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Urinary Schistosomiasis Prevalence and Diagnostic Performance of Reagent Strip at Point-of-Care

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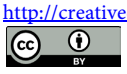

Abstract

Due to limited resources and experience, rapid diagnostic techniques are advocated in nations with a resource shortage when diagnosing schistosomiasis. We used rapid diagnostic tests to access the prevalence and intensity of schistosome infection in North Central, Nigeria. A total of 1951 participants were recruited for this study. The participants were screened for *S. haematobium* infection; haematuria and proteinuria were monitored in the recruited patients with a commercial reagent strip. Of the 1951 participants recruited for the study, 587 were found to be infected. Children aged 0 to 10 years showed the highest levels of haematuria with (100%) specificity. Meanwhile, other age groups (11 - 20, 21 - 30, 31 - 40 and above 40 years) had rates higher than 90%. The degree of haematuria increased with egg intensity. The same was seen in proteinuria, with a percentage of 41.9%. A significant difference ($p < 0.0001$) occurred across the infection categories in this study. The sensitivity of haematuria was highest (73.0%) in 11 - 20 age group, followed by 66.6% in 0 - 10 age group. Generally, the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the reagent strip were 67%, 97%, 89%, and 87% respectively. Although proteinuria's sensitivity was lower than that of other biomarkers, it outperformed haematuria in terms of specificity. Our research led us to the conclusion that using macrohaematuria in addition to the urine reagent strip test would improve the accuracy of the diagnosis of *S. haematobium* in rural endemic areas.

Keywords

S. haematobium, Haematuria, Proteinuria, Prevalence, Urine Analysis

1. Introduction

The link between schistosomiasis and poverty cannot be overstated, with sub-Saharan Africa accounting for the vast majority of infections [1]. More than 250 million people are infected with schistosomiasis worldwide; a larger percentage is located in the African region, where the per capita income is low compared to other developed nations across the world. An untreated disease has large long-term economic effects due to high morbidity, and it is estimated that each year, more than 1.4 million disability-adjusted life years (DALYs) are lost as a result [2]. In the African region, two prominent species (*Schistosoma haematobium* and *Schistosoma mansoni*) are associated with schistosome infection [3]. While *S. mansoni* is poorly reported in Nigeria, *S. haematobium* is reported from all six geo-political zones [4], where bulinid snails are the intermediate hosts. Meanwhile, Nigeria had the highest burden of infection among the estimated cases of urinary schistosomiasis encountered in Africa, closely followed by Tanzania Republic [5] [6]. The prevalence and intensity of the disease in Nigeria continue unabated, and regarding the control measures, there is continuous neglect by policymakers. Furthermore, the practise of people of all ages visiting rivers for domestic and recreational purposes has not decreased. The infection status and prevalence of schistosomiasis are high in children and adolescents [7] compared to adults [8]. Moreover, infection is also high in males compared to females in some regions where males visit river bodies more than females [9]. However, some are of the opinion that infection rates in females are higher compared to males because females are involved in washing clothes, utensils, and farm products in river bodies.

The World Health Organization (WHO) has used rapid diagnostic tests before treatment to reduce the global malaria burden [10]. The same strategy is being used to combat schistosomiasis. Meanwhile, the control of the malaria burden through diagnosis and treatment received more attention compared to schistosomiasis, because the latter is often referred to as a neglected tropical parasitic disease. Although the detection of schistosomiasis is done through the urine filtration or sedimentation method with high specificity, the sensitivity of this method is low [11]. Besides, the time spent on this process is high, especially when there is a need for a quick diagnosis. Furthermore, urine filtration or sedimentation methods require a well-trained microscopist who can differentiate between the ova of *Schistosoma haematobium* and those of other species or some materials that are found inside the counting chamber. In addition, due to the inadequate provision of basic infrastructure, such as electricity, in most sub-Saharan African countries, the use of a microscope during schistosome diagnosis could be impaired or delayed when there is a need for on-the-spot detection of schistosome infection.

Recently, it has been suggested that rapid diagnostic tests be used, particularly at the point-of-care and in large-scale surveillance studies [12], or when an immediate result is required following a control intervention in endemic areas.

