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Detection of Urinary Analytes due to *Schistosoma haematobium* Infection among School Children for Possible Application in Screening for Urinary Schistosomiasis

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Abstract

Urinary schistosomiasis is a disease that has persisted for a long time among children in Nigeria and other African countries. The persistence of schistosomiasis in people living in rural communities is attributed to the abundance of unsafe water bodies where both children and adults engage in activities such as irrigation, bathing, fishing, washing, or fetching water for domestic use. The lack of adequate community-based screening and treatment of infected cases has contributed to the continuous spread of schistosomiasis from endemic communities to new areas. Urinalysis can serve as a rapid method for screening for urinary schistosomiasis among populations in remote locations, enabling immediate treatment and the prevention of its transmission. In this study, 600 school children who gave their consent were enrolled across six Local Government Areas in Kaduna State, Nigeria. A fresh 10mL urine sample was collected from each subject, and eleven analytes were immediately determined upon submission using urine reagent test strips (SG11100-Uric 11V, Guilin Zhonghui Technology Co., Ltd, China). The urine samples underwent centrifugation at 3000 revolutions per minute for 5 minutes, and the sediment from each sample was examined microscopically for Schistosoma haematobium eggs. The data were analyzed statistically at a 95% confidence interval using IBM SPSS Version 23. The overall prevalence of urinary schistosomiasis among the children in the six LGAs of Kaduna State was 6.8%, with heavy infections at 2.2% and light infections at 4.7%. Significant associations were found between urinary schistosomiasis and the presence of leukocytes (18.5%, x²=47.596, df=1, P=0.000, OR=8.822), proteinuria (23.1%, x²=17.287, df=1, P=0.000, OR=4.959), and micro-hematuria (49.3%, x²=213.184, df=1, P=0.000, OR=63.695). This study demonstrates that urinalysis can be effectively utilized for screening urinary schistosomiasis in remote communities where advanced techniques like microscopy may not be available, facilitating prompt treatment initiation. Therefore, early detection and treatment are crucial in preventing the chronicity and community spread of the disease.

Keywords: Urinalysis, urinary schistosomiasis, early detection, rural communities, children

INTRODUCTION

Schistosomiasis is considered one of the most common neglected tropical diseases affecting humans. It is caused by blood flukes of the genus *Schistosoma*. The disease can manifest acutely in individuals infected for the first time, while those living in endemic regions may experience a mild or chronic form of the illness (WHO, 2022). It is most prevalent in rural areas and certain urban centers of tropical and subtropical countries. Communities lacking access to safe drinking water and proper sanitation are particularly susceptible to schistosomiasis (Osada and Kanazawa, 2011; Shiff, 2015; WHO, 2022).

Schistosomes are complex multicellular blood flukes (Edward and Andrew, 2002; Bishop *et al.*, 2016). Six species of *Schistosoma* can infect humans, namely *Schistosoma* haematobium, *Schistosoma* mansoni, *Schistosoma* japonicum, *Schistosoma* intercalatum, *Schistosoma* mekongi, and Schistosoma guineensis (Jamjoom, 2006; WHO, 2022).

There are two forms of schistosomiasis: urinary schistosomiasis and intestinal schistosomiasis.

The species of schistosome that infects a host and its predilection site define the form of the disease. However, both forms of the disease are commonly referred to as bilharzia, bilharziasis, or snail fever (Jamjoom, 2006; Nour, 2010). Schistosomiasis mainly affects children who engage in indiscriminate water activities. Adults practicing unsafe irrigation farming systems are also at risk of infection (Kanwai et al., 2011; Ibironke et al., 2012; Elele and Ewurum, 2013; Bishop, 2017; WHO, 2022). The only known route of schistosome infection is through skin penetration by cercariae (WHO, 2022). When humans engage in activities involving infested water, cercariae are attracted by warmth and lipids on the skin (Sakanari and Mckerrow, 2010).

