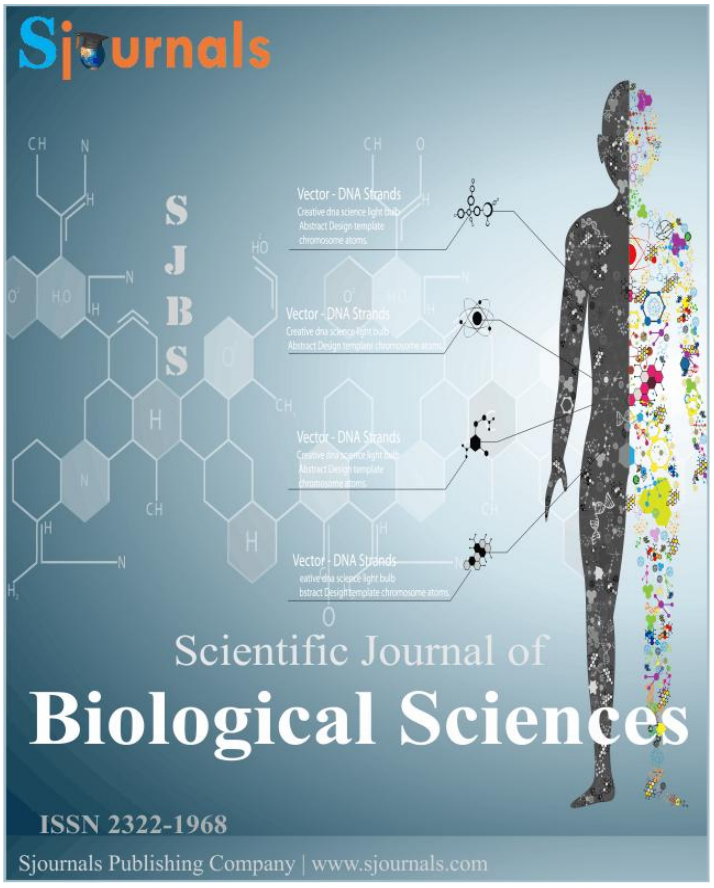

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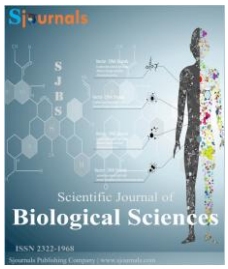

Original article

Comparative studies of immunochromatographic strips and microscopy in the diagnosis of urinary schistosomiasis in selected children in Zaria, Nigeria

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ABSTRACT

Schistosomiasis remains endemic in many developing countries due to poor hygiene and access to potable water sources. The study was conducted to determine the prevalence of urinary schistosomiasis in selected children. A total of 100 urine samples were collected randomly from the selected population and screened using the CCA rapid immunochromatographic test strips and microscopy for the ova of the *Schistosoma haematobium*. It was observed at the end that out of the 100 samples examined, the prevalence was found to be 8(8%) for microscopy and 14(14%) for immunochromatographic test strips. The observation shows similar prevalence of *S. haematobium* infection in children; with the highest prevalence occurrences in male children. The age group 9-11 years had the highest prevalence rate of 23.8% and 38.1% for microscopy and immunochromatographic test strips respectively and males were more infected than females. Of 48 male subjects examined, 5(10.4%) were positive for microscopy while 10(20.8%) were positive for strips. Of 52 females, 3(5.8%) were positive for microscopy while 4(7.7%) were positive for strips. For 70%, their source of water for domestic purpose was bore hole, 20% was from the well, 4% from river/stream and 6% were from other sources. A total of 10% did swim regularly in nearby water bodies and 6% of the children go fishing.The risk factors associated with *S. haematobium* in this survey were drinking water from lakes,

swimming in lakes or ponds and presence of snails (*Bolinus*) in such lakes. Sanitary control measures should be put in place in the areas so as to prevent people from defecating in the open.

