J Parasit Dis (Oct-Dec 2016) 40(4):1193–1198
DOI 10.1007/s12639-015-0648-2

ORIGINAL ARTICLE

Circulating cathodic antigen cassette test versus haematuria strip test in diagnosis of urinary schistosomiasis

Azza S. El-Ghareeb · Ghada S. Abd El Motaleb · Nevien Maher Waked · Nancy Osman Hany Kamel · Nagwa Shaban Aly

Received: 24 May 2014 / Accepted: 20 January 2015 / Published online: 14 February 2015

Abstract Urinary schistosomiasis caused by *Schistosoma haematobium* constitutes a major public health problem in many tropical and sub-tropical countries. This study was conducted to evaluate circulating cathodic antigen cassette test and haematuria strip test for detection of *S. haematobium* in urine samples and to evaluate their screening performance among the study population. Microscopy was used as a gold standard. A total of 600 urine samples were examined by microscopy for detection of *S. haematobium* eggs, screened for microhaematuria using Self-Stik reagent strips and screened for circulating cathodic antigen (CCA) using the urine-CCA cassette test. The specificity of CCA, microhaematuria and macrohaematuria was 96.4, 40.6 and 31.2 % respectively while the sensitivity was 88.2, 99.3 and 100 % respectively which was statistically significant ($P < 0.001$). These findings suggest that using of urine-CCA cassette test in diagnosis of urinary schistosomiasis is highly specific (96.4 %) compared with the highly sensitive haematuria strip test (100 %). The degree of agreement between microscopic examination and CCA detection was 99.3 % with highly statistically significant difference ($P < 0.001$). The combination of two techniques could potentially use for screening and mapping of S. *haematobium* infection.

Keywords *Schistosoma haematobium* · Haematuria · Circulating cathodic antigen

A. S. El-Ghareeb (✉) · N. S. Aly
Department of Parasitology, Benha Faculty of Medicine, Benha University, Benha, Egypt
e-mail: dr_azzaelghareeb@yahoo.com

N. S. Aly
e-mail: drnagwashaban@gmail.com

G. S. Abd El Motaleb
Department of Pediatrics, Benha Faculty of Medicine, Benha University, Benha, Egypt
e-mail: ghad2a@yahoo.com

N. M. Waked
Department of Pediatrics, Faculty of Medicine, October 6 University, 6th of October City, Egypt
e-mail: nevien.waked.95@gmail.com

N. Osman Hany Kamel
Department of Parasitology, Faculty of Medicine, October 6 University, 6th of October City, Egypt
e-mail: nancyosman7@hotmail.com

Introduction

Schistosomiasis remains a significant public health problem worldwide, with an estimated 207 million people infected (Steinmann et al. 2006; Utzinger et al. 2009). *Schistosoma. haematobium* infections can be diagnosed by several approaches, including detection of schistosome eggs in urine and rapid tests such as urine reagent strips for detection of microhaematuria (Mott et al. 1985). Haematuria, a common symptom of urogenital schistosomiasis, occurs when *S. haematobium* eggs induce inflammation and blood vessel rupture (Coon 2005). From the time of Hippocrates into the urine examination was thought to be an important diagnostic procedure (Koss and Hoda 2012). Reagent dipsticks are available to test for haematuria, which is can be used as a proxy for infection (Bosompem et al. 2004). The CCA rapid diagnostic test is an immunochromatographic dipstick that detects the presence *Schistosome* antigens (proteoglycans), as released from feeding worms, in host urine (Kremsner et al. 1993). Prior applications of this test in school-aged children have yielded diagnostic sensitivities of between 56.3 and 96.3 %

and specificities as high as 93.9 % (Legesse and Erko 2008). As there is no standard reference test for urogenital schistosomiasis (Koukounari et al. 2009).

The present study aimed to evaluate the diagnostic performance of the commercially available urine circulating cathodic antigen (CCA) cassette test and haematuria strip test for diagnosis of urinary schistosomiasis in school-aged children.