Urinary schistosomiasis affects children in various ways: it is a risk factor for renal dysfunction (Kayange *et al.*, 2015), causes anemia (Bishop *et al.*, 2016), growth stunting (Oyibo *et al.*, 2011), and impairs cognitive ability in children (Engels *et al.*, 2002; Kanwai *et al.*, 2011).

Laboratory detection of urinary schistosomiasis is typically done through the microscopic identification of ova with terminal spines in urine sediment, considered the gold standard (Cheesbrough, 2009; Ibironke et al., 2012; Barsoum, 2013). However, the efficacy of microscopy in screening for the disease can be hindered by the absence of schistosome ova during the early stages of infection or in cases of low infection rates. In many remote areas, there is a lack of rapid mass screening methods, delaying early treatment interventions and contributing to the continued spread of the within communities. Despite disease advancements in science and technology in the 21st Century, inadequate access to clean water remains a significant issue in some regions. The absence of safe water sources forces individuals to utilize contaminated alternatives such as slow-flowing streams, rivers, ponds, and large puddles for various domestic and agricultural activities. These water bodies are at a persistent risk of infestation by snails and cercariae (Bishop, 2017).

Urinalysis is useful in screening for various renal or kidney diseases, metabolic disorders, urinary tract infections, and monitoring patients with diabetes (Steggall, 2007; Zamanzad, 2009). It is a simple test that does not require advanced tools, making it particularly valuable for screening urinary schistosomiasis in remote rural communities where advanced diagnostics may

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be delayed or unavailable (Cheesbrough, 2009). The development of new diagnostic tools, drugs, and vaccines is crucial for the elimination of schistosomiasis (Brindley and Hotez, 2013). Creating awareness of schistosomiasis in rural communities is essential, as this population often migrates to urban areas. Infected individuals can unknowingly contribute to the spread of the disease by contaminating water bodies with snail hosts through the discharge of urine or feces (Bishop, 2017).

This study was aimed at detecting ova of Schistosoma haematobium by gold-standard microscopy among school children in Kaduna State, Nigeria; and to screen for urinary analytes that may serve as indices of the disease. Such indices can be validated and applied for community-based screening/surveys of urinary schistosomiasis in remote locations where microscopy may not be available or delayed. Validated urinarv indices of urinarv schistosomiasis can also be used in monitoring of treatment progresses. This study also aimed to create awareness on the danger of use of unsafe bodies of water among children across selected schools in the study area.

MATERIALS AND METHODS

Study Area and Population

The study was conducted across six Local Government Areas (LGAs), including two LGAs from each of the three senatorial zones of Kaduna State in Nigeria. The selected LGAs were Jaba and Kachia in the South senatorial zone, Kaduna South and Giwa in the Central senatorial zone, and Sabon Gari and Makarfi in the North population senatorial zone. The studv constituted a cross-section of 600 consented school children drawn from public secondary schools across the selected LGAs. Ethical approvals for this study were obtained from the Kaduna Health Ministry of (MOH/ADM/744/VOL.1/539) and the Kaduna State Ministry of Education, Science, and Technology (NCE/STAT.3/VOL.II). Approval letters were obtained from the Education Office of each LGA to each selected school. Consent of participant was obtained before each enrollment in the study. An awareness talk on schistosomiasis and the associated risk of unsafe water bodies was delivered during the school morning assembly, emphasizing the need to be screened for the infection in each selected school.

Collection of Urine Samples

The consented school children were instructed on how to obtain mid-stream to terminal urine samples. Each of the 600 children provided 10mL of urine sample collected in a sterile widemouth, screw-capped container. The samples were then covered with dark polythene bags, placed in cold containers, and transported to the Parasitology Laboratory at the Department of Microbiology, Ahmadu Bello University for examination.

