of- care test for the circulating cathodic antigen or POC-CCA (Coulibaly *et al,* 2013)

The CCA rapid diagnostic test is an immunochromatographic test detects the schistosome antigens (proteoglycans), as released from feeding worms, in host urine (Kremsner *et al,* 1993) and can differentiate between past and active infections, as the circulating antigens are probably present only with active infection (Doenhoff, 2004). Deribe *et al.* (2011) reported that *S. hematobium* was endemic in Alsafia and Abuselala South Darfur, on the Southern Egyptian borders, with a

higher prevalence among older children.

In Sub Saharan Africa, the circulating cathodic antigen point-of care-test (POC-CCA) was more accurate in detections of *S. mansoni* than microscopic Kato-Katz technique, but less known accuracy in diagnosing *S. haematobium* (Sanneh *et al*, 2017).

The present study aimed to evaluate the diagnostic performance of the commercially available (POC-CCA) test compared to traditional microscopic examination as golden test and to identify *S. haematobium* infection risk factors among outpatient children, Sohag University Hospitals.

Materials and Methods

Ethical considerations: The study was app roved by the Medical Research Ethical Committee, Faculty of Medicine, Sohag University, with the IRB registration No (Soh-Med-22-02-31). Written informed consents were obtained from parents or guardians of the children, after clarifying the aims and procedures of the study.

Study area: This study was carried out in Sohag Governorate, Upper Egypt. It covers the Nile Valley extent with a total area of 1547 km^2, with estimated 5,706,510 people.

Study design: A cross-sectional study was conducted on 100 outpatient children aged 3 to 15 years attended Sohag University Hospitals from November 2021 to end of September 2022. They were subjected to a detailed medical questionnaire including name, age, sex, and residence, exposures to water canal, history of previous schistosomiasis, and history of burning micturition.

Laboratory examination: Early urine samples were collected from each child in labeled covered cartoon boxes and transferred to Medical Parasitology laboratory to be examined by sedimentation centrifugation test (Cheesbrough, 2006). Eggs counted/10ml of urine were classified as 1-50 light infected children and more than 50 for heavy one.

A total of 10ml urine was tested by the POC-CCA cassette test (Rapid Medical Diagnostics; Pretoria, South Africa) for detection of an active *S. haematobium* infection according to the manufacturer's procedures. Two drops of fresh urine were added to each cassette. The test result was reported between 20 and 25min. after adding the sample. The control line became pink to valid the test; any other color was considered invalid and recorded as either negative, trace or positive. Also, weak pink line was considered as positive (Ashton *et al*, 2011).

Statistical analysis: Data were analyzed by IBM SPSS Statistics for Windows version 25.0, expressed as mean ±SD, number and percentage. Chi-square and Fisher's exact tests were used to compare appropriate qualitative variables. Odds ratios along with 95% confidence interval (95% CI) estimated the association strength between schistosomiasis infection and risk factors. Microscopic examinations of urine samples were used as the golded standard to assess sensitivity, specificity, PPV, NPP, accuracy and area under the curve (AUC) of POC-CCA test. The agreement between microscopic examination and the test was assessed by Cohen's kappa test as follows: k = 0 or no agreement; k= 0-0.20 or poor agreement; k= 0.21-0.40 or fair agreement; k= 0.41-0.60 or moderate agreement; k = 0.61-0.80 or substantial agreement; and k = 0.81-1.0 or perfect agreement. The P-value was considered significant if it was < 0.05.

Results

Of one hundred children, 57% were males and 43% females with aged ranged from 3 to 15 (9.1±3.1) years, 68% were in rural areas and 32% in urban ones. *S. haematobium* infected children by microscopy was 23/100 (23%). Risk factors for *S. haematobium* infection included increasing age (OR= 6.9-8.3, P < 0.04-0.02), male (OR= 3.5, P< 0.01), living in rural areas (OR= 4.1, P < 0.02), exposure to water canal (OR= 26.4, P < 0.0001), history of schistosomiasis (OR= 3.3, P < 0.01) and burning micturition (OR= 7.4, P< 0.0001) or hematuria (OR= 10.1, P < 0.0001).

