Tropical Medicine and International Health

VOLUME 9 NO 9 PP 991–996 SEPTEMBER 2004

Applicability of a monoclonal antibody-based dipstick in diagnosis of urinary schistosomiasis in the Central Region of Ghana

Kwabena M. Bosompem[1], Osei Owusu[1], E. O. Okanla[1] and Somei Kojima[2]

1 *Parasitology Unit, Noguchi Memorial Institute for Medical Research (NMIMR), Accra, Ghana*
2 *Asian Centre for International Parasite Control, Mahidol University, Bangkok, Thailand*

Summary We tested a rapid visually read monoclonal antibody (MoAb) based dipstick assay for specific diagnosis of urinary schistosomiasis against microscopy and the use of haematuria and proteinuria in a schistosomiasis haematobia endemic area in the Central Region of Ghana. The study group consisted of 141 school children (83 males, 58 females) aged 8–19 years. A total of 129 of 141 (91.5%) submitted stool samples, and 7.8% had *Schistosoma mansoni*, 55% had hookworms and 6.2% had tapeworms. The presence of *S. mansoni* and intestinal parasites did not appear to influence the results of the MoAb-dipstick assay. The urinary schistosomiasis prevalence by MoAb-dipstick (78%) was higher ($P < 0.05$) than the estimate by microscopy (60.3%), microhaematuria (27%) and proteinuria (30.5%). The MoAb-dipstick correctly identified 98.8% of microscopically confirmed cases and missed one (1.3%). The dipstick was also positive for 26 of 56 (46.4%) egg-negative individuals, thereby giving a sensitivity of 98.8% and a specificity of 53.6%. On the other hand, microhaematuria and proteinuria were 38.8% and 30.6% sensitive, and 91.1% and 69.6% specific, respectively. Microhaematuria and proteinuria were less sensitive ($P < 0.05$) than both microscopy and MoAb-dipstick.

keywords urinary schistosomiasis, monoclonal antibody, diagnosis, dipstick

Introduction

Human schistosomiasis remains one of the most important diseases in the tropics. In Africa the disease is caused mainly by *Schistosoma haematobium* and *S. mansoni* which are responsible for urinary and intestinal schistosomiasis, respectively. Together, the two parasites infect about 131 million people in Sub-Saharan Africa alone. In 1990, schistosomiasis was estimated to be responsible for the loss of 1.5 million Disability Adjusted Life Years and the mortality was estimated to exceed 100 000 per year (TDR 1997).

Diagnosis of schistosomiasis is, however, beset with several problems. The infections are generally chronic and characterized by the presence of parasite eggs in stool or urine, hence routine diagnosis is microscopical although the method is limited in sensitivity due to great fluctuation of egg output. Furthermore, urinary and intestinal schistosomiasis now overlap in distribution in hyperendemic areas, in several countries including Ghana, resulting in mixed infections in affected communities. This situation calls for more sensitive and preferably schistosome species-specific diagnosis. Less sensitive diagnostic assays are unsuitable for evaluation of control programmes such as morbidity reduction by chemotherapy, which normally lead to reduction in prevalence and increase in the number of low-intensity infections.

The most promising alternative diagnosis so far developed for schistosomiasis are schistosome circulatory antigen detection assays (De Jonge *et al.* 1989; Deelder *et al.* 1989; Kremsner *et al.* 1994) and the recently introduced *S. haematobium* species-specific dipstick-ELISA (Bosompem *et al.* 1996a, 1998). This is because they are more sensitive and detect circulatory and/or urinary antigens using non-invasive membrane-based assays which are better suited to field use in endemic areas (Peters 1976; Webbe & El Hak 1990; Bosompem *et al.* 1996b). Interestingly, only the urinary schistosomiasis dipstick-ELISA (Bosompem *et al.* 1996b) has been successfully adopted for application in routine diagnosis in the field. So far it has been tested in the Greater Accra, Eastern and Volta Regions of Ghana and found to be highly sensitive and specific. Nevertheless, there is a need to test this new promising assay in other geographical areas to determine its range of applicability. Hence we evaluated the urinary schistosomiasis dipstick in the Central Region of Ghana.

