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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

LEGESSE M.* & ERKO B.*

Summary:

The sensitivity, specificity, positive and negative predictive values of a reagent strip test for the diagnosis of schistosomiasis mansoni by detecting circulating cathodic antigen (CCA) in urine were evaluated using 184 stool and urine samples collected from schoolchildren living in relatively low endemic area of schistosomiasis mansoni in Ethiopia. A combined result of stool samples processed by Kato and formol-ether concentration methods was used as gold standard. The results showed that detection of CCA in urine using reagent strip test was slightly higher than the combined results of the stool techniques (65.2 % *vs* 42.4 %, $p > 0.05$) in suggesting the prevalence of the disease. The sensitivity, specificity, positive and negative predictive values of the reagent strip test were 76.9 %, 43.4 %, 50 % and 71.9 %, respectively. The result of egg counts using Kato method suggested that detection of urine CCA could be used to indicate the intensity of infection. Nevertheless, like that of stool examination, the reagent strip test was found to be less sensitive in case of light to moderate infections. About 23.1 % of the study children who were excreting the eggs of the parasite were found negative by the reagent strip test. The relative insensitivity of a reagent strip test in low intensity of infection necessitates for the development of more sensitive assay that can truly discriminate schistosome-infected from non-infected individuals.

KEY WORDS : *S. mansoni*, CCA, urine, reagent strip test, Ethiopia.

Résumé : ÉVALUATION SUR LE TERRAIN D'UN KIT DE DÉTECTION DANS LES URINES DES ANTIGÈNES CIRCULANTS CATHODIQUES POUR LE DIAGNOSTIC DE LA BILHARZIOSE À *SCHISTOSOMA MANSONI* DANS UNE ZONE DE FAIBLE ENDÉMICITÉ EN ÉTHIOPIE

La sensibilité, la spécificité, les valeurs prédictives, négatives et positives d'un kit pour le diagnostic de la bilharziose à Schistosoma mansoni *par détection des antigènes circulants cathodiques (ACC) dans les urines ont été évaluées dans 184 échantillons de selles et d'urines provenant d'enfants éthiopiens scolarisés dans une zone d'endémicité relativement basse. Le "gold standard" était le résultat combiné de l'examen des échantillons de selles par les techniques de Kato et de concentration formol éther. La sensibilité du kit détectant les ACC dans les urines était légèrement supérieure à la sensibilité de la combinaison des deux examens de selles (65,2 % vs 42,4 %, p > à 0,05). Les sensibilités, spécificités, valeurs prédictives positives et négatives du test étaient respectivement de 76,9 %, 43,4 %, 50 % et 71,9 %. La numération des œufs effectuée par la méthode de Kato suggère que la détection des ACC urinaires pourrait être utilisée pour évaluer l'intensité de l'infection. Cependant, comme pour l'examen de selles, le kit a été moins sensible en cas d'infestation légère ou modérée. Environ 23,1 % des enfants étudiés qui excrétaient des œufs du parasite étaient négatifs avec le test. Le manque de sensibilité du test en cas de faible infestation nécessite le développement d'un kit plus sensible qui pourrait véritablement discriminer les individus infectés des non infectés.*

MOTS CLÉS : S. mansoni, *diagnostic, ACC, urine, Éthiopie.*

INTRODUCTION

Diagnosis of intestinal schistosomiasis by demonstrating the parasites' eggs in feces using convectional techniques like Kato and formol-ether concentration has been claimed for its low sensitivity in case of low intensity of infection, post-treatment situations as well as for egg output fluctuation (De Vlas & Gryseels, 1992; Gryseels & De Vlas, 1996; Engels *et al.*, 1996; Kongs *et al.*, 2001). Detection of specific antibodies induced against the different stages of the parasite using immunological techniques has been suggested as a solution to minimize the risk of low sensitivity of the parasitological techniques (Rossi *et al.*, 1991; Hamilton *et al.*, 1999; Van Lieshout *et al.*, 2000). However, its less specificity to differentiate light infection from heavy infection or active infection from persistence of antibodies after treatment as well as its high cost made the techniques inadequate for epidemiological studies and control programs (Spencer *et al.*, 1991; Doenhoff *et al.*, 1993).