1. Introduction

Parasitic infections caused by pathogenic protozoa affect over a billion people worldwide and impose substantial health and economic burden on tropical less developed countries where they are prevalent (Ochoa et al., 2001). Schistosomiasis is the most frequent parasitic disease that remains endemic in 76 countries of the tropical and subtropical countries of Asia, Africa and South America with an estimated 207 million persons infected with the disease. Africa accounts for more than 90% of current cases of schistosomiasis (Engels, 2002; WHO, 2010; Gryseels, 2012). It is also the most prevalent of the waterborne diseases and poses one of the greatest risks to health in rural areas of developing countries (Ogbe, 2002). The WHO 2004 report indicated that Schistosomiasis remains second only to malaria in respect to its socio-economic effect on the prevailing population (WHO, 2004). Although it has low mortality rate, schistosomiasis remains a chronic disease leading to damage of the urinary bladder, impaired growth and cognitive development in children mostly infected (Emejula et al., 1999). John et al., (2006), reported that in sub-saharan Africa, roughly 70 million persons suffer from *Schistosoma haematuria*, 18 million from associated bladder wall pathology while 10 million from hydronephrosis. Reports in Nigeria indicate that schistosomiasis due to *S. haematobium* is widespread, constituting a public health problem particularly in children (Sulayman et al., 2009; Fana et al., 2010; Akinboye et al., 2011) with a high prevalence in five states that include Benue, Niger, Katsina (Malumfashi), Anambra and Kwara States (Bello et al., 2002). It has been estimated that 150,000 people die each year from resultant renal failure while an unknown but significant number die from bladder and other genitourinary cancers (John et al., 2006). Transmission occurs in stagnant or slow moving fresh water teaming with *Bulinus* snail that serve as the intermediate host and important in the life cycle of the parasite (Abolarinwa, 2000). Transmission rates to populations that have frequent exposure to water (e.g fishermen, farmers, working in irrigation canals, women fetching water for use and patients who swim regularly) are significantly high (Robert, 1993). The anthropogenic creation of new snail habitat through building dams or irrigation canals may increase rates of transmission to nearby human population (CDC, 2006). *S. heamatobium* is transmitted by cercariae penetrating the skin when bathing, washing clothes, fishing or engaged in agricultural work or other activities involving contact with contaminated water (Cheesbrough, 1998). In most endemic areas, a large proportion of patients and teenagers become infected and reinfected (Cheesbrough, 1998). The most common method employed in its diagnosis is identification of the characteristic terminal spined ova in urine (CDC, 2006), in biopsies of the bladder, rectum or vaginal wall (CDC, 2006). History of residence in an endemic area, presence of swimmers' itch and terminal haematuria anaemia, high eosinophil levels, and low blood platelets level are other useful informations considered (Brown and Neva, 1983). Immunodiagnosis may be useful for demonstration of active chronic urinary schistosomiasis specifically the circum oval precipitation test (COP) in which precipitate is formed around the eggs containing live miracidia after incubation in the serum of infected individuals (Piekarski, 1989). The enzyme linked immunosorbent assays (ELISA) is also widely used in diagnosis for the detection of circulating anodic antigen (CAA) and circulating cathodic antigen (CCA) in serum and urine samples of infected persons (Nmorsi et al., 2004). ELISA serology may be positive in the second (acute) stage of disease i.e. 3-8 weeks after exposure (Chindawana and Taylor, 1998). The aim of this study therefore is to determine the prevalence of urinary schistosomiasis in children using the CCA rapid immunochromatographic test strips and microscopy.

2. Materials and methods

2.1. Study area and sample size

The study was conducted Samaru, located in Sabon Gari Local Government Area of Kaduna State. Samaru is a densely populated semi-rural and semi-urban community with about 4 (four Public Primary Schools and several

private schools. It is an area known for acute shortage of potable water and most depend on borehole and wells for their water supply. There are two large dams, the ABU water treatment Dam and the University farm irrigation Dam where occasionally children go for bathing and other activities. Mollusc controls are not routinely carried out on these dams and could be a risk factor for the children. Prior to sample collection, a structured questionnaire was administered on the participants to obtain demographic and risk factors that might be associated with the disease. A total of 100 urine samples were samples randomly collected from both male and female children between the age group of 3-14years that received treatment at Samaru clinic and used for the study. The samples were collected in the morning in sterile screw cap bottles. The urine samples collected were transported to the Department of Microbiology Laboratory Ahmadu Bello University using black polythene bag placed in a box to prevent the ova from hatching.

