

Urine colour as a rapid assessment indicator in evaluating the prevalence of *Schistosoma haematobium* infection in two endemic areas of Benue State-Nigeria.

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Abstract

Schistosomiasis is a formidable public health problem, especially in sub-Saharan Africa where the majority of cases reside. In the context of having interest upon chemotherapy control, rapid, cheap and fast diagnostic tools and assay play an important role in assessing where treatment methods should be concentrated. We examined whether urine colour observation was correlated with intensity of infection in urinary schistosomiasis as measured by the gold-standard parasitological diagnosis. Using this tool and other proven field diagnostic (reagent strips), we examined 750 urine samples collected from school children and communities in two endemic areas of Benue State-Nigeria. Our findings demonstrate that urine colour observation was significantly associated with infection intensity ($r = 0.72$, $p < .01$). Given that parasitological examination is laborious, we showed that urine colour observation was significantly correlated with the indirect diagnosis method, Proteinuria ($r = 0.75$, $p < .01$) and Haematuria (0.52 , $p < .01$) widely used at the present time. We suggest that urine colour observation may be useful for diagnostic purposes, and for monitoring and evaluating treatment programs over time. Furthermore, we recommend that additional research should be done to further elucidate the relationship between this technique and other diagnostic methods.

INTRODUCTION

Schistosomiasis is the most prevalent parasitic infection in the world after malaria, with nearly 200 people infected, and 650 million currently at risk in the 76 countries where the disease is endemic¹. Of those, 20 million are believed to suffer from serious clinical disease, and 120 million are symptomatic². Despite past successes and renewed interest in the control of schistosomiasis within the last decade, the absolute number of people infected with the disease has increased in the last 50 years³, with approximately 85% of cases currently living in sub-Saharan Africa.

Identification of cases or communities for treatment with *Schistosoma haematobium* infection is usually based on microscopic detection of eggs in urine. The prevalence of an infection in a particular population varies directly with the sensitivity of the diagnostic techniques used. This dictates the need for a simple, fast, cheap and reliable diagnostic method for the detection of infected persons. The rapid diagnostic technique recommended by the National Expert Committee on the Control of Schistosomiasis (NECCS) is the indirect reagent strip technique for schistosomiasis⁴.

Proteinuria and haematuria are recognized clinical features of *S. haematobium* infection⁵. Many epidemiological studies have been conducted to investigate the characteristics of these methods to measure urinary schistosomiasis; this usually involved comparing the outcomes with intensity of infection.

In this study, we objected at comparing the merits of urine colour observation with other diagnostic techniques in order to justify the choice of the technique with those of reagent strips and egg count method, and if this could be used as a rapid screening technique for the assessment of urinary schistosomiasis in endemic areas.

MATERIALS AND METHODS

STUDY AREA

The study was carried out in Buruku and Katsina-Ala Local Government Areas of Benue State-Nigeria between November 2008 and March 2009. The selection of the areas was based on reports from local hospitals, clinics and health centers where cases of urinary schistosomiasis were reported. Agriculture and fishing are the major occupations

and sources of economic survival in the area.

STUDY POPULATION AND SAMPLES COLLECTION

Prior to the commencement of the research, ethical approval was sought from the Local Government chairmen and Local Government Education Authorities of both areas. Parents of the school children were duly informed on the significance of the study.

Urine samples were collected from communities (250), primary school children (250) and secondary school children (250). About 20 ml of clean-catch, midstream urine samples were collected in a 20 ml capacity autoclaved wide mouthed, leak, proof universal containers by subjects who were carefully instructed. Samples were obtained between 10:00 hrs and 14:00 hrs of the day, according to the method of ⁶⁷. Samples were noted for urine colour and specimens were appropriately labeled with identification numbers and placed in a cooler. Where delay in transportation of specimens to laboratory was inevitable, ordinary household bleach was added to the urine samples to preserve any schistosome ova present. ⁶⁷

INDIRECT REAGENT STRIP TECHNIQUE

Reagent strips (Medi-Test Combi 9) were dipped into the urine inside the universal containers. Microhaematuria and proteinuria were evaluated according to the manufacturer's instructions.