ELISA based detection of circulating antigens, CAA or CCA in serum or urine has been shown to be highly sensitive and specific in the diagnosis of active schistosomiasis (Deelder *et al.*, 1989; Van Lieshout *et al.*, 1992; De Clercq *et al.*, 1997). Moreover, these circulating antigens have been correlated with the intensity of infection and morbidity of the disease (Van Lieshout *et al.*, 1992; Polman *et al.*, 1995; Hassan *et al.*, 1999). Because of less field applicability of the ELISA-based method for the diagnosis of schistosomiasis at a community level, reagent strip/dipstick based assay has

* Aklilu Lemma Institute of Pathobiology, Addis Ababa University, P.O. Box 1176, Addis Ababa, Ethiopia.
Correspondence: Mengistu Legesse.
Fax: 00 251 1 2755296 – E-mail: dlegessem@yahoo.com

been developed for the diagnosis of both intestinal and urinary schistosomiasis by detecting CCA of the parasites in urine of infected individuals regardless of species specificity, according to the information provide by the manufacturer (B.V. European, Veterinary Laboratory, The Netherlands) and Van Dam *et al.* (2004). The test has been found to be highly sensitive and specific in detecting CCA in the urine of *Schistosoma mansoni* -infected individuals in high endemic areas of the disease (Van Etten *et al.*, 1994; Van Dam *et al.*, 2004; Stothard *et al.*, 2006). On the other hand, the test was found to be valueless in the diagnosis of schistosomiasis haematobium (Stothard *et al.*, 2006). Moreover, detection of circulating antigens using dipsticks/reagent strip tests has been complained for its low sensitivity in the case of light infection (Stothard *et al.*, 2006; Legesse & Erko, 2007). Therefore, the present study has focused on the evaluation of the efficiency of reagent strip test in diagnosis of light infection with *Schistosoma mansoni* by detecting CCA in urine in relatively low endemic area of the disease.

MATERIALS AND METHODS

Study area and study population

In June 2007, a cross-sectional study on *Schistosoma mansoni* infection was conducted in Kemmie primary schoolchildren using convectional stool examination techniques and rapid reagent strip test. Kemmie primary school is found at an altitude of about 1,600 m above sea level to the Southeast of Lake Langano, 210 km to the Southeast of Addis Ababa, in an area where urinary schistosomiasis was not present. The study area has been described in detail elsewhere (Erko *et al.*, 2001).

Selection of study population and Data collection

After explaining the aim of the study and obtaining informed consent from the Director of the study school, a total of 184 subjects (97 male and 87 female, age range from 5-22, years, mean 11) were randomly selected out of a total of 580 students. Then, a disposable small piece of clean plastic sheet and cup were given to each individual (guardian/parent in case of under 12 years children) to provide fresh stool and urine samples, respectively.

Stool specimens were processed using Kato technique, template-delivering 41.7 mg of stool sample as previously described (Ebrahim *et al.*, 1997). The slide was microscopically examined on the spot for the eggs of *S. mansoni* and other intestinal helminthes. Egg count was performed for *S. mansoni* and the intensity of infection was expressed as egg count per gram (EPG) of stool for each subject (WHO, 2002).

About 1 gm of the same stool sample was also transferred into plastic vials containing 10 % formalin and transported to laboratory. The samples were processed by the formol-ether concentration method and qualitatively examined for *S. mansoni* and other helminthes eggs.

Urine specimens were immediately tested using Schistosomiasis One Step Test for the detection of Schistosoma CCA according to the manufacturer protocol (B.V. European, Veterinary Laboratory, The Netherlands). The positivity of the urine samples by the reagent strip was classified as strong positive and weak positive. Then, strong positive samples were scored as 1 while weak positive as 2.

Data analysis

Data were analyzed using SPSS, Version 10.0. Chi-square test was used to determine proportion of difference in positivity. Differences were considered significant when the P-value was less than 0.05. The reagent strip was evaluated in terms of sensitivity, specificity, positive and negative predictive values. A combined result of stool samples processed by Kato and formol-ether concentration methods was used as gold standard to evaluate the sensitivity, specificity, positive and negative predictive values of the reagent strip test.

Ethical consideration

The study was ethical approved by the Ethical Clearance Committee of Aklilu Lemma Institute of Pathobiology. The aim of the study was explained to the Director of the school, children and parents and verbal consent was obtained. Those individuals who were found positive for schistosomiasis mansoni and other intestinal helminths were treated with appropriate doses of praziquantel and albendazole, respectively.

RESULTS

Stool examination methods

Of the total 184 stool samples, 67 (36.4 %) and 44 (23.9 %) were found to be positive for *S. mansoni* egg by Kato and formol-ether concentration methods, respectively. However, 78 (42.4 %) samples were found to be positive by the combination of the Kato and formol-ether concentration results, whereas 106 (57.6 %) samples were negative by both methods.