Subjects and methods

Study type cross sectional analytic study

Study place this study was conducted in Benha city, Qualyobia Governorate and October city, Giza Governorate, Egypt. Samples were examined in Department of Parasitology, Faculty of Medicine, Benha University.

Study group a total of 600 urine samples were collected from children aged 5–12 years attending ten primary schools.

Collection of urine samples a sample of about 20 ml contains both mid stream and last drops of urine were collected in 50 ml capacity clean plastic container labeled with his/her name and date of collection. Samples were obtained between 10.00 am and 2.00 pm. Samples with visible haematuria were noted. The specimens were placed in a cold box with ice packs, immediately after collection. They were processed 1–2 h of collection. In situations where delay in transportation of specimens to laboratory was inevitable, ordinary household bleach was added to the urine samples (ratio; 1 ml bleach: 50 ml urine) to preserve any *Schistosome* ova present (Cheesbrough 1998).

Samples examination each sample was examined by:

(1) Physical examination for, aspect and colour to detect macrohaematuria.

(2) Microscopic examination for *S. haematobium* ova (Baker et al. 1985).
10 ml of the urine was centrifuged at 1500 r.p.m for 5 min. The supernatant was discarded to leave sediment which was transferred to the centre of a clean grease-free glass slide to which was added a cover slip. This was mounted on a light microscope and examined at 10× objective to identify *S. haematobium* ova

(3) Filtration of urine (Houmsou et al. 2011)
10 ml of urine was taken and filtered through an 8-um polycarbonate membrane in a filter holder with the help of a forceps. The filter holder was placed on a slid. A drop of lugol's Iodine was added and the slide was examined under microscope using 10× and 40× objective lenses. The number of eggs was counted per 10 ml of urine and intensities of infection were classified as 1–10, 11–49 and >50 eggs for light, moderate and heavy infections respectively.

(4) Examination for microhaematuria (Mott et al. 1985):

(a) A Self-StikR reagent strips (Chung Do.Pharm.Co. Ltd. Seoul, Korea) was carefully dipped into the bottle containing urine for 5 s. The resulting change in colour of the strip was compared with manufacturer's colour chart to estimate the amount of blood in the urine.

(b) Circulating cathodic antigen (CCA) urine cassette test (Ashton et al. 2011): CCA tests (Rapid Medical Diagnostics; Pretoria, South Africa) were conducted according to the manufacturer's instructions on single urine samples collected from 96 pupils (this group contains both cases positive by microscopic examination & those with activity related to water lakes). CCA results were graded according to test band strength, where weak positive was defined by the control band being darker than test band, while a strong positive was defined by a test band darker or the same colour as the control band.

Statistical analysis

Data were tabulated and analyzed using SPSS version 16 software (SPSS Inc, Chicago, ILL Company). Fisher's exact test and student "*t*" tests were used as tests of significance. Kappa test was used to assess the agreement degree between microscopic and serological methods. The accepted level of significance in this work was stated at 0.05 ($P < 0.05$ was considered significant).

Table 1 Gross haematuria among the studied samples

	S. haematobium egg by microscopic ex		Total
	Negative	Positive	
Haematuria			
No			
Count	568	22	590
% Within *S. haematobium* egg	100.0 %	68.8 %	98.3 %
Yes			
Count	0	10	10
% Within *S. haematobium* egg	0.0 %	31.2 %	1.7 %
Total			
Count	568	32	600
% Within *S. haematobium* egg	100.0 %	100.0 %	100.0 %