Some of the characteristics that are measured during a rapid assessment of *S. haematobium* are proteinuria, haematuria, and leukocyturia. The observation of red-urine (haematuria) is considered one of the signs of schistosome infection. When the prevalence of haematuria is greater than 30% in a population, this is considered a high risk by the WHO [13], and praziquantel is administered to the patients [2]. Proteinuria is one of the useful diagnostic indicators of *S. haematobium*, and it forms a close association with the intensity of schistosome infection in active transmission sites [14].

Several researchers have studied the haematological, biochemical, and immunological parameters in infected individuals to determine the extent of changes in the host's biological system [8] [15] [16]. Leder and Weller [17] reported that urinary schistosomiasis was a major cause of anaemia in endemic communities. The anaemic condition was attributed to blood loss from terminal haematuria and the parasite's continuous feeding on blood glucose. In Koulikoro, a similar report discussed the relationship between *S. haematobium* intensity and disease burden, where blood in the urine was the most frequent clinical symptom [18]. A prevalence of 58.1% was recorded using microhaematuria as an indicator in Southwestern Nigeria [19], while in Northern Nigeria [20], macrohaematuria (75.0% - 97.3%) and proteinuria (65.0% - 79.6%) were observed among patients, with the highest prevalence in age groups less than 35 years. The ability of the reagent strip to detect microhaematuria in an infected asymptomatic patient has been well acknowledged and ascribed an advantage over the urine filtration method [21] [22]. Another advantage of the reagent strip is its low cost (about USD 0.25) for each strip [23]; hence, it is affordable for use in all medical facilities. As a result, we investigate the prevalence of schistosomiasis and the performance of rapid diagnostic tests in Kwara State, North Central Nigeria.

2. Materials and Methods

2.1. Study Site

The study involved sixteen Local Government Areas where the knowledge of schistosome infection is low; the earliest population in the area was pegged at 3,192,900. The study area had four major ethnic groups (Yoruba, Nupe, Baruba, and Fulani) with distinct socio-cultural differences. The presence of basic infrastructure is lacking, and the major source of water is from river bodies.

2.2. Study Design

A cross-sectional study was carried out involving the first and second phases in 2019. The initial phase was done to identify communities with schistosomiasis in all 16 LGAs of Kwara State. The second phase involved the random recruitment of participants from the communities that had reported cases of schistosome infection (two communities with the highest, and a community with the lowest prevalence and mean population egg load (MPEL).

2.3. Data Collection

The sample size was calculated using Fisher's method [24]. Using a prevalence of 44.1% in a survey of urinary schistosomiasis in southwestern Nigeria [25], it was determined to have a 95% confidence level and a ±5% margin of error. A minimal sample size of 378.8 was determined. A pre-labeled screw-capped plastic urine container was provided to each of the 1951 participants, and their demographic data was collected, which tallied with the number on their plastic container for the avoidance of any mix-up during data entry. Midday urine samples (between 10 and 15 hours) from all the participants were observed for schistosome infection.

2.4. Inclusion and Exclusion Criteria

Participants in the research had ages ranging from 0 to more than 40. Menstruating, postmenopausal, pregnant, and mature males with a history of uropathy were not included in urine sample collection. Additionally, people who took schistosomiasis medication three weeks before and during the data gathering, as well as children who were critically ill at the time the data were collected, were excluded.

2.5. Determination of Blood and Protein in Urine

The presence of blood in the urine (haematuria) and protein in the urine (proteinuria) were rapidly determined in freshly passed urine samples using the semi-quantitative method. Each urine sample was examined visually for gross haematuria and chemically tested for microhaematuria and proteinuria using commercial reagent strips (Medi-test Combur-9; Analyticon Biotechnologies, Lichtenfels, Germany) following the manufacturer's instructions. In brief, a urine strip was dipped into a freshly passed urine sample for 30 seconds, thereafter, the strip was removed and the colour change was read-off. The result in each sample was scored on a scale of 0 (negative), +1 (trace or $\leq 10 \times 10^6$ erythrocytes·l^{-1}), +2 (moderate or $\leq 50 \times 10^6$ erythrocytes·l^{-1}) or +3 (heavy or $\leq 250 \times 10^6$ erythrocytes·l^{-1}). Similarly, protein levels were scored from 0 (negative), +1 (trace or ≤0.3 g albumin·l^{-1}), +2 (moderate or ≤1.0 g albumin·l^{-1}), or +3 (heavy or ≤3 g albumin·l^{-1}) [26].