Materials and methods

Study design

In order to identify a suitable study site for evaluation of the dipstick-ELISA in the Central Region of Ghana, a questionnaire survey was conducted in the Cape Coast District. Typical urinary schistosomiasis symptoms (haematuria and dysuria) were used as indicators for the presence of the disease. Urine samples were collected from 90 individuals with symptoms and 51 asymptomatic subjects and analysed for *S. haematobium* eggs by microscopy for microhaematuria and proteinuria by reagent strips, and for antigen by dipstick-ELISA. Stool specimens were also collected from both symptomatic and asymptomatic subjects and analysed for intestinal parasites. Another urine sample was collected from individuals who were *S. haematobium* antigen positive but egg negative and tested by both microscopy and dipstick. The relative sensitivity and specificity of the dipstick-ELISA was calculated using microscopy as gold standard.

Study area

The study was conducted at Kuase Municipal Authority School, which serves about 12 villages covering an area of about 20 km^2. The principal source of water in the area is the Brimsu River on the outskirts of the villages. The vegetation along the river banks consists of grass and a few trees, and there are large amounts of decaying plant leaves and twigs as well as aquatic plants (*Ceratophylum* sp. and *Pistia* sp.) preferred by the schistosomiasis host snails *Bulinus globosus*.

Collection and analysis of urine and stool specimen

Fifty to 100 ml of urine were obtained from each of 141 school children involved in the study. Urine was collected between 11:00 and 14:00 hours. The samples were tested for microhaematuria and proteinuria using haemacombrix urine analysis reagent strips (Millipore Corporation, Bedford, MA, USA) and for *S. haematobium* antigen by the monoclonal antibody (MoAb) dipstick-ELISA. The urine samples were then centrifuged separately at 320 ***g*** and the entire sediment examined by microscopy for *S. haematobium* eggs.

Individuals who gave urine specimens were also given containers to produce stool samples. Stool specimens were transported to the laboratory on ice and stored at 4 °C. They were examined within 2 weeks for *S. mansoni* and intestinal parasite ova using the formol-ether concentration technique (Garcia & Shimuzu 1981).

Monoclonal antibody

The IgG1 MoAb used in this study was generated following immunization of BALB/c mice with precipitated proteins from *S. haematobium* infected human urine (Bosompem *et al.* 1996a). The antibody was shown to bind a 29 kDa *S. haematobium* species-specific peptide present in the egg stage of both Ghanaian and Egyptian strains of the parasite, and it did not show cross-reactivity with *Necator americanus* (hookworm) egg antigens in the microplate-ELISA (Amanor *et al.* 1996).

Dipstick ELISA procedure

The MoAb dipstick-ELISA procedure described by Bosompem *et al.* (1998) was used. Briefly, polyvinylidene diflouride membrane strips wetted by immersion in methanol were incubated in test urine for 30 min at room temperature (21–25 °C) to capture antigen. The strips were rinsed twice with Tris-Buffered Saline (TBS) (50 mM Tris and 150 mM NaCl, pH 7.4) and then blocked by incubation for 30 min in 5% skimmed milk diluted in TBS. This was followed by 1 h incubation of the strips in a reagent mixture consisting of *S. haematobium* species-specific MoAb and goat anti-mouse immunoglobulin conjugated to horseradish peroxidase in 0.1% skimmed milk in TBS. The strips were washed five times (2 min per wash) with excess TBS and then incubated for 1 min in substrate solution containing 0.05% (w/v) 3,3-diaminobenzidine, 0.15% (v/v) H_2O_2 and 5 mM $CO(NO_3).6H_2O$ in TBS. A bluish-black reaction represented a positive result; negative results remained colourless.