* Fisher's exact $P < 0.001$

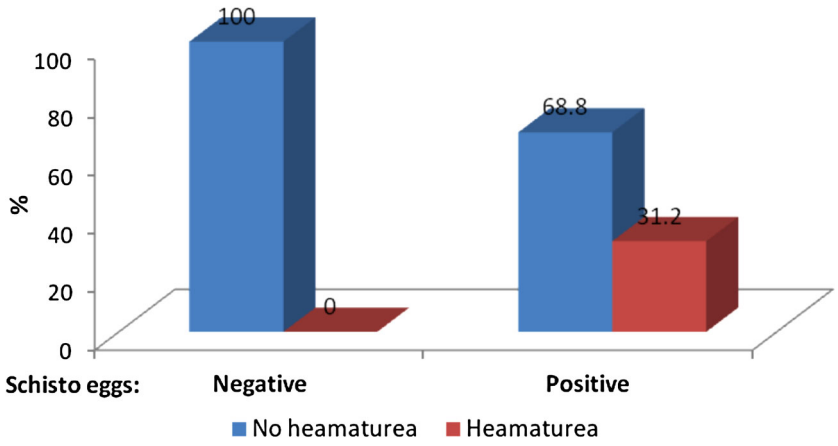

Fig. 1 Gross haematuria among the studied samples

Table 2 Microhaematuria among the studied sample

	Schisto egg		Total
	Negative	Positive	
Heamaturia			
No			
Count	568	19	587
% Within schisto egg	100.0 %	59.4 %	97.8 %
Yes			
Count	0	13	13
% Within schisto egg	0.0 %	40.6 %	2.2 %
Total			
Count	568	32	600
% Within schisto egg	100.0 %	100.0 %	100.0 %

* Fisher's exact $P < 0.001$

Results and discussion

Thirty-two cases (5.3 %) out of 600 cases were positive for *S. haematobium* eggs in urine by microscopic examination. Regarding haematuria, gross haematuria was highly statistically significant ($P < 0.001$*) positive in 10 (31.2 %) of *S. haematobium* positive cases. While, the remaining 22 (68.8 %) microscopic positive cases had no gross haematuria in urine. It was found that all negative *Schistosoma* cases had no gross haematuria (Table 1; Fig. 1). Microhaematuria as detected by reagent strips was highly statistically significant ($P < 0.001$*) positive in 13 (40.6 %) of *S. haematobium* positive cases, while, the remaining 19 (59.4 %) microscopic positive cases had no microhaematuria. It was found that there were 4 cases (0.7 %) microhaematuria positive and *Schistosoma* negative (Table 2; Fig. 2). Sensitivity and specificity of gross and micrhaematuria were 31.2 & 40.6 and 100 & 99.3 % respectively (Table 3; Fig. 3). The absence of microhaematuria in some infected individuals (false negative) could be the result of new infection in which tissues of the urinary bladder and kidney have not been damaged yet (Houmsou et al. 2011).

Variation in sensitivity and specificity of microhaematuria in urinary schistosomiasis has been reported in several studies conducted in different African settings. They have been varied from 41 to 93 % and from 67 to 99 % for