2.6. Prevalence and Intensity Study

All urine samples were screened quantitatively for *S. haematobium* eggs within 48 hours of collection using the urine filtration technique [27]. 10 ml of urine was drawn from the urine container into a syringe. Thereafter, a filter holder (Swinnex, 13 mm in diameter) fitted with a polycarbonate membrane filter (13 mm in diameter and 12 μm pore size, Millipore Company UK) was fitted into the syringe. The urine was filtered slowly through the filter membrane using the syringe plunger. The filter holder was thereafter unscrewed to carefully remove the filter onto a clean, degreased slide using a pair of forceps. A drop of physiological saline was then added before it was examined under a light compound microscope. The presence of eggs in urine confirms infection. According to

WHO guidelines, infection intensity was classified as heavy (>500 eggs/10mL), moderate (51 - 499 eggs/10mL), or light (<50 eggs/10mL).

2.7. Statistical Analysis

All variables were entered into an Excel spreadsheet, and the data was cleaned and analysed with IBM. SPSS 24 (International Business Machines' Statistical Package for Social Science, version 24) for Windows. A chi-square was used to test the difference in proportion, while an independent student's t-test was used to access the differences in the intensity of the infection. The difference in the intensity of infection and haematuria was calculated using a one-way analysis of variance. The geometric egg count analysis was based on the infected individuals in the population, while the mean population egg density was estimated as the infection burden in the studied population. Comparisons across groups were done using Fisher's exact test, and a p-value < 0.05 was considered significant. The performance of the urine reagent strip was determined (sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV)). The sensitivity of the urine reagent strip can be determined by comparing the percentage of positive results that were correctly detected to the standard test (urine filtration method). On the other hand, the proportion of negatives that were correctly identified when compared to the standard diagnostic test is called the specificity of the urine reagent strip. When the probability of a participant or patient having the infection is positive and the test confirms it, this is referred to as a PPV, whereas when the probability of the patient having the infection is negative and the test results show negative, this is referred to as NPV [28].

3. Results

Out of the 1951 participants, 587 tested positive for schistosome infection by the urine filtration method. The prevalence of schistosome infection and morbidity indicators (haematuria and proteinuria) among observed subjects in relation to egg intensity is shown in **Table 1**. The majority of the infected population carried a light burden of the infection. Only 6.6% of the infected individuals had heavy intensity, with 50.41% of the cases classified as heavy haematuria (+3) and 13.2% with gross haematuria. In any cases where there was no ova in the urine, there was no heavy and gross haematuria. The degree of haematuria increases with egg intensity. This pattern was also observed in proteinuria, with a percentage of 41.9%. A significant difference ($p < 0.0001$) occurred across the infection categories in this study.

The performance of the diagnostic reagent strip is shown in **Figure 1**. For visual examination, out of 1951 participants, only 32 tested positive for macrohaematuria. However, 26 of them were confirmed positive (**Figure 1**). The overall sensitivity for visual examination of urine samples was 4.5%; however, a high (99.6%) specificity was recorded for this method of diagnosis. Meanwhile, with the use of a commercial chemical reagent strip, a total of 393 participants were found infected out of the 587 positive cases with the aid of the microscopy me-

thod. The sensitivity, specificity, PPV, and NPV are 67%, 97%, 89%, and 87%, respectively. In this study, more males had infections compared to females (Figure 2). Infection was found in 393 males (67.1%), compared to 193 females (32.9%). Males had the highest rate (56.6%) of the total number of heavy infections. Similarly, the highest level of light infection was found in males with 78.0%, while females had 22.0% (≤49 eggs/10mL of urine).

