

Comparative Assessment of Urine Circulating Cathodic Antigen (CCA) Detection Cassette and Microscopy for the Diagnosis of Schistosomiasis in North Central Nigeria.

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Abstract

This study was carried out between May and December 2019 in four States (Benue, Kogi, Kwara and Niger) of North Central Nigeria to determine the prevalence of schistosomiasis (urinary and intestinal) among primary school pupils using the newly developed Schisto point-of-care (PoC) Urine Circulating Cathodic Antigen (CCA) detection cassette and microscopy in order to evaluate the performance of the CCA detection cassette test. One thousand, one hundred and seventy-six stool and urine specimens were collected from each participant and examined using urine CCA detection cassette test and microscopy (Kato-Katz method for stool and urine filtration techniques for urine specimens).

A total of 524 (40.9%) out of the 1,176 pupils sampled were tested positive using CCA detection cassette, while 381 (33.5%) pupils were positive using microscopy. The difference in the prevalence of schistosomiasis using CCA detection cassette and microscopy was statistically significantly ($p = 0.000$). The sensitivity and specificity of CCA detection cassette using latent class analysis (LCA) were 76.3% and 76.9% respectively, while the sensitivity and specificity of microscopy were 62.5% and 86.5% respectively. The prevalence of schistosomiasis in males and females was 42.6% and 38.2% respectively using CCA detection cassette, while microscopy method had a prevalence of 32.4% in males and 35.3% in females ($p = 0.693$). There were no significant differences in prevalence in both sexes using CCA detection cassette and microscopy.

It was concluded that the newly developed Urine CCA detection cassette having identified more schistosomiasis cases than the old microscopic methods, stands to be more promising for clinical and community diagnosis of schistosomiasis as compared to the old microscopic methods.

Key words: Comparative Assessment, Newly Developed, Urine Circulating Cathodic Antigen, Detection, Microscopy.

Introduction

Schistosomiasis is one of the most prevalent neglected tropical diseases (NTDs) and ranked second only to malaria among the parasitic diseases in terms of socio-economic and public health importance in tropical and subtropical areas by the World Health Organization and the Tropical Diseases Research (WHO – TDR)¹. It is a chronic, debilitating, progressive and granulomatous disease which is not immediately fatal but has enormous medical and public health significance, with an estimated 207 million people infected², and with about 700 million people worldwide at risk of the infection^{3,4}.

The disease is acquired only through direct contact with freshwater such as rivers, stream, ponds that has been contaminated with schistosomes eggs which biologically transform to infective cercariae by undergoing polyembryony. Some species of freshwater snail are exclusive intermediate host of *Schistosoma species* (*Biomphalaria spp* for *S. mansoni*, *Bulinus spp* for *S. haematobium* and *Onchomelania spp* for *S. japonicum*). Schistosomiasis being a disease associated with freshwater contact, is largely occupational disease of fishermen, farmers, miners and staff of bridge/dam construction companies. In endemic areas, children are at risk of acquiring the infection while swimming, playing, washing and carrying out other domestic activities in cercariae contaminated water bodies. Poverty, ignorance, lack of social amenities, poor hygiene, construction of water related schemes (e.g. dams) and lack of political will remain the main risk factors associated with this infection^{5,17,21}.

Diagnosis of this important parasitic infectious disease (Schistosomiasis) remains a great challenge with very cumbersome procedures globally. In Nigeria, like other tropical and sub-tropical countries, the diagnosis of Schistosomiasis (both Urinary and Intestinal) depends wholly on this cumbersome microscopy method which is very time consuming, expensive, with less sensitivity and requires high expertise^{5,6}. Although, several reports have shown low detection of the infection by microscopy in light

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intensity infections, microscopic detection of characteristics eggs of Schistosomes from stool and urine specimens remain the conclusive diagnosis of schistosomiasis. Today, the disease has been earmarked for global control and/or eradication by the WHO, however, the success of control and/or eradication of the schistosomiasis lies significantly on efficient and effective diagnostic procedures/methods.^{7,8,9.}

Recently, a newly developed 'Point of Care Urine Circulating Cathodic Antigen (CCA) Detection Cassette' for Rapid Diagnosis of Schistosomiasis has been introduced⁴.