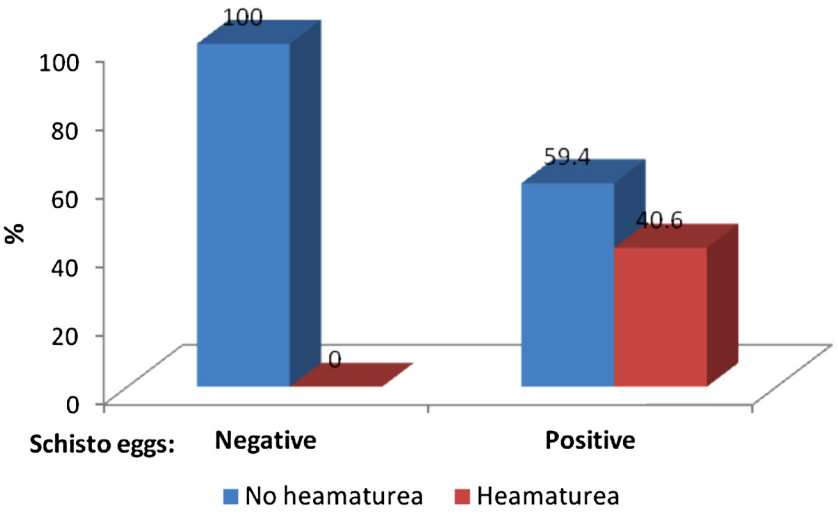

Fig. 2 Microhaematuria among the studied sample

Table 3 Haematuria and CCA test results

	CCA test		Total
	Negative	Positive	
Haematuria			
No			
Count	564	23	587
%	100.0 %	63.9 %	97.8 %
Gross			
Count	0	10	10
%	0 %	27.8 %	1.7 %
Reagent strips			
Count	0	10 + 3	10 + 3
%	0 %	36.1 %	2.2 %
Total			
Count	564	36	600
%	100.0 %	100.0 %	100.0 %

sensitivity and specificity respectively (Anosike et al. 2001, French et al. 2009, Robinson et al. 2009 and Houmsou et al. 2011). The positive predictive value (probability of infected children with *S. haematobium* eggs among those having microhaematuria) was higher in children having macrohaematuria (100 %) than microhaematuria (76.5 %). This indicates that almost all children with haematuria were indeed infected with *S. haematobium* eggs. The haematuria dipsticks proved sufficiently sensitive and specific method for diagnosis of *S. h.* infection.

The agreement between the microscopic examination and detection of Circulating Cathodic *Schistosoma* antigen dipstick test was 95.8 % (P = 0.044*) (Table 4), comparing predicatively of microscopic examination and (CCA) in diagnosis. The specificity of the CCA cassette test was 96.4 versus 96.7 % for the microscopy and sensitivity was 88.2 versus 76.5 % for the microscopy, with statistical significant, P < 0.001 (Table 5). Four samples were positive by CCA for *S. haematobium* eggs but negative by microscopic examination (Tables 1, 2 and 3).

Ruth et al. (2011) reported that the diagnostic accuracy of CCA urine cassette test was poor in detecting *S. haematobium* infections, with a sensitivity of 36.8 % and specificity of 78.9 %. Obeng et al. (2008) recorded that CCA urine cassette test gave low sensitivity (41 %) and high specificity (91 %). Artemis et al. (2009) reported that urine antigen detection test showed similar sensitivity to microscopy. One examined urine sample may not be the best indicator of infection status. This agreed with De Clercq et al. (1997) and Berhe et al. (2004) who suggested that daily fluctuation in egg excretion might be the cause. Besides, Stothard et al. (2006) and Ayele et al. (2008) reported CCA positive while, egg was negative cases. However, in the present study, differences in sensitivity were observed.

In conclusion, microhaematuria reagent strips and CCA cassette are rapid, cheap and easy methods in detection of *S. haematobium* infection. Combination of both techniques could potentially used for screening and mapping of *S. haematobium* infection.