Urogenital schistosomiasis among participants stratified by age: of all the age groups, 11 - 20 years had the highest infection status of 258 (44.0%), followed by 0 - 10 years. Meanwhile, heavy infection (≥500 eggs/10ml of urine) with haematuria was found in the 0 - 10 age groups and the 11 - 20 age groups. Participants over the age of 41 had the lowest level of infection, with no cases of heavy infection recorded (Figure 3).

Table 1. Correlation between results of haematuria and proteinuria, and microscopic examination.

Egg counts/10 ml of urine	Number examined (%)	Number of haematuria examined (%)					Number of proteinuria examined (%)			
		0	+1	+2	+3	Gh	0	+1	+2	+3
0	1364 (69.9)	96.6	2.9	0.7	0	0	97.5	1.5	0.9	0
1 - 49	214 (11.0)	22.0	55.1	6.5	16.4	0	22.0	75.7	2.3	0
50 - 499	244 (12.5)	31.2	30.3	18.4	37.3	3.7	10.3	56.1	26.2	7.4
≥500	129 (6.6)	0	3.9	32.6	50.4	13.2	8.5	8.5	40.3	41.9

Key: Gh = Gross haematuria, Haematuria score: 0 - (negative), +1- (trace or ≤10 × 10^6 erythrocytes·l^{-1}), +2 - (moderate or ≤50 × 10^6 erythrocytes·l^{-1}), +3 - (heavy or ≤250 × 10^6 erythrocytes·l^{-1}). Protein levels scored: 0 - (negative), +1 - (trace or ≤0.3 g albumin·l^{-1}), +2 - (moderate or ≤1.0 g albumin·l^{-1}), +3 - (heavy or ≤3 g albumin·l^{-1}).

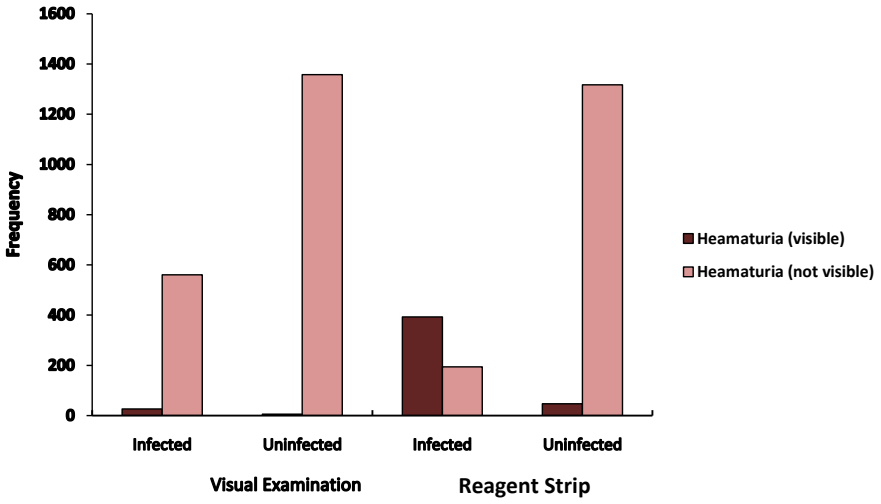

Figure 1. Sensitivity and specificity of haematuria test (visual or strip) for diagnosing urinary schistosomiasis. (Visual Examination: Sensitivity = 4.5%, specificity = 99.6%, PPV = 81%, NPV = 71%. Reagent Strip: Sensitivity = 67%, specificity = 97%, PPV = 89%, NPV = 87%).

The efficiency of the diagnostic reagent strip in relation to the participants is recorded in Table 2 and Table 3, respectively. The performance of haematuria in relation to age group reveals that the 0 - 10 age groups had the highest specificity (100%); while other age groups recorded greater than 90%. The sensitivity of haematuria on hand was highest (73.0%) in the 11 - 20 age groups, followed by 66.6% in the 0 - 10 age groups (Figure 3). The specificity was highest (100%) in the 0 - 10 age groups, with a general decrease in values as the age of participants increased, but the specificity of proteinuria performed better than that of haematuria in this study. Our result also showed that the sensitivity of proteinuria was lower than that of haematuria; however, the general sensitivity for proteinuria was 47.0% (Table 3).

