

Proteinuria as a Morbidity Marker of Urinary Schistosomiasis in School Children Living in Onchocerciasis Endemic Areas of Benue State, Nigeria

R.S. Houmsou^{*1}, E.U., Amuta², B.E., Wama¹, T.D., Hile³

¹Department of Biological Sciences, Taraba State University, P.M.B 1167, Jalingo, Taraba State, Nigeria

²Department of Biological Sciences, University of Agriculture Makurdi, Benue State, Nigeria

³Institut Supérieur des Sciences de la Santé Université Adventiste Cosendai, Nanga Eboko, Cameroun

^{*}rs.houmsou@gmail.com.

Abstract- Proteinuria is an indirect marker of urinary schistosomiasis in endemic areas but cases of misdiagnosis could occur in areas where other diseases that produce proteinuria overlap with urinary schistosomiasis. The study was conducted to assess whether proteinuria measured by urine reagent strips could be used as a rapid screening technique of urinary schistosomiasis in onchocerciasis endemic areas. Urine reagent strips and filtration technique were used to screen proteinuria and to determine *Schistosoma haematobium* eggs in 1,124 school children respectively. Our findings demonstrate that proteinuria positively correlated with intensity of infection ($\rho=0.71$, $p<0.01$), highly sensitive (95.7%) and moderately specific (67.7%). We suggest that measuring proteinuria using urine reagent strips may be useful for diagnostic purposes, monitoring and evaluating urinary schistosomiasis in onchocerciasis endemic areas. Furthermore, we recommend that additional research should be done to further elucidate the relationship between proteinuria and pathological changes of the urinary tract that may be attributed to infection with *Schistosoma haematobium* in onchocerciasis endemic areas.

Keywords- Proteinuria; Onchocerciasis; Schistosomiasis; Filtration; Strips

I. INTRODUCTION

Proteinuria and haematuria are indirect disease markers that are commonly used to identify individuals or communities at risk of *Schistosoma haematobium* infection in endemic rural African settings. Such approaches have been reported in several studies with significant results. Ugbomoiko *et al.* [1] reported sensitivities of 68.3% and 53.3% for microhaematuria and proteinuria respectively using urine reagent strips for rapid diagnosis of urinary schistosomiasis. However, the use of these markers could only estimate population with infection but do not differentiate *S. haematobium* infection from other diseases producing such symptoms. Additionally, the use of antimalarials or other drugs in individuals not infected with *S. haematobium* can also mimic the presence of proteinuria and haematuria in urine.

The presence of proteinuria has been observed in a proportion of onchocerciasis patients and in individuals receiving anti-filarial treatment [2, 3]. Amuta [4] and Buck *et al.* [5] observed mild proteinuria in onchocerciasis patients in Nigeria and Chad Republic respectively. Benue State has been reported as endemic for onchocerciasis over three decades [6]. During the last two decades tremendous efforts have been made to control the disease through mass distribution of Ivermectin, the anti-filarial drug.

Onchocerciasis and urinary schistosomiasis have been recently reported to prevail in Katsina-Ala and Buruku LGAs [7, 8]. As is considered above both diseases generate proteinuria. In order to differentiate between urinary schistosomiasis and onchocerciasis in areas where both diseases co-exist and where mass distribution of Ivermectin further complicates the picture, we aimed to test whether proteinuria detected by reagent strips could be used as a rapid screening tool for urinary schistosomiasis in onchocerciasis endemic areas. This was compared to filtration technique, the gold standard method, used for the quantification of *S. haematobium* eggs.

II. MATERIALS AND METHODS

A. Study Area

The study was conducted between November 2008 to September 2009 in Buruku and Katsina-Ala local government areas (LGAs) of Benue State. Buruku LGA is located at longitude 9°12'E and latitude 7°28'N, while Katsina-Ala LGA is located at longitude 9°16'E and latitude 7°10'N. Both areas are located within the guinea savanna of Nigeria. These areas are endemic for urinary schistosomiasis [7, 9]. Before commencement of the study, permission was sought from local government chairmen and local government education authorities of both areas. The climate of the areas is tropical with two seasons, the dry season

comprises the months from October to March and the rainy season goes from April to October. Agricultural activities such as crop farming, fishing and rearing of animals are the mainstay of the inhabitants.

B. Sample Collection and Examination

A total of 1,124 urine samples were collected from school children (6 primary and 4 secondary schools) aged 3-27 years between 10:00 and 14:00 hrs using universal bottles. Urine samples were rapidly tested on the field using Medi Test Combi 9 (Macherey-Nagel GmbH & Co.KG, Germany) reagent strips for the determination of proteinuria. Proteinuria was measured as mg/dl. The degree of proteinuria concentration was quantified as follows: 0 (negative), Ca.30 (+), Ca.100 (++) and Ca.500 (+++).