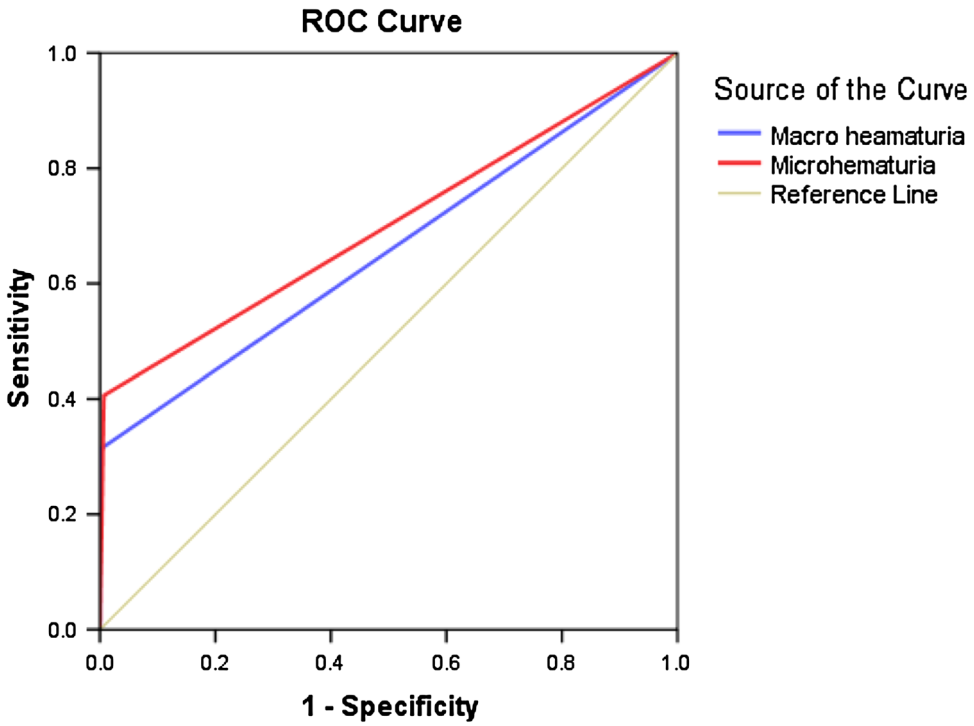

Fig. 3 ROC curve for validity of haematuria

Table 4 Agreement between microscopic examination and CCA detection

	Schistosoma egg		Total
	Negative	Positive	
Schistosoma antigen			
Negative			
Count	564	0	564
% Within schistosoma egg	99.3 %	0.0 %	94.0 %
Positive			
Count	4	32	36
% Within schistosoma egg	0.7 %	100.0 %	6.0 %
Total			
Count	568	32	600
% Within schistosoma egg	100.0 %	100.0 %	100.0 %

Kappa test = 0.938, * $P < 0.001$, Degree of agreement = 99.3 %

Table 5 Validity and predictivity of different methods for *S. haematobium* diagnosis

Variable	Sensitivity %	Specificity %	PPV%	NPV%	P
Microhaematuria	40.6	99.3	76.5	96.7	<0.001*
Macrohaematuria	31.2	100	100	96.2	0.003*
CCA	88.2	96.4	61.7	99.6	<0.001*
Microscopy	76.5	96.7	60.6	99.3	<0.001*

* The result is statistically of high significance

References

Anosike JC, Nwoke BEB, Njoku AJ (2001) Validity of haematuria in the community diagnosis of urinary schistosomiasis infection. J Helminthol 75:223–225

Artemis KJ, Webster P, Christl A, Donnelly BC, Bray JN et al (2009) Sensitivities and specificities of diagnostic tests and infection prevalence of *Schistosoma haematobium* estimated from data on adults in villages northwest of Accra in Ghana. Am J Trop Med Hyg 80(3):435–441

Ashton RA, Stewart BT, Petty N, Lado M, Finn T et al (2011) Accuracy of circulating cathodic antigen tests for rapid mapping of *Schistosoma mansoni* and *S. haematobium* infections in Southern Sudan. Trop Med Int Health. 16:1099–1103

Ayele B, Erko B, Legesse M, Hailu A, Medhin G (2008) Evaluation of circulating cathodic antigen (CCA) strip for diagnosis of urinary schistosomiasis in Hassoba school children, Afar, Ethiopia. Parasite 15:69–75

Baker FJ, Silveston R, Luck ED (1985). An introduction to medical laboratory, 6th ed. Burhen Work and Co.Ltd, pp 286–293

Berhe N, Medhin G, Erko B, Smith T, Gedamu S, Bereded D et al (2004) Variations in helminth faecal egg counts in Kato-Katz thick smears and their implications in assessing infection status with *Schistosoma mansoni*. Acta Trop 92:205–212