EGG COUNTS

Eggs were recovered from urine by the filtration technique. Filtration is the most sensitive, rapid, and reproducible technique for detecting and quantifying *S. haematobium* eggs in urine. Using blunt-ended (untoothed) forceps, a polycarbonate membrane filter (13 mm diameter and 13µm porosity) was placed carefully on the filter support of the filter holder (13 mm diameter) and attached to the end of a 10ml Luer syringe. The plunger was removed from the syringe before the syringe is filled to the 10ml mark with well-mixed urine after which the plunger was replaced. Holding the syringe over a beaker the urine was slowly passed through the filter. The filter holder is removed and unscrewed before a blunt-ended forceps is carefully used to remove the membrane filter. This was transferred face upwards (eggs on surface) to a slide before addition of a drop of Lugol's iodine with subsequent covering using a cover glass. Using the 10X objective with the condenser iris closed sufficiently to give good contrast, the entire filter was

examined systematically for eggs of *S. haematobium*. The number of eggs are counted and reported as egg per 10ml of urine, 1-10 eggs/10 ml urine was considered as light infection, 11-49 eggs/10 ml of urine as mild infection and >50 eggs/10 ml of urine as heavy infection.

RESULTS

Observations of urine colour were recorded from a total of 750 subjects aged 3-70 years (372 males and 378 females). Of these, 335 (44.66%) were positive for *Schistosoma haematobium* eggs using the filtration technique.

Urine was visually inspected and assigned a number (Figure 1). The urine colour chart ranges from 1 to 4, with 1 indicating urine free of any trace of microhaematuria or proteinuria (light-yellow) and 4 corresponding to dark red urine (bloody urine). Whereas number 2 and 3 grouped as brown colour correspond to visually discernable microhaematuria and proteinuria present in the urine.

Key:

- 1= Light yellow, normal colour of urine
- 2=Light-brown, urine with presence of low proteinuria and microhaematuria
- 3=Dark-brown, urine with medium presence of proteinuria and microhaematuria
- 4=Blood-brown, urine with visible haematuria

Figure 1

Fig 1: Urine colour related to the presence of proteinuria and haematuria



The relationship between urine colour and intensity of infection (egg count technique) is given in Figure 2. Out of 367 urine screened having brown colour, 293(39.06%) were found positive for *Schistosoma haematobium* eggs with 25.33%, 10.53% and 2.1% for light, mild and heavy infection respectively. Of the 27 screened having blood brown colour, 23(85.18%) were found with heavy infection. The least infection was found among those that were

screened having light-yellow urine colour. Of the 356 screened, 341(95.78%) counted negative for *Schistosoma haematobium* eggs, only 15 (2.00%) were found positive. A significant correlation was found between urine colour and intensity of infection ($r = 0.72$, $P < 0.01$).

Figure 2

Fig 2: Association between urine colour and intensity of infection ($r = 0.72$, P

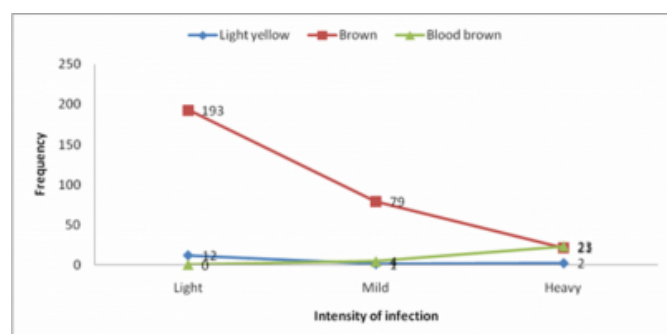
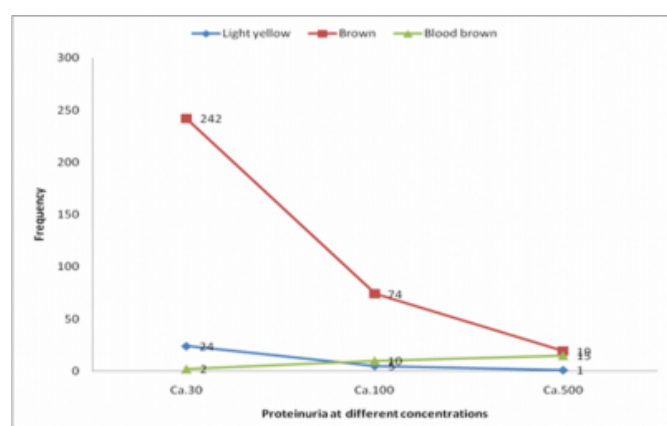