Reagent strip methods

Of the total 184 urine samples, 120 (65.2 %) were found to be positive for *S. mansoni* CCA by reagent

Method	No. of examined	No. of positive	% positives
Kato	184	67	36.4
Formol ether	184	44	23.9
Combination of Kato & Formol ether	184	78	42.4
Reagent strip	184	120	65.2
Combination of Reagent strip & Stool examination	184	138	75

Table I. – Prevalence of schistosomiasis mansoni in Kemmie primary schoolchildren as detected by various methods.

strip test which was significantly higher than prevalence detected by Kato alone (65.2 % *vs* 36.4 %, p = 0.003), but slightly higher than prevalence found by the combination of the Kato and formol-ether concentration results (65.2 % *vs* 42.4 %, p > 0.05). The prevalence of schistosomiasis mansoni in Kemmie primary schoolchildren as detected by Kato, formol-ether concentration, reagent strip, combination of Kato and formol-ether concentration or reagent strip test is shown in Table I.

SENSITIVITY AND SPECIFICITY OF THE REAGENT STRIP TEST

Of the total 184 samples examined 60 (32.6 %) samples were found to be positive by both stool examination and reagent strip methods, while 46 (25 %) samples were found to be negative by both tests. Eighteen (23.1 %) samples which were positive by stool examination were found negative by the reagent strip test. On the other hand, 60 (50 %) samples were found to be negative by stool examination techniques which were positive by reagent strip test (Table II). The sensitivity, of the reagent strip test was found to be 76.9 % while its specificity was 43.4 %. The positive and negative predictive values were 50 % and 71.9 %, respectively.

		Stool examination	
		Positive	Negative
Urine test	Positive	60	60
	Negative	18	46

Table II. – Comparison of urine test with stool examination methods in the diagnosis of schistosomiasis mansoni.

INTENSITY OF INFECTION

Of the total 67 positive subjects by Kato, 47 (70.1 %), 17 (25.4 %) and 3 (4.5 %) were found to have light, moderate and heavy infections, respectively. Among individuals with light infection, 10 (21.3 %), 31 (66 %) and 6 (12.8 %) subjects were found to be negative, weak positive and strong positive by reagent strip test, respectively. Similarly, of those subjects with moderate infection, 2 (11.8 %), 11 (64.7 %) and 4 (23.5 %) subjects were found to be negative, weak positive and strong positive by the reagent strip test, respectively. Among 65 subjects found negative by Kato 59 (90.8 %) were found to be weak positive by reagent strip test. Moreover, most of the 12 subjects who were positive by Kato, but negative by the reagent strip test had an egg count less 100 per gram of stool sample. Most of the individuals found negative, light and moderate infections by Kato were detected as weak positive by reagent strip test (Fig. 1).

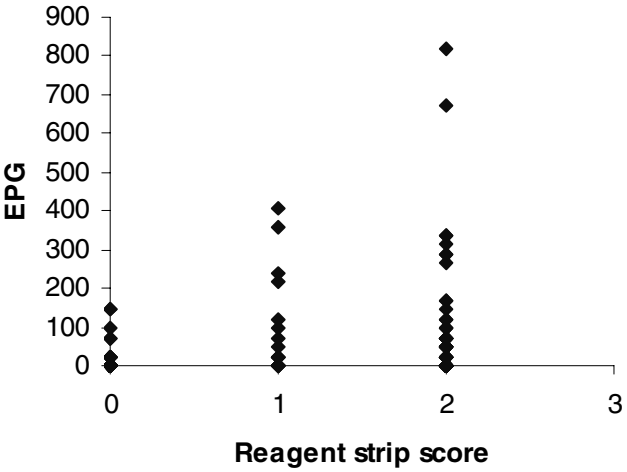

Fig. 1. – Association of reagent strip score with the intensity of *S. mansoni* infection.

OTHER INTESTINAL HELMINTHES

Among the total subjects, 102 (55.4 %), 17 (9.2 %), 10 (5.4 %), 25 (13.6 %) and 10 (5.4 %) were positive for hookworm infection, trichuriasis, ascariasis, taeniasis and *Hymenolepis nana* in that order. Thirty four (33.3 %) subjects who were negative for schistosomiasis mansoni infection by stool examination but had hookworm were found positive by reagent strip. On the other hand, 37 (36.3 %) subjects who had hookworm were found to be negative by reagent strip test.