This study aims to determine and compare the sensitivity and reliability of the newly introduced Circulating Cathodic Antigen (CCA) detection cassette method with the known gold standard Microscopic methods (urine filtration procedure for urinary schistosomiasis and kato-katz technique for intestinal schistosomiasis) for diagnosis of Schistosomiasis (both Urinary and Intestinal)

Materials and Methods

The study was carried out in ten(10) randomly selected primary schools in four (4) local government areas in each of the four (4) states (Benue, Kogi, Kwara and Niger) in North Central Nigeria. While Kwara State bordered Benin Republic in the east with River Niger in the North and Kogi state in the South, Niger State harbors River Niger and Kainji dam, the Nigerian biggest hydro-electric dam and the biggest in West Africa. The state also bordered the Federal Capital of Nigeria, Abuja. Benue State borders the Republic of Cameroun in the West and River Benue in the North and Kogi State is where these two rivers meet in Nigeria. It also shares boundary with Abuja. These rivers with many tributaries and distributaries present very favorable ecological sites for the breeding of the snail intermediate host which aid the transmission of Schistosomiasis¹³.

North Central Nigeria lies within a region described as tropical climate and is characterized by double rainfall maxima and has tropical wet and dry climate. Both seasons last for about six months.

Ethical Issues

Ethical approval for the research work was obtained from the Ethical Review Committee of the Faculty of Basic Medical Sciences, University of Ilorin, Ilorin, Nigeria. Consent was sort and obtained from the Ministries of Health and Education from the four states as well as their State Universal Basic Education Boards (SUBEBs) and the parents and or guardians of the pupils to carry out the study.

Data Collection using standardized questionnaire

With the help of Headteachers who had been trained, pre-tested and standardized questionnaires

were administered to collect data such as name, age, sex, weight, religion, ethnicity, duration of stay in community, presence of blood in stool and urine, water contact habits, and history of deworming of the pupils in each of the selected schools. The height and weight of each pupil were also measured and recorded.

Sample Size and Sampling Technique

The sample size was calculated using the modified Fischer's sample size formulae¹⁴. For a population > 10,000. A total of 1,176 school pupils, participated in the study.

Sampling Technique

Schools that are less than 300 meters away from river, stream or pond were deliberately picked from each local government area because the disease to be studied is associated with fresh water contact. A school and pupils within inclusion criteria were then selected from the deliberately picked schools by simple random technique. Ten primary schools were selected from each State.

Inclusion and Exclusion Criteria

Inclusion criteria

- All the pupils that attended school during the period of sample collection and fall within age range of 5-14 years
- Pupils that are willing to participate in the study

Exclusion Criteria

Pupils who are less than 5 years and those more than 14 years. Those who fall within the age bracket but refused to participate were exempted.

Sample Collection(stool and urine)

The samples were collected during school hours(10:00 – 14:00) because *Schistosome* eggs particularly *S. haematobium* eggs are at peak of excretion within this period¹⁵, and each pupil under thorough supervision, maximum cooperation and reliability with assistance of their class teacher was provided with a clean labeled plastics universal bottle with screw cap, a disposable plastic cup, a sheet of cardboard paper and a wooden spatula. Both the stool and urine samples were simultaneously collected from the pupils with the help of their class teachers.

The stool specimens after collection were preserved by adding 10ml of 10% formalin¹⁷ using 10ml disposable syringe as a standard procedure and covered tightly with screw cap.

100µl of urine (with aid of disposable calibrated micropipette) was drawn from each plastic cups immediately after submission and used for rapid presumptive detection of schistosomiasis using Circulating Cathodic Antigen (CCA) detection cassette for schistosomiasis.

Disposable wooden spatulas were then used

carefully to stir the urine specimens in plastic cups to enhance equal distribution of *S. haematobium* eggs and 10ml of the urine was taken using 10ml disposable syringe and dispensed into clean labeled plastic universal containers. The urine specimens were preserved by adding 10ml of 70% alcohol to the 10ml of urine in each universal container and covered tightly with the screw cap.

Urine examination

Urine specimens were examined macroscopically for visible haematuria and turbidity. The intensity of the haematuric/turbid urine was recorded as light (+), moderate (++), heavy (+++) and non-haematuric (-).

(a) Rapid presumptive test for schistosomiasis using CCA cassette

100µl of urine was drawn from each specimen with aid of disposable calibrated micropipette and dropped on the sample chamber of the CCA cassette. The solution was then allowed to run over the strip where the

antigen-antibody complex attaches to the monoclonal antibody immobilized at the test line for 20 minutes. The presence or absent of schistosomiasis was recorded as Positive (+ve) or Negative (-ve) respectively.

(b) Microscopic examination of urine

The preserved urine specimens were proceeded according to standard procedure^{17, 18, 19}

Data Analysis

Data obtained from questionnaires and laboratory analysis were entered into Microsoft excel and analyzed using Statistical Package for Social Sciences (SPSS) version 21.00 California, USA. Statistical analysis was carried out at the significance level of $p < 0.005$. Chi square and Student t-test were used to compare the prevalence.