Urinalysis

The urine samples of the children underwent immediate urinalysis in the field. Eleven urinary analytes were assessed using urine reagent test strips (SG11100-Uric 11V, Guilin Zhonghui Technology Co., Ltd, China). A separate strip was immersed in each urine sample and promptly removed, with excess urine removed by gently running the strip against the container's rim. Horizontal positioning of the strip was maintained to prevent crosscontamination of chemicals between adjacent reagent pads. Results were interpreted by comparing the color changes on the reagent pad for each analyte with the corresponding colorcoded chart within the specified time outlined in the manual attached to the strip-vial. The detection of leukocytes required a 2-minute interval, while nitrite, urobilinogen, protein, and pH required 60 seconds each. Specific gravity, ketones, bilirubin, glucose, and ascorbic acid required 45 seconds each. The results for all eleven analytes were documented for each sample tested.

Microscopic Detection of Schistosoma haematobium

The urine samples were removed from the cold containers and allowed to attain room temperature. Each 10mL sample was gently shaken before loosening the screw-cap, then transferred into labeled centrifuge tubes. Centrifugation was done at 3000 revolutions per minute (rpm) for 5 minutes, and the supernatant was discarded. The sediment retained at the bottom of the centrifuge tube was collected using a Pasteur pipette. A wet mount of the urine sediment was prepared, and a coverslip was applied over it for examination under a compound light microscope with 10× and 40× objectives to identify characteristic ova of Schistosoma haematobium (with terminal spines) (Cheesbrough, 2009; Bishop et al., 2016).

The entire sediment of each sample was examined to determine the intensity of infection in terms of egg count per 10mL of urine. In cases where the entire sediment could not fit in a single wet mount, multiple wet mounts were prepared, and the egg counts were cumulatively recorded (Bishop et al., 2016; Bishop and Akoh, 2018). A color atlas of Parasitology was used as reference for identifying the ova of а Schistosoma haematobium. A light infection was characterized by a count of <50 ova per 10mL of urine, while a heavy infection was defined by a count of ≥50 ova per 10mL of urine (Cheesbrough, 2009; Bishop et al., 2016).

Statistical analysis

Chi-square (x^2) and odds Ratio (OR) analyses, conducted using IBM SPSS version 23, were utilized to assess any significant correlations in the prevalence of urinary schistosomiasis across different school levels among the study participants. Additionally, the x^2 and OR tests were employed to investigate potential associations between urinary analytes and urinary schistosomiasis within the subjects, along with the risk of urinary abnormalities attributed to the infection. The findings were summarized and illustrated through charts and tables.

RESULTS

Infection with *Schistosoma haematobium* was detected among the school children by evidence of ova that were golden-yellow with terminal spines (Figure 1). The overall prevalence of urinary schistosomiasis in this study was 6.8%. Children in senior secondary school had a higher prevalence of *Schistosoma haematobium* infections (8.9%) than those in junior secondary school (5.9%). However, the difference was not statistically significant (P = 0.178), but the senior students were more at risk (OR = 1.554) of acquiring the infection than the junior counterparts (Table 1).

Intensity of the infection was categorized into two groups. Light infections, with a count of <50 ova/10mL of urine, were observed in 28 (4.7%) cases, while heavy infections, defined by a count of \geq 50 ova/10mL of urine, were present in 13 (2.2%) cases, as illustrated in Figure 2.

A urinalysis profile of the school children is presented in Table 2. Children whose urine contained leukocytes had a higher occurrence of *Schistosoma haematobium* infection (18.5%)

than those whose urine did not contain leukocytes (2.5%). A significant association was found between urinary schistosomiasis and the presence of leukocytes in the children's urine (P = 0.000). The risk of leukocyturia was higher among children with urinary schistosomiasis (OR = 8.822).

Nitrite and bilirubin were not detected in any of the infected subjects; however, urobilinogen (7.7%, OR = 1.147), ketones (14.8%, OR = 2.519), and ascorbic acid (3.2%, OR = 0.419) were present in the urine of the infected children. Nonetheless, these analytes did not show a statistically significant association with urinary schistosomiasis (P > 0.05), as indicated in Table 2.