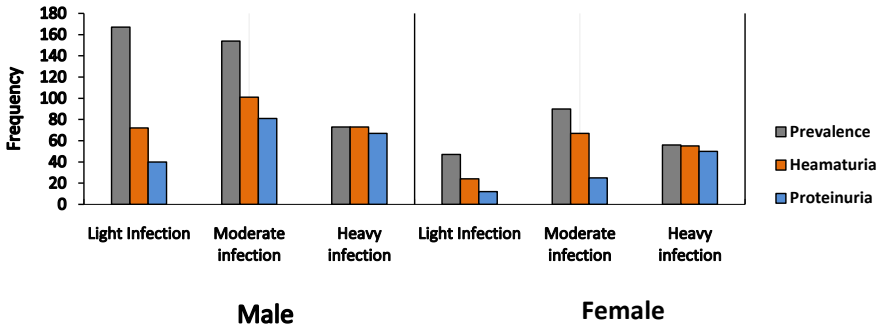

Figure 2. Sex differences in the positive rate of *Schistosoma haematobium* infection examined by microscopy, haematuria and proteinuria.

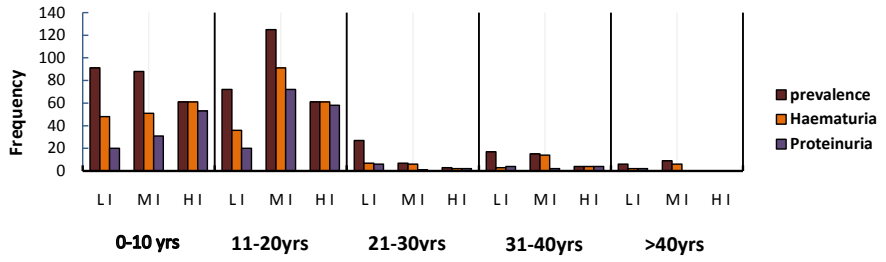

Figure 3. Rate of infection of *Schistosoma haematobium* in relation to age. (L I = Light infection ≤ 49 eggs/10ml of urine, M I = Moderate infection (50 - 499 eggs/10ml of urine, H I = Heavy infection (≥500 eggs/10ml of urine).

Table 2. Age-related haematuria for urinary schistosomiasis: sensitivity, specificity and predictive values.

Age	Number examined	Sensitivity (%)	Specificity (%)	Likelihood ratio Positive test	Likelihood ratio Negative test	Diagnostic odds ratio
0 - 10	677	66.9	100	∞	0.3	∞
11 - 20	734	73.0	96.8	22.8	0.3	76
21 - 30	191	40.5	94.2	7.0	0.6	11.7
31 - 40	224	58.3	93.1	8.5	0.5	17
41≤	125	53.3	90.9	5.6	0.5	11.2
Total	**1951**	**66.7**	**96.5**	**19.1**	**0.4**	**47.6**

Table 3. Age-related differences in the sensitivity, specificity, and predictive values of proteinuria for urinary schistosomiasis.

Age	Number examined	Sensitivity (%)	Specificity (%)	Likelihood ratio Positive test	Likelihood ratio Negative test	Diagnostic odds ratio
0 - 10	677	43.3	100	∞	0.6	∞
11 - 20	734	58.3	98.5	38.9	0.4	97.3
21 - 30	191	24.3	96.1	6.2	0.79	7.8
31 - 40	224	26.3	94.3	4.6	0.78	5.9
41≤	125	13.3	93.6	2.1	0.9	2.3
Total	**1951**	**47.0**	**97.9**	**22.4**	**0.5**	**44.8**

4. Discussion

Infections with *Schistosoma haematobium* were found to be prevalent in the current investigation, which was not unexpected. This is because our initial observations of human behaviour in these communities revealed that there is constant contact with river bodies for domestic purposes. Also, a significant number of the people who reside in these communities engage in farming and use water from river bodies after they are done with farming activities.