DISCUSSION

Demonstrating eggs in stool samples using Kato or formol-ether concentration method is the most common way to diagnosis active intestinal schistosomiasis. However, stool examination techniques have been criticized mainly for their low sensitivity in case of low intensity of infection and day to day egg output fluctuation (De Vlas & Gryseels, 1992; Gryseels & De Vlas, 1996; Engels *et al.*, 1996; Ebrahim

et al., 1997; Kongs *et al.*, 2001). Reagent strip/dipstick based assay has been developed and found to be highly sensitive and specific in detecting CCA in urine in active *Schistosoma mansoni* infected individuals even at a community level (Van Etten *et al.*, 1994; Van Dam *et al.*, 2004, Legesse & Erko, 2007). Like that of stool examination, in some published studies this assay was found to be less sensitive in case of low intensity of infection (Stothard *et al.*, 2006; Legesse & Erko, 2007). In the present study, a reagent strip based assay developed by Van Dam *et al.* (2004) was evaluated for its sensitivity in the detection of CCA in urine of *Schistosoma mansoni* infected children in relatively low endemic area of the disease in Ethiopia (Erko *et al.*, 2001). Similar to our previous report (Leggesse & Erko, 2007) and observations by others (Van Dam *et al.*, 2004; Stothard *et al.*, 2006), the result revealed that detection of CCA in urine using this reagent strip test is more sensitive than the conventional stool examination techniques like Kato and formali-ether concentration in such low endemic areas (Table I). Nevertheless, the present observed sensitivity (76.9 %) of the test is found to be lower than the sensitivity (82.1 %) of the test previously observed in relatively high endemic area of the disease in Ethiopia (Legesse & Erko, 2007). This sensitivity is also lower than the sensitivity result reported from high endemic areas of schistosomiasis mansoni elsewhere (Van Dam *et al.*, 2004).

ELISA based detection of CAA or CCA in serum or urine has been shown to be highly sensitive and specific in the diagnosis of active schistosomiasis (Deelder *et al.*, 1989; van Lieshout *et al.*, 1992; De Clercq *et al.*, 1997). Study by Van Lieshout *et al.* (1995) suggested that detection of CCA in urine using ELISA is as sensitive as the parasitological method in demonstrating low intensity of infection. Fillie *et al.* (1994) found higher sensitivity of serum CCA and CAA in heavily infected individuals than in low to moderate infections. Stothard *et al.* (2006) observed a low sensitivity of urine CCA in detecting light infection with *S. mansoni* in children using dipstick method. Pervious study in Ethiopia also showed that a considerable number of individuals (13.2 %) who had light to moderate infections were found to be negative by reagent strip test (Legesse & Erko, 2007). In the current study, although the reagent strip test has shown higher prevalence of schistosomiasis mansoni infection than stool examination methods, about 23.1 % (18 out of 78) of the study subjects who were excreting eggs of the parasite, but had light infection were found negative by the reagent strip.

A prevalence of a disease could affect the predictive values of a test. In areas where the prevalence of schistosomiasis mansoni is high, the positive predictive value of a reagent strip test was found to be high, while the negative predictive value was low (van Dam *et al.*, 2004; Leggese & Erko, 2007). In this study, the observed low positive predictive value (50 %) and relatively a high negative predictive value (71.9 %) of the reagent strip test could express the low prevalence of the disease in the study area as previously reported (Erko *et al.*, 2001). Measurement of CCA either using ELISA or reagent strip/dipstick in urine or serum of *S. mansoni* infected individuals has been shown to be a promising method to estimate the intensity of infection (van Lieshout *et al.*, 1995; van Dam *et al.*, 2004; Stothard *et al.*, 2006; Leggese & Erko, 2007). In consistent with this previous report, most of the study subjects (65.5 %) who were found to be weak positive by the reagent strip test had light to moderate infections as expressed by the Kato method.

Cross-reactivity of antibodies from individuals harboring hookworm or *Ascaris lumbricoides* against *Schistosoma mansoni* antigens has been concerned (Correa-Oliveira *et al.*, 1988). Since, more than half of the study children had hookworm infection in this study, the frequency of positivity of the reagent strip test in those subjects found negative for *Schistosoma mansoni* infection by stool examination, but had hookworm was looked at. The result has not implicated that infection with hookworm can affect the diagnostic efficiency of the reagent strip test, since almost similar proportion of children (who had no schistosomiasis by stool examination, but had hookworm) was found to be positive and negative by reagent strip test.

CONCLUSION

Despite the many advantages of currently used reagent strip test like field applicability, ease in collecting many urine samples and testing within a short time which facilitate the field activities, the results of this study revealed a relatively reduced sensitivity of reagent strip test in detecting urine CCA in low endemic area of schistosomiasis mansoni. Since sensitivity of a test is an important requirement that truly shows a diseased subject, the relative insensitivity of the reagent strip test necessitates for the improvement of its sensitivity or the development of more sensitive assay that can truly discriminate schistosome-infected from non-infected individuals.

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