Results

A total of 1,176 pupils in 40 selected primary schools (10 from each of the four States) were



Fig. 1a. CCA positive Test

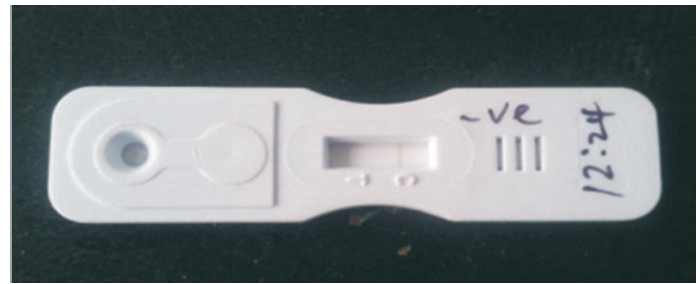


Fig. 1b. CCA Negative Test



Fig. 2a: Macroscopic Examination of Urine Samples showing Different Levels of Haematuria among Schistosomiasis Positive Urine Specimens.

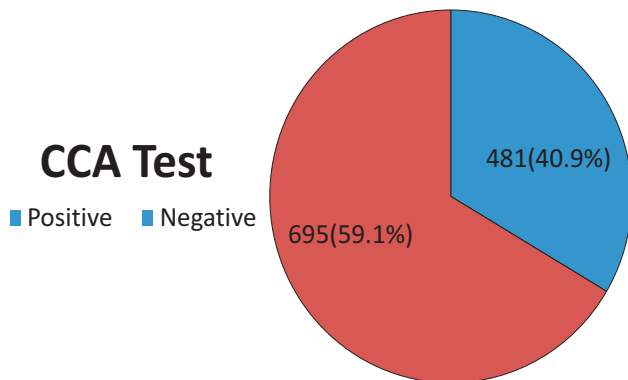


Fig. 2b

Microscopy

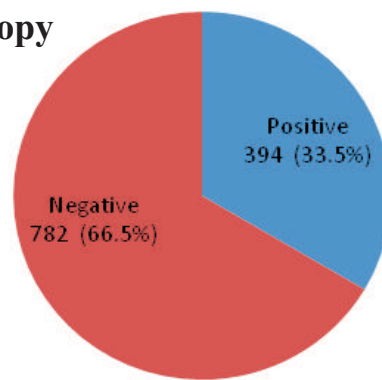


Fig. 2c

Figs. 2b & 2c: Prevalence of Schistosomiasis using the CCA and the Microscopy Methods Respectively.

Table 1: Prevalence of both Urinary and Intestinal Schistosomiasis by Age Using Urine Microscopy, Stool Microscopy and CCA Test Kit.

Age (Years)	Stool Microscopy		Total	Urine Microscopy		Total	CCA Test		Total
	Positive(%)	Negative(%)		Positive(%)	Negative(%)		Positive(%)	Negative(%)	
5-9	72 (9.4)	696(90.6)	768	262 (34.1)	506(65.9)	768	327 (42.6)	441(57.4)	768
10-14	27 (6.6)	381 (93.4)	408	132(32.4)	276(67.6%)	408	154 (37.7)	254(62.3)	408
Total	99 (8.4)	1077(91.6)	1176	394(33.1)	782(66.5)	1176	481 (40.9)	695(59.1)	1176

The difference between the detection of schistosomiasis by Microscopy and CCA Test Kit is statistically significant ($P=0.011$).

Table 2: Prevalence of Schistosomiasis (Urinary and Intestinal) by Gender, Age and Weight using CCA Test and Microscopy.

Variable	Microscopy			CCA		
	Positive (%)	Negative (%)	Total	Positive (%)	Negative (%)	Total
Gender						
Males	234 (32.4)	488 (67.6)	722	308 (42.7)	414 (57.3)	722
Females	160 (35.2)	294 (64.8)	454	173 (38.1)	281 (61.9)	454
Total	394 (33.5)	782 (66.5)	1,176	481 (40.9)	695 (59.1)	1,176
Age (Years)						
5-9	274 (35.7)	494 (64.3)	768	327 (42.6)	441 (57.4)	768
10-14	120 (29.4)	288 (70.6)	408	154 (37.7)	254 (62.3)	408
Total	394 (33.5)	782 (66.5)	1,176	481 (40.9)	695 (59.1)	1,176
Weight (Kg)						
<26	154 (39.1)	240 (60.9)	394	167 (42.4)	227 (57.6)	394
26-33	112 (23.2)	370 (76.8)	482	165 (34.2)	317 (65.8)	482
34-41	118 (43.7)	157 (56.3)	270	125 (46.3)	145 (53.7)	270
>41	10 (33.3)	20 (66.7)	30	24 (80.0)	6(20.0)	30
Total	394 (33.5)	782 (66.5)	1,176	481 (40.9)	695 (59.1)	1,176