Sensitivity and specificity

Sensitivity, the ability of a test to detect true positives, was calculated as shown below:

$$\text{Sensitivity (\%)} = \frac{\text{Number of individuals positive by the test that were also positive by microscopy}}{\text{Total number of individuals testing positive by microscopy}} \times 100.$$

Specificity, the ability of a test to detect true negatives, was calculated thus:

$$\text{Specificity (\%)} = \frac{\text{Number of individuals negative by the test that were also negative by microscopy}}{\text{Total number of individuals testing negative by microscopy}} \times 100.$$

Results

Urinary schistosomiasis prevalence by symptomatology, microscopy and dipstick-ELISA

Table 1 and 2 summarize the results of the questionnaire. Ninety (63.8%) of 141 individuals interviewed reported urinary schistosomiasis symptoms (haematuria and/or dysuria). Fifty-one (36.2%) were asymptomatic and 34 (37.8%) answered yes to blood in urine (haematuria), 12 (13.3%) had only dysuria, and 44 (48.9%) had both haematuria and dysuria (Table 1). In all, 61 of 90 (67.8%) of the symptomatic individuals were confirmed to be infected by microscopical demonstration of *S. haematobium* eggs (Table 1). However, the MoAb-dipstick detected more positives (74, 82.2%) in the same group. This included all (100%) of the microscopically confirmed cases and 13 others who were *S. haematobium* egg negative (Table 1). One (7.7%) of the dipstick-positive but egg-negative individuals had haematuria whilst five of 13 (38.5%) had proteinuria.

Of the 51 asymptomatic individuals, 24 (47.1%) were confirmed to be infected with *S. haematobium* by microscopy (Table 1). Again the MoAb-dipstick detected more infections 36/51 (70.6%). This included 23 of 24 (95.8%) of the microscopically confirmed infections. Four (30.8%) of 13 dipstick-positive/*S. haematobium* egg-negative individuals had proteinuria but none had haematuria. In all, the MoAb-dipstick detected 84 of 85 (98.8%) of the parasitologically confirmed *S. haematobium* infections and missed one.

Results of stool examination

The results of stool examinations are summarized in Table 2. Of 141 individuals who gave urine specimens, only 129 (91.5%) submitted stool samples, and 10 (7.8%) contained *S. mansoni* eggs. In addition, ova of intestinal helminth parasites were identified. These included 71 (55%) hookworm and eight (6.2%) tapeworm (*Taenia* sp.) single infections, and a total of eight (6.2%) mixed infections (Table 2). Table 2 shows the probable influence of intestinal parasitism on the MoAb-dipstick results. Fifty-two (73.2%) of the 71 hookworm-infected individuals tested positive by the MoAb-dipstick. Forty-one of these (78.8%) were positive for *S. haematobium* eggs as determined by microscopy. As shown in Table 2, the MoAb-dipstick detected 40 of 41 (97.6%) of the hookworm/*S. haematobium* mixed infections and missed one. On the other hand, 18 of 30 (60%) of the hookworm infections that were *S. haematobium* egg negative were also negative by MoAb-dipstick. All the subjects who were infected with *S. mansoni* or tapeworms and were positive for *S. haematobium* urinary antigens also had eggs of the parasite (Table 2). Interestingly, one hookworm and two *S. mansoni* infected individuals who required repeated microscopy to demonstrate *S. haematobium* eggs were identified by the dipstick.

Prevalence of urinary schistosomiasis and sensitivity and specificity of the tests

Table 3 summarizes the urinary schistosomiasis prevalence as determined by microscopy, MoAb-dipstick,

Table 1 Urinary schistosomiasis infection rates in different symptomatic groups as determined by various tests

Symptomatic status	Number involved	Number positive by various tests			
		Microscopy	MoAb-dipstick	Micro-haematuria	Proteinuria
Asymptomatic	51	24 (1)†	36 (13)‡	4 (2)‡	10 (8)‡
Haematuria	34	24	31 (7)‡	12 (1)‡	13 (4)‡
Dysuria	12	3	5 (2)‡	3 (1)‡	4 (2)‡
Haematuria/ dysuria§	44	34	38 (4)‡	19 (1)‡	16 (3)‡
Symptomatic total¶	90	61	74 (13)‡	34 (3)‡	33 (9)‡

† Number negative by MoAb-dipstick.
‡ Number negative for *S. haematobium* eggs by microscopy.
§ Group of individuals with both haematuria and dysuria.
¶ Excluding asymptomatic individuals.