2.2. Urine microscopy to determine the presence of schistosome ova

Diagnosis by microscopy as described by Piekarski (1989) was employed. About 10ml of a duly labeled urine sample was centrifuged at 2000-3000 rpm for 5 minutes in order to concentrate egg of the schistosome. The supernatants were discarded leaving about 0.2ml behind in each tube (Feldmeir et al., 1982). The re-suspended deposit/ sediment were each transferred onto different slides with the aid of a Pasteur pipette which was rinsed in between transfers. Covers slips were then placed on the slide and observed microscopically for eggs of *S. haematobium*

2.3. Determination of urine circulating cathodic antigen

A one-step, genus specific CCA (circulating cathodic antigen) strip was used. The CCA Strip was opened and placed on a flat surface, and then a drop of fresh urine was added to the sample zone of the test, followed by addition of two drops of schistosomiasis buffer on the same zone. Within 25mins, result was read from the test zone after comparing it to the control zone band and recorded as negative, weak positive and strong positive.

2.4. Determination of the sensitivity and specificity of the circulating cathodic antigen test using microscopy as standard

Sensitivity (also called the true positive rate) is a measure of the proportion of positives that are correctly identified while Specificity (also called the true negative rate) measures the proportion of negatives that are correctly identified.

Sensitivity or true positive rate (TPR) = TP/ (TP+FN)*100
Specificity or true negative rate (TNR) = TN/ (TN+FP)*100
Positive predictive value (PPV) = TP/ (TP+FP)*100
Negative predictive value (NPV) = TN/ (TN+FN)*100
Accuracy = (TP+TN)/ (TP+FP+FN+TN)*100
TP- True Positive; TN- True Negative; FP- False Positive; FN- False Negative

3. Results

The prevalence of *Schistosoma haematobium* among children as determined by microscopy and the CCA strips was found to be 8% and 14% as presented in Fig. 1. Of 48 male subjects examined, 5(10.4%) were positive for microscopy while 10(20.8%) were positive for strips. Of 52 females, 3(5.8%) were positive for microscopy while 4(7.7%) were positive for strips. There was no statistical significance between the infection and gender. (P=0.475, P=0.058). The age range 9-11 years had the largest number of positive cases for both microscopy and strips where there is significant association (P<0.05) between the infection and ages (P=0.020, P=0.004 respectively) (Table 4.1). It was found that the occupation of the children's parents was significantly associated with the prevalence of the infection for microscopy (P=0.048) but not with the CCA (P=0.272) as presented in table 1.

Symptom such as pain while urinating Presence of blood in urine joint and muscle pain and fever were significantly associated with the disease while abdominal pain was not (Table 2).

Similarly, swim regularly in nearby water bodies and fishing by the children were the risk factors found to be significantly associated with the infection in the study area (Table 3).

3.1. Sensitivity of the circulating cathodic antigen test as compared to microscopy

The result showed that the circulating cathodic antigen test had a sensitivity and Specificity of 100% and 93.9% respectively. The positive and negative predictive values were 57.1% and 100% with an accuracy of 94.3%.

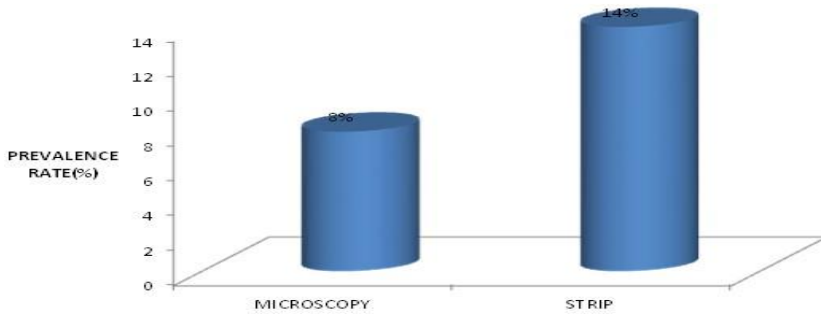

Fig. 1. The Prevalence of Urinary Schistosomiasis among the study population using Microscopy and CCA Chromatographic Test Strips.

Table 1
Association between Demographic Factors and Number Tested Positive for Urinary Schistosomiasis.