Urine samples from children with pH values of 5 and 8 showed infection rates of 7.5% each for *Schistosoma* haematobium. Samples with pH values of 7 and 6 had infection rates of 6.5% and 6.1%, respectively. Samples with a pH of 9 did not show any infections, but this relationship was not statistically significant (P > 0.05), as shown in Table 2.

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Presence of protein in urine (i.e., proteinuria) was more frequent among children infected with *Schistosoma haematobium* (23.1%) than in those without the infection (5.7%). The relationship between proteinuria and urinary schistosomiasis among the school children was statistically significant (P = 0.000), with a higher risk of proteinuria due to underlying urinary schistosomiasis (OR = 4.959).

Micro-haematuria was more frequently detected among children infected with Schistosoma (49.3%) compared to uninfected subjects (1.5%). The presence of micro-haematuria (P = 0.000) was a significant indicator of underlying schistosomiasis in the study participants, with a higher risk observed among those infected with Schistosoma (OR = 63.695) as detailed in Table 2.

Urine with specific gravity of 1.005 were most detected with *Schistosoma haematobium* (15.9%), followed by 9.0% of the infection in urine with specific gravity of 1.010. The least occurrence of the parasite (4.1%) was in urine with specific gravity of 1.015, but the relationship had no statistically significance (P = 0.135) in Table 2.

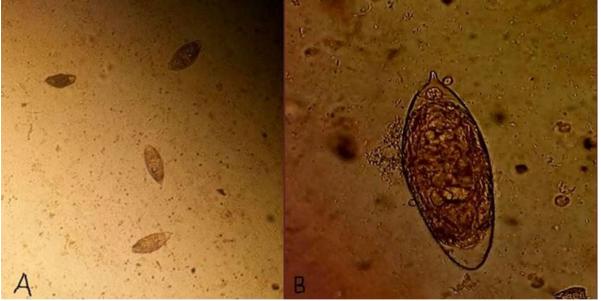


Figure 1: Microscopic appearance of ova of *Schistosoma haematobium* in urine sediment of two school children. (A) = ova from heavy infection $(100\times)$; (B) ovum from light infection $(400\times)$.

Secondary level	Number	Number	x ²	df	P-value	Odd ratio
	examined	positive (%)				(OR)
Junior	408	24(5.9)	1.811	1	0.178	0.643
Senior	192	17(8.9)				1.554
Total	600	41(6.8)				

Table 1: Distribution of Urinary Schistosomiasis based on School Level of the Children

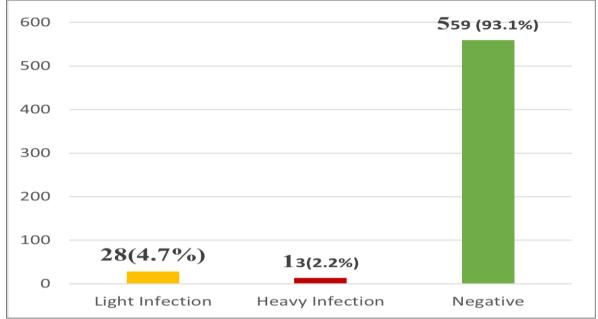


Figure 2: Overall Intensity of Urinary Schistosomiasis among School Children in Kaduna State, Nigeria