- More males were infected than females ($P= 0.042$)
- The difference in infection using both methods, by age group, is statistically insignificant ($P=0.000$)
- Weight loss and the presence of schistosomiasis does not indicate a particular pattern.

examined for both urinary and intestinal schistosomiasis. The overall prevalence of urinary schistosomiasis was 40.9% using CCA detection cassette and 33.1% using microscopy method. In the case of intestinal schistosomiasis, only the microscopic method was able to identify the prevalence of intestinal schistosomiasis to be 8.4%. (Figs: 1a&b, 2a, b, c and Table 1)

The distribution of schistosomiasis based on gender varies significantly within the two methods ($P>0.05$). Whereas 35.2% of females were tested positive for schistosomiasis using the conventional microscopy method, the CCA test recorded 38.1% and as high as 42.7% in males. (Table 2).

The trend of infection according to age group was the similar using the two methods but with higher percentage in the CCA test. Microscopy recorded 35.7% among the age group of 5-9 years while CCA test recorded 42.6%. On the other hand, 29.4% and

37.7% were recorded in the age group of 10-14 years by microscopy and CCA tests respectively. (Table 2).

The pattern of infection in relation to weight however showed no particular arrangement as 43.7% was recorded in the higher weight of 34-41 kg and 39.1% in the >26 kg weight using the microscopy method and 46.3% in the 34-41kg weight and as low as 34.2% in the 26-35kg weight using the CCA test. (Table 2). In table 3, where a combined result using the two methods showed a 40.9% prevalence in both microscopy and CCA tests, 33.1% were positive only by microscopy as confirmed by the CCA test and only 10.5% were positive by CCA test but negative by microscopy.

In relation to haematuria, 30.1% of non-haematuric urine were positive for schistosomiasis using CCA test and 23.7% using microscopy. By both methods, 100% of the haematuric urines were positive for schistosomiasis. (Table 4).

Table 3. Positivity and Sensitivity of CCA and Microscopy for Detection of Schistosomiasis N= 394

Positivity Level	Methods		
	CCA	Microscopy	Total
True Positive by all (A)	+	+	481 (40.9%)
Positive by microscopy only (B)	-	+	Urine-394 (33.1%) Stool- 99 (8.4%)
Positive by CCA only (C)	+	-	124 (10.5%)

A= Urine specimens positive for both CCA and Microscopy Methods

B= Urine and Stool samples positive by microscopy only.

C= Urine samples not positive by microscopy but positive by CCA.

Table 4. Prevalence of Schistosomiasis by Haematuria using CCA Test Kit and Microscopy

Urine Macroscopy	CCA Test			Microscopy		
	Positive (%)	Negative (%)	Total	Positive (%)	Negative (%)	Total
Normal urine	247 (31.1)	54 (68.7)	788	187 (23.7)	601 (76.3)	788
Light haematuria	187 (54.8)	154 (45.2)	341	160 (46.9)	181 (53.1)	341
Moderate haematuria	40 (100)	0 (0.0)	40	40 (100.0)	0 (0.0)	40
Heavy haematuria	7 (100)	0 (0.0)	7	7 (100)	0 (0.0)	7
Total	481	695	1,176	394	782	1,176

The higher the haematuria, the higher prevalence using both methods.

Discussion

The overall prevalence of schistosomiasis by CCA detection Cassette Test Kit (40.9%) and microscopy (33.5%) as established from this study indicates that the disease is still endemic among the vulnerable primary school age pupils in North Central Nigeria. Reports from other studies in the same region had shown high prevalence of schistosomiasis^{20,21,2,7,10,22}.

While previously, it had been only the use of microscopy in the diagnosis of both urinary and intestinal schistosomiasis, the newly developed Urine Circulating Cathodic Antigen Detection Kit has detected more cases of true positive results as compared to the conventional microscopy methods. Also, another highly advantageous point of this kit is that it was able to detect the circulating antigens from both the intestinal urinary schistosomiasis at once. The limitation however is that for now, this kit has not been able to differentiate *S. mansoni* (intestinal schistosomiasis) induced antigens from those of *S. haematobium* (urinary schistosomiasis). However, successes recorded by the detection of schistosomiasis through CCA test has outweighed the limitation as mentioned above. Interestingly, 481 (40.9%) out of the 1,176 samples examined was positive for CCA test kit as compared to 394 (33.1%) from the usual microscopy methods. It was a clear demonstration of the promise in the use of the newly developed CCA method.