Haematuria and proteinuria due to urogenital schistosomiasis and their consequent induction of anaemia, particularly in children in endemic areas, have been documented in the literature [7] [29] [30]. In this study, the intensity of haematuria and proteinuria parallels the prevalence and intensity of the infection. The population of infected individuals excreting blood in urine in the study areas was 86.9%, while proteinuria was 91.7%. Ugbomoiko and his colleagues [31] reported a prevalence of 89.5% and 77% haematuria among *S. haematobium* infected individuals in Ogun and Osun communities, respectively. In the LGAs, the percentage prevalence of haematuria and proteinuria varied with the infected population; 6.6% of the infected individuals had heavy intensity. Of these, 50.4% of the cases were classified as heavy haematuria (+3) and 13.2% as gross haematuria. This finding is in agreement with other reports [30] [31]. They opined that haematuria and proteinuria are indicators for urogenital schistosomiasis infection. Clinical and pathological conditions associated with urogenital schistosomiasis include long term morbidity such as anaemia, which could result from bleeding (haematuria) from the urinary tract due to worm invasion and movements; iron deficiency, a sequel to nutritional impairment such as nutrient malabsorption and digestive disorders; schistosomal-induced kidney damage; fibrosis of the bladder and urethra; hydronephrosis; and bladder cancer [32]. The infection has also been identified as a key predisposing agent for Human Immunodeficiency Virus (HIV) transmission, which hastens the progression of the disease [33]. The infection is also associated with placental inflammation that results in poor delivery outcomes because of placental incompetency [32].

In this study, age-related infection was higher in males compared to their female counterparts. Series of studies regarding the age of participants have con-

firmed higher infection rates in males [7] [34], but other studies did not reveal any age-/sex-related cases among their study population [35]. This significant difference in age-related cases showed that both genders had differences in water contact patterns, with males, having a higher water contact compared to females.

The diagnostic accuracy of rapid assessment methods based on macrohaematuria, microhaematuria, and proteinuria has been widely studied [14] [31]. Overall, low sensitivity (4.5%) was observed with visual examination for gross haematuria. However, it had high (99.6%) specificity. The high specificity of the visual technique was recorded in Osun and Ogun States, southwestern Nigeria [14] [31]. With the use of commercial reagent strips, sensitivity was observed to vary with the intensity of infection and age. The age group 10 - 20 years recorded the highest reagent strip sensitivity value, while the older age groups recorded low reagent strip sensitivity values. This suggests that chemical reagent strips are more effective for testing urogenital schistosomiasis in individuals younger than 20 years old. This was similar to other observations [14] [36], which reported high sensitivity of reagent strips in schoolchildren of the age group 6 - 15 and pregnant women of the age group 18 - 20 respectively. Both microhaematuria and macrohaematuria are associated with schistosome infection in endemic areas. We noticed that the use of a single chemical reagent strip could be considered a limitation during this study. The storage method, effect of heat, and humidity were not monitored during this study, which could have contributed to the reduced sensitivity during our study. We could not use polymerase chain reaction to detect the infection status in this study. Hence, patients with a prepetent infection could not be diagnosed.

5. Conclusion

Therefore, we draw the conclusion that, given the high specificity associated with haematuria shown in this investigation, it could play a significant role in the primary diagnostic approach for schistosomiasis in endemic locations, and that all patients who exhibit it should be treated. We advise using it in conjunction with microhaematuria and macrohaematuria in the diagnosis of schistosome infection because of the commercial reagent strip's high specificity for *S. haematobium*, which was used in this study. This is especially important in low-resource countries, which are common in the African region.

Author Contributions

JOS designed and carried out the experiment; wrote the manuscript; carried out the data analysis. SUU designed and carried out the experiment. OGO carried out the data analysis; wrote the manuscript. AOB carried out the data analysis.

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Institutional Review Board Statement

Ethical approval (NERC/ASN/2014/011) was obtained from the Research and Ethical Committee of the Faculty of Life Sciences, University of Ilorin, and Kwara State Ministry of Health Ethical Review Committee. We also informed community leaders about the study and got their cooperation.

Informed Consent Statement

Informed consent was obtained from every adult participant and assent from the parent/caregivers of each child before they were enrolled in the study.

Data Availability Statement

Data included in the article.

Acknowledgements

We appreciate the cooperation in data gathering provided by the Kwara State Ministry of Health. We also thank all participants, their guardians, and village chiefs for allowing us to conduct this study in their neighbourhoods.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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