Table 2 Probable influence of intestinal parasitism on the MoAb-dipstick assay

Parasites	Number examined	Number positive	Microscopy positive†	MoAb-dipstick positive
Hookworm	129	71	41 (1)‡	52 (12)§
Taenia sp.	129	8	5	6 (1)§
S. mansoni	129	10	6	8 (2)§
Hookworm/ *Taenia*¶	129	4	2	3 (1)§
Hookworm/ *S. mansoni*¶	129	4	2	4 (1)§
Total		97	56 (1)‡	73 (17)§

† Microscopical demonstration of *S. haematobium* eggs.
‡ Number negative by MoAb-dipstick.
§ Number negative for *S. haematobium* eggs.
¶ Mixed infections.

microhaematuria and proteinuria. The prevalence estimated by microscopy was significantly lower ($P < 0.05$) than that by MoAb-dipstick, yet both prevalences were higher ($P < 0.05$) than the estimates by microhaematuria and proteinuria.

The sensitivity and specificity of the MoAb-dipstick, microhaematuria and proteinuria compared with microscopy as a gold standard test are also shown in Table 3. Only one of 85 microscopically confirmed infections was missed by the MoAb-dipstick, thereby giving a relative sensitivity of 98.8% (84/85). The relative sensitivities of microhaematuria (38.8%) and proteinuria (30.6%) were lower ($P < 0.05$) than that of the MoAb-dipstick. On the other hand, the relative specificity of the MoAb-dipstick was low (53.6%). There was no significant difference ($P > 0.05$) between the specificity of the MoAb-dipstick (53.6%) and that of proteinuria (69.6%). However, microhaematuria had a significantly higher specificity 91.1% ($P < 0.001$) than both the MoAb-dipstick and proteinuria (Table 3).

Disparities between microscopy and the MoAb-dipstick

Thirty individuals were initially MoAb-dipstick positive but *S. haematobium* egg negative. Of these 20 submitted another urine sample for repeat examination and four of 20 (20%) were confirmed to be infected microscopically. In another disparity, urine from four other subjects which contained *S. haematobium* eggs tested negative for the parasite's antigens by the MoAb-dipstick. Repeated examination of fresh urine from the same individuals, however, showed dipstick-positive results for three.

Discussion

Detection of schistosomal antigens in human specimens is the best of the alternative diagnostic methods so far introduced for urinary schistosomiasis (Kremsner *et al.* 1994; Bosompem *et al.* 1996b). This approach has several advantages depending on the nature of the antigen being detected. Thus, it can be used to diagnose infection, ascertain the severity of pathologic manifestations in the lower renal tract, monitor the impact of chemotherapy in infected individuals, and investigate ongoing transmission in areas endemic for schistosomiasis.

The objective of this study was to evaluate a newly developed MoAb-based dipstick assay for urinary schistosomiasis (Bosompem *et al.* 1998) in an endemic area in the Central Region of Ghana. The rationale behind this evaluation was to compare the MoAb-dipstick with other schistosomiasis diagnostic methods, namely, parasite egg detection by microscopy and the use of microhaematuria and proteinuria.

The demonstration of both *S. haematobium* and *S. mansoni* infections by microscopy in Kuase, Cape Coast District, where the study was conducted, suggests that the area is hyperendemic for schistosomiasis. In earlier studies evaluating the MoAb-dipstick no *S. mansoni* was encountered, hence the specificity of the assay for *S. haematobium* under field conditions was not determined (Bosompem

Table 3 Prevalence of urinary schistosomiasis as determined by the various tests, and their sensitivity and specificity

Test	Number tested	Number positive	Number negative	Prevalence (%)	Sensitivity†	Specificity†
Microscopy	141	85	56	60.3*	100	100
MoAb-dipstick	141	110 (26)‡	30 (1)§	78.0**	98.8	53.6
Microhaematuria	141	38 (5)‡	103(52)§	27.0***	38.8	91.1
Proteinuria	141	43 (17)‡	98 (59)§	30.5**	30.6	69.6

Prevalence values with different asterisks are significantly different ($P < 0.05$).
† Sensitivity or specificity based on microscopy as a gold standard.
‡ False positives.
§ False negatives.

et al. 1996b, 1998). In this study, the observation that 20% of *S. mansoni* infections were MoAb-dipstick positive although they were *S. haematobium* egg negative may suggest cross-reactivity. However, *S. haematobium* infection in 33.3% of the *S. mansoni* infected individuals required repeated urine examination to confirm, thereby suggesting that the 20% discordant results were due to low sensitivity of the microscopical method rather than cross-reactivity. Indeed, Bosompem *et al.* (1998) found that as many as eight repeated microscopic examinations were necessary to demonstrate *S. haematobium* eggs in some low intensity infections that were detected by the dipstick. In addition, Amanor *et al.* (1996) showed that the MoAb used in the dipstick does not cross-react with crude *S. mansoni* adult worm and egg antigens.