Positive infections by POC-CCA test were 19/100(19%), with sensitivity of 56.5%, spe-

cificity of 92.2%, PPV of 68.4%, NPV of 87.7%, accuracy of 84%, and AUC of 0.744. Kappa value was 0.519 or fair agreement between microscopy & POC-CCA with high significance (P< 0.0001). POC-CCA, its value increased with infection intensity, in light cases was 52.6% and in heavy increased to 75% without significance (P < 0.412).

Details were given in tables (1, 2, & 3) and figures (1 & 2).

Table 1: Distribution of *S. haematobium* infection among 100 children and risk factors

Variations	No. Examined	Infected (%)	Odds ratio (95%)	P value
Age: 3-5years	21	1 (4.8%)	1	
:6-10	35	9 (25.7%)	6.9 (0.8-59.2)	<0.04*
:11-15	44	13 (29.5%)	8.3 (1.01-69.1)	<0.02*
Male	57	18 (31.6%)	3.5 (1.1-10.4)	
Female	43	5 (11.6%)	1	<0.01*
Residence				
Rural	68	20 (29.4 %)	4.1(1.1-14.7)	<0.02*
Urban	32	3 (9.3%)	1	
Exposure to water canal				
Yes	57	22 (38.6%)	26.4 (3.3-205.8)	<0.0001*
No	43	1 (2.3%)	1	
Past infection				
Yes	31	12 (38.7%)	3.3 (1.2-8.7)	<0.01*
No	69	11 (15.9 %)	1	
Burning micturition				
Yes	49	19 (38.8%)	7.4 (2.3-24.01)	<0.0001*
No	51	4 (7.8 %)	1	
Hematuria				
Yes	27	15 (55.6 %)	10.1(3.5-29.2)	<0.0001*
No	73	8 (11%)	1	

*P < 0.05 significant.

Table 2: Efficacy of POC-CCA cassette tests in comparison to microscopic examination techniques as golden test

POC-CCA tests	Microscopic results			Sensitivity	Specificity	PPV	NPP	Accuracy	AUC
	Positive	Negative	Total						
Positive	13	6	19	56.5%	92.2%	68.4%	87.7%	84%	0.744
Negative	10	71	81						
Total	23	77	100						

P value = .0001*, Kappa = 0.519**

Table 3: Relation between *S. haematobium* by POC CCA and microscopic intensity of infections.

POC-CCA tests	Microscope light (1-50eggs /10ml)		Microscope heavy (>50 eggs/10 ml)		Total	P value
	No.	%	No	%		0.412
Positive	10	52.6	3	75	13	
Negative	9	47.4	1	25	10	
Total	19		4		23	

Discussion

No doubt, the schistosomiasis is still a problem among risky outdoor playing children, and improved diagnostics, especially the development of inexpensive POC test is a major focus of the new 2021–2030 roadmap to eliminate neglected tropical diseases (WHO, 2020).

In the present study, prevalence of *S. haematobium* was 23% by microscope. This agreed with El-Kady *et al***.** (2020), who in Qena reported 24% *S. haematobium* infection, but was higher than by Dyab *et al*. (2021), who in Aswan reported 4.7% infections. El Baz *et al.* (2003) in El Fayoum Governorate reported that *S. haematobium* was 7.9%, but without *S. mansoni*. Barakat (2013) reported that in the Middle and Upper Egypt, there was consistent reduction in the prevalence of *S. haematobium* except in the governorates of Sohag, Qena, and Aswan but, foci of *S. mansoni* were in the governorates of Giza, Fayoum, El-Menia and Assiut.

In the present study, the 10-15 years aged children had a significantly high S. *haematobium* infection rate followed by the 5-10 years aged ones and the least infection rate was among those aged 3-5 years (OR=6.9, 8.3 respectively). The results agreed with Yameny (2017) who found that age group

from 11-20 years showed higher prevalence (10.3%) than those aged < 11 years (8.8%). This may be explained by the fact that the elder age group was more likely to contact infections from the environment.