Generally, diagnosis of schistosomiasis like

other tropical disease has remained very cumbersome, time consuming and demands laboratory experience for accurate detection of infection¹⁵, however, with less than two minutes, the results from highly sensitive, less cumbersome can be read. This method involves less expertise, therefore, the development of CCA test is heartwarming for rapid diagnosis.

Comparison between result of CCA detection cassette and Microscopy shows that CCA (40.9%) appears to be more sensitive than Microscopy (33.5%). Using Pearson Chi-Square, there is high significant difference ($P = 0.000$) between performance of CCA detection cassette and microscopy. The sensitivity and specificity of CCA detection cassette using latent class analysis (LCA) were 76.3% and 76.9% respectively, whereas the sensitivity and specificity of microscopy were 62.5% and 86.5% respectively. This agrees with the findings of *Fernanda*²³ who analyzed the sensitivity and specificity using the two methods. In the first analysis, they compared the results of the detection of *S. mansoni* eggs in the stool using the Kato-Katz method. The sensitivity and specificity of the POC-CCA assay using latent class analysis (LCA) were 68.1% and 72.8%, respectively, whereas the sensitivity and specificity of the Kato-Katz method using LCA were 25.6% and 94.6%, respectively. The result of the study shows that sensitivity of CCA detection cassette is higher than its specificity which means it is highly sensitive but less specific. In contrast, specificity of microscopy is higher than its sensitivity, which means its

highly specific but less sensitive. Shane has reported that the sensitivity of the POC-CCA assay was 94.2% and the specificity was 59.4% in Kenya²⁴.

Statistical analysis shows that a total of 124 (10.5%) pupils were tested positive (for schistosomiasis) using CCA detection cassette test and negative using microscopy. In this case, immature (Schistosomula) worms during early stage of re-infection may be releasing antigens (such as CCA) that are detectable by CCA detection cassette, before eggs are excreted in stool/urine of infected person. This, probably could make some people tested positive using CCA detection cassette and negative using microscopy. According to studies^{25,26,27} immature worms during the acute phase or in recently re-infected cases of schistosomiasis may produce worm antigens (e.g., CCA) before eggs are excreted in the stool. This process may result in a positive result using the POC-CCA assay and a negative result using the Kato-Katz technique.

As previously reported^{17,21} this study has also shown that the most vulnerable age group is between the active age bracket of 5-9, years with a high prevalence of 9.8% (using stool microscopy for intestinal schistosomiasis), 34.1%, using microscopy for urinary schistosomiasis and 42.6% for the both urinary and intestinal schistosomiasis using the CCA test kit. In most literature^{28,23,29} the pattern is the same as compared with this study and a higher prevalence is demonstrated in the use of the CCA Test kit method.

Previous studies show that male pupils were found to be more infected than the female pupils of the same age group^{6,7,8}, this study, by the use of microscopy has demonstrated a slight difference in which there was 35.2% prevalence in females as compared to 32.4% male prevalence. Using CCA test, more males (42.7%) were infected than their female counterparts (38.1%). In all cases, it is suggested that the rate of water contact activities ultimately determines the rate at which both genders are infected. For instance, in 2015, a high prevalence of urinary schistosomiasis in males was attributed to high farming activities and the tendency of boys to play more in water as compared to girls³⁰. Whereas some findings^{16,17} showed a high prevalence of the disease among the females as compared to the males. In our case, we have generally higher males been infected than females, and this is likened to other findings^{6,7,8}.

For weight (in Kg), it is generally believed that the higher the weight, the less the rate of the detecting the presence of schistosome infection, however, the result from this study, in which pupils with higher weight were also reported to have higher infection rate is an indication of the high sensitivity of CCA detection method. The difference between the two methods were found to be statistically significant in detecting schistosomiasis.

Our study found relation between haematuria and prevalence of schistosomiasis as earlier reported by Edungbola and others¹⁸. Although the ability of this method to detect the schistosomiasis positivity for both *S. mansoni* and *S. haematobium* is novel, it does not distinguish between these two most common species when both are found in a patient.

We therefore recommend that the CCA test could be routinely used as alternative for both clinical and community diagnosis of schistosomiasis. More research work should be carried out to advance this method so that a single detection should differentiate *S. mansoni* and *S. haematobium* infections.

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