Bosompem *et al.* (1996b, 1998) reported a high relative sensitivity and specificity, 99.1% and 98.3%, respectively, for the MoAb-dipstick as compared with microscopy. However, in this work, the specificity (56.3%) of the MoAb-dipstick was significantly lower ($P < 0.05$) than that of microscopy although the sensitivity was still high (98.8%). The low relative specificity recorded by the MoAb-dipstick in this study may be explained by either a high false positivity rate or low sensitivity of the microscopical technique. Indeed, the second explanation is more likely to be true, as Bosompem *et al.* (1998) required up to eight repeated microscopical examination to confirm some low-intensity *S. haematobium* infections; and in this study 20% of 66.7% of the MoAb-dipstick positive/egg negative individuals who submitted a second urine specimen were confirmed to be infected.

Bosompem *et al.* (1998) extensively investigated the phenomenon whereby some microscopically confirmed *S. haematobium* infections tested negative for the parasite antigens by MoAb-dipstick. They observed that the phenomenon was not associated with a particular intensity of infection (low or high) and explained that those discordant reactions could be due to the absence of detectable antigen in test urine from some patients with peculiar immunological reactions which could lead to masking of the diagnostic epitope during the course of an infection. This conclusion was partly based on the results of an earlier study (Bosompem *et al.* 1996a) in which schistosome antigens were reported to occur in infected human urine mainly as immune complexes together with human immunoglobulins and complement. The occurrence of one of 85 (1.2%) of the egg positive/MoAb-dipstick negative phenomenon in this study therefore confirms earlier findings and could be explained similarly.

Interestingly, both microscopy and MoAb-dipstick detected infections in the asymptomatic group (47.1% and 70.6%, respectively). The significantly higher ($P < 0.05$) prevalence of urinary schistosomiasis obtained by MoAb-dipstick compared to the other tests (microscopy, microhaematuria and proteinuria) may be explained by earlier observations (Bosompem *et al.* 1998). In a longitudinal study they found prevalence variability in the estimates of both microscopy and MoAb-dipstick. However, using a cohort of infected individuals the dipstick was shown to detect more of the infected persons on all occasions and it was the only assay that ever estimated correctly the true prevalence of 100% (Bosompem *et al.* 1998). Consequently, it was concluded that in an endemic setting the dipstick is more likely to correctly estimate higher prevalence than microscopy. It is, however, necessary to confirm the observations made by Bosompem *et al.* (1998) in other geographical areas.

In conclusion, this study has shown that the urinary schistosomiasis MoAb-dipstick is indeed applicable in the Cape Coast geographical area of Ghana. The assay showed a high sensitivity and it did not cross-react with other intestinal parasites. Furthermore, the non-invasive nature of the dipstick assay together with its ease of application in this field makes it a desirable method in mass surveys.

Acknowledgements

We wish to express our sincere gratitude and deep appreciation to Mrs Irene Ayi, W.K Anyan, J.R.K. Asigbee and J. Otchere for their technical assistance. We are also especially thankful to Prof. F.K. Nkrumah of the Noguchi Memorial Institute For Medical Research, University of Ghana, Legon.

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Authors

K. M. Bosompem (corresponding author), **Osei Owusu** and **E. O. Okanla**, Parasitology Unit, Noguchi Memorial Institute for Medical Research, PO Box LG 581, Legon, Ghana. Tel.: +233 21 501178/9; Fax: +233 21 502182; E-mail: Kbosompem@noguchi.mimcom.net

S. Kojima, Asian Centre for International Parasite Control, Mahidol University, 120–126 Rajvithi Road, 10400 Bangkok, Thailand.