In the present study, the male children were more infected than females (OR=3.5). This agreed by El-Khoby *et al* (2000), who reported that male was a risk factor for *S haematobium* infection (OR=1.9), spending more time outdoors, playing and swimming in water canal than females.

The current study revealed the patients in rural areas were 4.1 times more prone to the infection than those living in urban areas. Which was supported by Hammam *et al* (2000) who revealed that the prevalence of *S. haematobium* among children was significantly associated with residing in rural regions in Qena, Upper Egypt (OR=1.49).

In the present study, children exposed to water canal, burning micturition and hematuria were schistosomiasis patients compared to their counter parts (OR= 26.4, 3.3, 7.4 & 10.1 respectively). This agreed with El-Khoby *et al* (2000) in nine governorates of Egypt reported risk factors for *S. haematobium* infection (OR=2.1, 1.9, 1.5 & 3.6 respectively), and with Hammam *et al*. (2000) in Qena, who reported (OR= 3.1, 2.6, 1.5 & 3.6 respectively).

In the present study, diagnostic performance of POC CCA versus microscopic examination showed that sensitivity, specificity, PPV & NPV of tests were 56.5%, 92.2%, 68.4% & 87.7% respectively with an accuracy of 84% & AUC of 0.744. These data agreed with Obeng *et al* (2008) in Ghana, Ayele *et al* (2008) in Ethiopia, Ashton *et al* (2011) in Sudan, Sanneh *et al* (2017) in Gambia and Yameny (2017) in Egypt, they reported POC CCA gave low sensitivity versus microscopy (41%, 52%, 36.8, 47.7%, & 56%) respectively and low specificity (91%, 63.8%,78.9%, 75.8%, & 76%) respectively. But, this disagreed with Midzi *et al* (2009) in Zimbabwe and El-Ghareeb *et al* (2016) in Giza; they detected *S. haematobium* with higher sensitivity 88.2%, 88.2%, and specificity 95.8%, 96.4% respectively.

Ashton *et al* (2011) reported that CCA test was more sensitive in detecting *S. mansoni* than *S. haematobium* 89.1% versus 36.8% respectively. Also, Colley *et al*. (2013) and Straily *et al*. (2022) in schistosomiasis endemic area found that POC-CCA tests were more sensitive than Kato-Katz thick smears in diagnosing *S. mansoni*; 86% versus 62% respectively among school children.

In the present study, the sensitivity of POC CCA increased with intensity of infection and the sensitivity of the test in heavy infected patients (75%) was more than the sensitivity in patients with light infection (52.6%) however it was not significant (P< 0.412). The present data agreed with Ayele *et al* (2008) in Ethiopia and Ashton *et al*. (2011) in Sudan who found that most false-negative POC CCA results were for *S. haematobium* light intensity and the strong positive test were for heavy infection intensity. Besides, Yameny (2017) reported that the POC CCA sensitivity in urine heavily infected patients (66.7%) was more than that in patients with light infection (54.5%), but without significant (P= 0.575). The false-negative data may be attributed to fact that the female worms, main source of circulating cathodic antigen (Grenfell *et al*, 2013), and in experimentally infected baboon model, CCA was only detectable with more than 50 worms **(**Cai *et al*, 2021). Also, false-positive samples may be due to daily fluctuation in egg excretion (Berhe *et al* 2004) and during acute or active infection, immature worms produce worm antigens (CCA) before eggs excretion, with positive circulating antigen assay without eggs in urine (Lambertucci *et al*, 2013).

Conclusions

The POC-CCA assay compared to microscopic examination showed low sensitive and costly expensive tool to diagnose urinary schistosomiasis. Positive children were successfully treated as out-patients.

Authors' declarations: They reported that

they neither have conflict of interest nor received any funds**.**

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Explanation of figures

Fig. 1: (ROC) curve analysis of POC CCA versus microscopy in diagnosis of *S. haematobium* showed AUC = (0.744).

Fig 2: POC-CCA tests: A= positive, B= trace and C= negative.