		Microscopy				Strips			
Demographic factors	**No examined**	**No (%) positive**	**No (%) negative**	**χ^2**	**P-value**	**No (%) positive**	**No (%) negative**	**χ^2**	**P-value**
Age (years)									
0-5	6	0(0.0)	6(100)			0(0.0)	6(100.0)		
6-8	21	0(0.0)	21(100)	9.831	0.020*	1(4.8)	20(95.2)	13.422	0.004*
9-11	21	5(23.8)	16(76.2)			8(38.1)	13(61.9)		
>12	52	3(5.8)	49(94.2)			5(9.6)	47(90.4)		
Gender									
Male	48	5(10.4)	43(89.6)	0.732	0.475	10(20.8)	38(79.2)	3.580	0.058
Female	52	3(5.8)	49(94.2)			4(7.7)	48(92.3)		
Occupation									
Civil Servant	28	4(14.3)	24(85.7)			5(17.9)	23(82.1)		
Farmer	31	4(12.9)	27(87.1)	6.081	0.048*	6(19.4)	25(80.6)	2.605	0.272
Unemployed	41	0(0.0)	41(100)			3(7.3)	38(92.7)		

χ^2 =Chi square, *=Significant association at P≤0.05.

Table 2
Association between Symptoms and Number Tested Positive for Urinary Schistosomiasis.

		Microscopy				Strips			
Symptoms	**No examined**	**No (%) positive**	**No (%) negative**	**χ^2**	**P-value**	**No (%) positive**	**No (%) negative**	**χ^2**	**P-value**
TFPWU									
Yes	7	5(71.4)	2(28.6)	41.144	0.000*	5(71.4)	2(28.6)	20.618	0.000*
No	93	3(3.2)	90(96.8)			9(9.7)	84(90.3)		
TEBIN									
Yes	2	2(100.0)	0(0.0)			2(100.0)	0(0.0)		
No	90	6(6.7)	84(93.3)	23.913	0.000*	12(13.3)	78(86.7)	13.621	0.001*
No response	8	0(0.0)	8(100)			0(0.0)	8(100.0)		
THAP									
Yes	20	3(15.0)	17(85.0)			4(20.0)	16(80.0)		
No	65	5(7.7)	60(92.3)	2.644	0.267	10(15.4)	55(84.6)	3.143	0.208
No response	15	0(0.0)	15(100)			0(0.0)	15(100.0)		

TFJMP									
Yes	10	4(40.0)	6(60.0)			4(40.0)	6(60.0)		
No	85	4(4.7)	81(95.3)	15.601	0.000*	10(11.8)	75(88.2)	6.781	0.034*
No response	5	0(0.0)	5(100)			0(0.0)	5(100.0)		
THV									
Yes	6	3(50.0)	3(50.0)	15.298	0.000*	3(50.0)	3(50.0)	6.871	0.009*
No	94	5(5.3)	89(94.7)			11(11.7)	83(88.3)		

Key: TFPW –Those that feel pain while urinating; TEBIN- Those that experience blood in urine; THAP- Those that have abdominal pain; TFJMP- Those that feel joint and muscle pain; THV- Those that have fever.

Table 3
Association between Risk Factors and Number Tested Positive for Urinary Schistosomiasis.

Risk factors	No examined	Microscopy				Strips			
		No (%) positive	No (%) negative	χ^2	P-value	No (%) Positive	No (%) negative	χ^2	P-value
SWFDP									
Borehole	70	5(7.1)	65(92.9)			11(15.7)	59(84.3)		
Well water	20	3(15.0)	17(85.0)	2.271	0.518	3(15.0)	17(85.0)	1.815	0.612
River/Stream	4	0(0.0)	4(100.0)			0(0.0)	4(100.0)		
Others	6	0(0.0)	6(100.0)			0(0.0)	6(100.0)		
TSIR									
Yes	10	5(50.0)	5(50.0)	26.630	0.000*	5(50.0)	5(50.0)	11.960	0.001*
No	90	3(3.3)	87(96.7)			9(10.0)	81(90.0)		
TGF									
Yes	6	3(50.0)	3(50.0)			3(50.0)	3(50.0)		
No	92	5(5.4)	87(94.6)	15.377	0.000*	11(12.0)	81(88.0)	7.103	0.029*
No response	2	0(0.0)	2(100.0)			0(0.0)	2(100.0)		

Key: SWFDP- Source of water for domestic purpose; TSIR- Those that swim in the river; TGF- Those that go fishing.