Bosompem KM, Bentum IA, Otchere J, Anyan WK, Brown CA, Osada Y, Takeo S, Kojima S, Ohta N (2004) Infant schistosomiasis in Ghana: a survey in an irrigation community. Trop Med Int Health 9:917–922

Cheesbrough M (1998) District laboratory practice in tropical countries. Part1. Cambridge University Press, London

Coon DR (2005) Schistosomiasis: overview of the history, biology, clinicopathology, and laboratory diagnosis. Clin Microbiol Newsl 27:163–168

De Clercq D, Sacko MP, Vercruysse J, Landoure A, Diarra A et al (1997) Circulating anodic and cathodic antigen in serum and urine of mixed *Schistosoma haematobium* and *S.mansoni* infections in office du Niger, Mali. Trop Med Int Health 2:680–685

French MD, Rollinson D, Basáñez MG, Mgeni AF, Khami IS, Stothard JR (2009) School-based control of urinary schistosomiasis on Zanzibar, Tanzania: monitoring microhaematuria with reagent strips as a rapid urological assessment. J Pediatr Urol 3:364–368

Houmsou RS, Kela SL, Suleiman MM (2011) Performance of microhaematuria and proteinuria as measured by urine reagent strips in estimating intensity and prevalence of *Schistosoma haematobium* infection in Nigeria. Asian Pac J Trop Med 4:997–1000

Koss LG, Hoda RS (2012) Koss's cytology of the urinary tract with histopathologic correlations. Springer, New York. doi: 10.1007/978-1-4614-2056-9

Koukounari A, Webster JP, Donnelly CA, Bray BC, Naples J, Bosompem K, Shiff C (2009) Sensitivities and specificities of diagnostic tests and infection prevalence of *Schistosoma haematobium* estimated from data on adults in villages northwest of Accra, Ghana. Am J Trop Med Hyg 80:435–441

Kremsner PG, de Jonge N, Simarro PP, Muhlschlegel F, Mir M et al (1993) Quantitative determination of circulating anodic and cathodic antigens in serum and urine of individuals infected with *Schistosoma intercalatum*. Trans R Soc Trop Med Hyg 87:167–169

Legesse M, Erko B (2008) Field-based evaluation of a reagent strip test for diagnosis of *Schistosomiasis mansoni* by detecting circulating cathodic antigen (CCA) in urine in low endemic area in Ethiopia. Parasite 15:151–155

Mott KE, Dixon H, Osei-Tntu E, England EC, Tekle A (1985) Evaluation of reagent strips in urine tests for detection of

Schistosoma haematobium infection: a comparative study in Ghana and Zambia. Bull WHO 63:125–133

Obeng BB, Aryeetey YA, de Dood CJ et al (2008) Application of a circulating-cathodic-antigen (CCA) strip test and real-time PCR, in comparison with microscopy, for the detection of *Schistosoma haematobium* in urine samples from Ghana. Ann Trop Med Parasitol 102(7):625–633

Robinson E, Picon D, Sturrock HJ, Sabasio A, Lado M, Kolaczinski J et al (2009) The performance of haematuria reagent strips for the rapid mapping of urinary schistosomiasis: field experience from southern Sudan. Trop Med Int Health 14(12): 1484–1487

Steinmann P, Keiser J, Bos R, Tanner M, Utzinger J (2006) Schistosomiasis and water resources development: systematic review, meta-analysis, and estimates of people at risk. Lancet Infect Dis 6:411–425

Stothard JR, Kabatereine NB, Tukahebwa EM, Kazibwe F, Rollinson D et al (2006) Use of circulating cathodic antigen (CCA) dipsticks for detection of intestinal and urinary schistosomiasis. Acta Trop 97:219–228

Utzinger J, Raso G, Brooker S, de Savigny D, Tanner M et al (2009) Schistosomiasis and neglected tropical diseases: towards integrated and sustainable control and a word of caution. Parasitology 136:1859–1874