Urinalysis	Category	Number	Number	x ²	df	Р	OR
parameter		examined	positive (%)				CI=95%
Leukocytes	Absent	438	11(2.5)	47.596	1	0.000	0.113
	Present	162	30(18.5)				8.822
Nitrite	Absent	595	41(6.9)	0.370	1	0.543	0.931
	Present	5	0(0.0)				0.000
Urobilinogen	Normal	561	38(6.8)	0.048	1	0.826	0.872
-	High	39	3(7.7)				1.147
Protein	Absent	561	32(5.7)	17.287	1	0.000	0.202
	Present	39	9(23.1)				4.959
рН	5	174	13(7.5)	0.863	4	0.930	
	6	214	13(6.1)				
	7	46	3(6.5)				
	8	160	12(7.5)				
	9	6	0(0.0)				
Micro-haematuria	Absent	533	8(1.5)	213.184	1	0.000	0.016
	Present	67	33(49.3)				63.695
Specific gravity	1.005	44	7(15.9)	8.418	5	0.135	
	1.010	122	11(9.0)				
	1.015	49	2(4.1)				
	1.020	99	5(5.1)				
	1.025	82	5(6.1)				
	1.030	204	11(6.8)				
Ketones	Absent	573	37(6.5)	2.829	1	0.093	0.397
	Present	27	4(14.8)				2.519
Bilirubin	Absent	578	41(7.1)	1.675	1	0.196	0.929
	Present	22	0(0.0)				0.000
Glucose	Absent	600	41(6.8)		Co	nstant	
	Present	0	0(0.0)				
Ascorbic acid	Absent	537	39(7.3)	1.480	1	0.224	2.389
	Present	63	2(3.2)				0.419

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Urine samples from children containing ketones showed a higher occurrence of *Schistosoma haematobium* (14.8%) compared to urine samples without ketones (6.6%). The likelihood of finding the parasites in ketone-containing urine was approximately 2.5 times higher than in urine samples not containing ketones. However, the relationship between *Schistosoma haematobium* infection and the presence of ketones in the urine was not statistically significant (P = 0.093) as shown in Table 2.

There was no detection of glucose in the urine samples of all the school children who participated in this study, irrespective of their *Schistosoma haematobium* infection status (Table 2). A lower detection of *Schistosoma haematobium* (3.2%) was observed in the urine of children containing ascorbic acid, while the infection was higher in urine without ascorbic acid (7.3%). However, the relationship was not statistically significant (P = 0.224) as indicated in Table 2.

DISCUSSION

Microscopic detection of golden-yellow, oval eggs with terminal spines in urine sediment confirmed the presence of urinary schistosomiasis among the school children in this study. This method has long served as the gold standard for the diagnosis of schistosomiasis (Ibironke et al., 2012). When an individual has heavy Schistosoma haematobium infections, it indicates multiple infections or re-infections due to repeated exposures to infested water. Most cases of chronic complications of schistosomiasis are usually associated with heavy infections. Complications of schistosomiasis vary depending on the number of tissue-entrapped ova, predilection site, duration, and intensity (Leder and Weller, 2011).

The frequency of light infections among the adolescents was twice as prevalent as that of infections. heavv Heavv infection with Schistosoma haematobium necessitates repeated exposure to the infective stage of the parasite (cercariae) when in contact with infested water (Sakanari and Mckerrow, 2010; Bishop and Akoh, 2018). Schistosomes do not proliferate in human hosts. A high intensity of infection increases the severity the of pathological damage to the urinary system, especially the bladder. This is attributed to hypersensitivity reactions to entrapped ova in venules of the bladder (Cheesbrough, 2009).

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Children in the senior secondary school were more infected than those in the junior section. Those in the senior section were relatively older. Several studies had proven that older children had an increased tendency to go out freely and participate in water-contact activities (Bigwan et al., 2013; Omenesa et al., 2015; Geleta et al., 2015; Bishop and Ahmadu, 2018). Another reason could be due to the fact that some of the older children might have been participating actively in irrigation farming, while others assisted their parents on the farms by fetching water from cercarial-infested streams to irrigate crops. These practices could predispose them to repeated infections and may develop a high intensity of the infection. Though the distribution of urinary schistosomiasis based on their school level was not statistically significant, individuals can become infected regardless of class or age as long as there is body contact with infested water.

Urinary schistosomiasis affects the normal functioning of the urinary system. There was a significant association between urinary schistosomiasis and the presence of leukocytes in the urine of the children ($P \le 005$). The presence of leukocytes in Schistosoma-positive urine was due to the persistent loss of blood through the damaged tissues of the urinary system. Normal urine should be negative for leukocytes; their presence can indicate urinary tract infections (especially those caused by Schistosoma haematobium) or other renal diseases (King, 2011).