Figure 3 illustrates the significance and association of proteinuria to urine colour. It was observed that of the 367 screened having brown colour of urine, 335(44.66%) had proteinuria with a break down of 32.30%, 9.9% and 2.5% for Ca.30, Ca.100 and Ca.500 respectively. Out of 27 screened having blood-brown colour of urine, 15 (55.55%) had proteinuria at Ca.500 and 10 (37.03%) at Ca.100. 24(3.2%) having light-yellow colour were observed having proteinuria at Ca.30. However, a significant association was observed between proteinuria and urine colour($r = 0.75$, $P < 0.01$)

Figure 3

Fig 3: Association between urine colour and proteinuria ($r = 0.75$, P

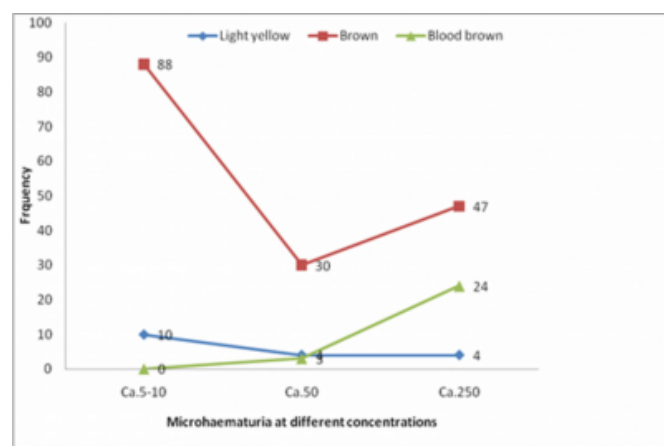


The significance and relationship of microhaematuria to urine colour is shown in Figure 4. A significant association was observed between urine colour and microhaematuria($r = 0.52$, $P < 0.01$). Out of the 367 screened having brown colour

urine, 165(44.95%) were found positive for microhaematuria; 23.97%, 8.17% and 12.80% for Ca.5-10, Ca.50 and Ca.250 respectively. Twenty seven (27) that were screened having blood-brown urine were all positive for microhaematuria with 11.11% and 88.88% for Ca.50 and Ca.250 respectively.

Figure 4

Fig 4: Association between microhaematuria and urine colour($r = 0.52$, P



DISCUSSION

The focus of this study was to determine if urine colour observation was useful as a rapid operational field indicator for evaluating infection status with *Schistosoma haematobium* in comparison with other commonly used direct (filtration technique) and indirect (reagent strips) diagnostic methods.

We have demonstrated that assessing urine colour through simple observation significantly estimated the prevalence of infection and correlated with infection intensity as measured by egg counts (44.66%) (Filtration technique) known as the gold standard test. This shows that urine colour observation, if used as rapid screening tool in an endemic area is capable of assessing the endemic community for urinary schistosomiasis prevalence.

In addition to being a useful rapid field diagnostic for *Schistosoma haematobium* infection, urine colour as assessed by observation may also prove to be an indicator of morbidity through proteinuria and microhaematuria. Both proteinuria and microhaematuria correlated well with urine colour, $r = 0.75$ and $r = 0.52$ respectively at 0.01 significance level. The brown colour of urine should be the result of excreted protein and red blood cells into the urine from the damage of urinary tract and kidney. Inconclusive evidence

suggests that *Schistosoma haematobium* affects the glomeruli, the units of the kidney that function to separate out wastes and extra fluid from the blood. When the glomeruli are damaged, protein and often red blood cells leak into the urine as this might be the case in this study. Although, at the present time the precise origin and clinical significance of the proteinuria observed in *S.haematobium* infection remains unknown⁸. Nonetheless^{9, 10, 11} claimed an association between glomerulonephritis, the inflammation of the membrane tissue of the kidney and *S.haematobium* in human.¹² reported that glomerulonephritis is highly prevalent in areas of the tropic where urinary schistosomiasis is also common, however its relationship to *S.haematobium* remain unclear.¹³ showed that infected hamsters and not control animals develop significant glomerular damage related to the presence and intensity of *Schistosoma haematobium* infections.

CONCLUSION

Urine colour observation is strongly correlated with egg counts and infection intensity as measured by parasitological diagnosis. Urine colour observation is reasonably an easy indicator that can assess urinary schistosomiasis rapidly in the field setting of endemic areas. This has a potentially role to play in a mass treatment programme for the reduction of infection and morbidity associated with urinary schistosomiasis. Assessing urine colour among communities and school children has diagnostic and evaluative potentials for strengthening control program and clarifying the burden of disease attributable to *Schistosoma haematobium* infection.

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