4. Discussion

Diagnosis of human schistosomiasis is very central to making a decision on individual case management, at all stages of control programs and for comparing control programs (Feldmeier et al., 1993; Sturrock, 2001). Although the most reliable way to diagnose urinary schistosomiasis is the detection of eggs in urine by filtration method, failure to recover eggs does not rule out the possibility of infection for a number of reasons. Hence, a diagnostic tool should be sensitive enough to detect all infected individuals irrespective of duration of infection. The sensitivity of the strips compared to microscopy was a 100% while the specificity was 93.9% and the accuracy was 94.3%. This shows that the use of CCA strips for the diagnosis of urinary schistosomiasis are quiet effective. In previous studies, sensitivity for circulating antigens in general has been reported to be between 65 and 85% (De jonge et al., 1991; van Lieshout et al., 1992; Stothard et al., 2006). The positive predictive value was rather low but since the predictive values depend on the prevalence and intensity of infection, in higher endemic settings the positive predictive value may be different.

A prevalence rate of 8% and 14% for urinary schistosomiasis was observed for microscopy and the CCA strips respectively among the children that visited the Samaru clinic, Zaria within the study period. According to the classification of the WHO Expert Committee on the control of schistosomiasis (WHO, 1985) prevalence greater than 25% are moderate while those below are low. The rapid assessment indicators of morbidity used in these study, which are the visual observation for eggs of *S. haematobium* microscopically and the diagnostic reagent strip gave different results. The diagnostic reagent strip technique gave a prevalence of 14% while the observation for eggs of *S. haematobium* microscopically produced a prevalence of 8% for the same group of people. The absence of schistosome eggs in urine samples after centrifugation may be due to the presence of light infections where eggs may be difficult to detect (Garcia and Bruckner, 1997). Mafe (1997) indicated that the diagnostic reagent strip test can be used to detect all infected persons who are at risk of urogenital disease. The test is very

suitable for mass screening for urinary schistosomiasis being very fast, producing immediate results. The higher prevalence of the infection in the older age-groups is consistent with previous reports of Chandiwana (1987), Mafiana and Omotayo (1994) and Emejulu and Co-workers (1994). The higher infection rate in older age groups might be a reflection of susceptibility to *S. haematobium* and/or of increased contact with infected water through swimming and fishing (Mafiana and Omotayo, 1994). Prevalence was higher in males and this could be attributed to the fact that boys engage more in swimming in ponds of stagnant water than girls. Such practices could increase the risk of infection. The age group 9-11 years had the highest prevalence.

Response to questions posed to the children indicated that feeling pain while urinating, experiencing blood in urine, having fever, swimming in the river and fishing in the river are highly significant symptoms and risk factors associated with urinary schistosomiasis. This report is similar to that observed by Udonsi (1990) in Igwun river basin of Nigeria. Contrary to this report are those of Ekejindu et al., (2002).

5. Conclusion

A prevalence rate of 8% and 14% for urinary schistosomiasis was observed for microscopy and the CCA immunochromatographic strips respectively. Major risk factors that contributed to the prevalence of urinary schistosomiasis in this study were swimming in the river and going fishing.

In conclusion, the study has identified an intermediate level of knowledge, attitude and practice on the transmission of urinary schistosomiasis among children in Samaru, Zaria which were some of the factors among others that contributed to the prevalence of urinary schistosomiasis among school going children aged between 3 and 14 years. This medium level of knowledge about urinary schistosomiasis will need to be addressed with intensive health education both at school and community level. Such health education should focus on the cause and modes of transmission as well as the health impacts associated with urinary schistosomiasis infection. The role of MDA (Ministries, Departments and Agencies) should be addressed so that all school children should participate during administration of the drugs since it will help to cure the disease for those with the infection and thus reduce the transmission of the disease to others. Provision of safe recreational water sources for children and pipe borne water supply for house hold use are suggested urgent interventions.

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