Nitrite and bilirubin were not detected in children infected with Schistosoma haematobium. The very few samples positive for nitrite indicated bacteriuria, as bacteria can break down nitrate to nitrite in urine (Encyclopedia of Surgery, 2018; Mayo Clinic, 2021). The presence of bilirubin suggests a likelihood of hepatic disease, but it was not detected in any cases of urinary schistosomiasis in this study. Although a larger proportion of the infected children had high levels of urobilinogen, it was not statistically associated with urinary schistosomiasis, but could have been due to other gastrointestinal problems. Bacteria in the gastrointestinal tract are known to convert conjugated bilirubin into urobilinogen (Encyclopedia of Surgery, 2018; Mayo Clinic, 2021).

Many of the subjects infected with Schistosoma excreted ketones in their urine, with over 2.5 times the likelihood of detecting ketonuria among them compared to uninfected

individuals. Normal urine is not supposed to contain ketones, as they are a product of fatty acid breakdown in the body (Encyclopedia of Surgery, 2018; Mayo Clinic, 2021). Therefore, it can be inferred that the presence of urinary schistosomiasis could have led to an increased accumulation of ketones in the body.

There were significant detections of microhaematuria and proteinuria in the urine of Schistosoma-infected children compared to those not infected, and these analytes support the evidence of visible haematuria reported among them ($P \le 0.05$). Urinary schistosomes cause damage to the venous plexus of the bladder, leading to direct blood loss into the urine. Micro-haematuria and proteinuria can serve as (pre-)diagnostic indicators of urinary schistosomiasis in endemic areas (Morenikeji et al., 2014). In cases where visible haematuria is not observed due to light infection, screening for micro-haematuria can be a proxy for detecting urinary schistosomiasis, particularly in endemic areas. Haematuria is considered one of the classical signs of the disease.

Specific gravity of urine of the children in this study ranged from 1.005 - 1.030. The kidneys help to concentrate urine in order to conserve water to keep the body hydrated (Encyclopedia of Surgery, 2018; Mayo Clinic, 2021). Most of the Schistosoma haematobium infections were found in children whose urine had specific gravity of 1.005 and 1.010, which were below normal range. It is evident therefore, that in schistosomiasis urinarv there is higher concentration of solutes in urine. These solutes included leukocytes, red blood cells, ketones and high levels of urobilinogen detected in urine of infected subjects (Encyclopedia of Surgery, 2018; Mayo Clinic, 2021).

Α major challenge in the control of schistosomiasis is the lack of effective diagnostic methods for the detection of the early infection phase, especially when the schistosomes are yet to reach maturity or release their ova in urine. The application of urinalysis in screening for urinary schistosomiasis will help detect early infection for the initiation of treatment to prevent the onset of pathologic lesions due to tissue-entrapped ova (Zhang et al., 2015; Weerakoon et al., 2015; Onile et al., 2017). It is better to detect urinary schistosomiasis at its early phase for the initiation of treatment and other interventions rather than waiting for overt symptoms to appear. It might be too late to reverse the pathological damage due to ova of the parasites entrapped *in situ*.

CONCLUSION

School-based awareness campaign on schistosomiasis was created across the selected schools in each LGA during the study. All the infected children were referred to health facilities for proper medical attention.

Children in senior secondary had a higher burden and risk of urinary schistosomiasis than their junior counterparts. The overall prevalence of urinary schistosomiasis in this study was 6.8%, with a higher occurrence of light infections (4.7%) than heavy infections (2.2%). Significant physiological indices of urinary schistosomiasis identified included leukocyturia, proteinuria, micro-haematuria, and ketonuria.

Therefore, the detection of urinary indices, especially during surveys or community-based screenings for urinary schistosomiasis, will aid in the early identification of infections, allowing for prompt treatment initiation and preventing additional damage to the urinary system of affected children.

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