Immediately after testing with the reagent strips, 1 ml of ordinary household bleach was added to each collected urine sample to preserve any ova present and then these were taken to the laboratory within 2 hours for parasitological examination. Ten (10) ml of urine was taken and filtered through a 12 μ m polycarbonate membrane in a filter holder. With the help of forceps, the filter was removed from the filter holder and placed on a slide. A drop of Lugol's iodine was added and the slide was examined under microscope using x10 and x40 objective lenses. The number of eggs was counted per 10 ml of urine and intensities of infection were classified as 1-10 eggs, 11-49 eggs and ≥ 50 eggs for light, moderate and heavy infections respectively. The slides were prepared and double cross-checked by two researchers for accuracy of the eggs' counts.

C. Statistical Analysis

Collated data were double entered in Microsoft Excel and analysed in PASW (Predictive Analysis Software) version 18.0. Associations between variables were tested using Spearman correlation (ρ) at $p < 0.01$ significance level.

The diagnostic performance of proteinuria was assessed by calculating sensitivity and specificity using the following formulae [10].

Sensitivity = $a / a + b$, with a = True positive

b = False negative

Specificity = $c / c + d$, with c = True negative

d = False positive

III. RESULTS

Table 1 shows the relationship between proteinuria and *S. haematobium* eggs count using filtration technique among school children in Katsina-Ala and Buruku LGAs of Benue State. It was observed that of the 462 screened having no trace (negative) of protein in their urine, 13(2.8%) and 7(1.5%) had light and moderate intensity of infection respectively. Of the 317 screened having proteinuria at Ca.30 mg/dl, 155(48.9 %) were devoid of eggs in their urine and light infection recorded the highest rate with 118(37.2%). Of the 225 screened having proteinuria at Ca.100 mg/dl, moderate intensity recorded the highest rate with 84(37.3%), followed by light infection, 57(25.3%) and heavy infection, 28(12.4%). For those that had proteinuria at Ca.500 mg/dl, moderate and heavy infections had the highest rates with 54(45.0%) and 52(43.3%) respectively, and only 9(7.5%) had light infection. A significant association was observed between proteinuria and intensity of infection using the filtration technique ($\rho = 0.71, p < 0.01$)

TABLE 1 RELATIONSHIP BETWEEN PROTEINURIA AND INTENSITY OF *SCHISTOSOMA HAEMATOBIMUM* EGGS AMONG SCHOOL CHILDREN IN KATSINA-ALA AND BURUKU LGAS OF BENUE STATE

Proteinuria (mg/dl)	number/intensity of eggs per 10 ml of urine (%)				Total
	Negative	1-10 eggs	11-49 eggs	> 50 eggs	
Negative	442(95.7)	13(2.8)	7(1.5)	0(0.0)	462
Ca.30	155(48.9)	118(37.2)	36(11.4)	8(2.5)	317
Ca.100	56(24.9)	57(25.3)	84(37.3)	28(12.4)	225
Ca.500	5(4.2)	9(7.5)	54(45.0)	52(43.3)	120
Total	658(58.5)	197(17.5)	181(16.1)	88(7.8)	1124

Key: 1-10eggs/10 ml of urine = light infection, 11-49 eggs/10 ml of urine = moderate infection, ≥ 50 eggs/10 ml of urine = heavy infection

Table 2 compares proteinuria results to the presence of *S. haematobium* eggs using the filtration technique; those individuals with both proteinuria and eggs of *S. haematobium* (true positives) totaled 446(67.4%). Conversely, 216(32.7%) of the subjects were found to be devoid of *S. haematobium* eggs (false positives). Twenty, 20(4.3%) tested positive for *S. haematobium* eggs, but showed no sign of proteinuria (false negatives). As observed in the present study, the ability of proteinuria to identify all those with urinary schistosomiasis (sensitivity) was 95.7%, while its ability to correctly sort out those without urinary schistosomiasis (specificity) was 67.7%.

TABLE 2 COMPARISON OF PROTEINURIA RESULTS TO THE PRESENCE OF *S. HAEMATOBIIUM* EGGS USING FILTRATION TECHNIQUE

Presence of proteinuria	Presence of eggs in urine		
	Negative (%)	Positive (%)	Total (%)
Negative	442(95.7) ^c	20(4.3) ^b	462(41.1)
Positive	216(32.6) ^d	446(67.4) ^a	662(58.9)
Total	658(58.5)	466(41.5)	1,124

Keys: a = True positive, b = False positive, c = True negative, d = False negative

IV. DISCUSSION AND CONCLUSION

The results of this study demonstrate the feasibility of using proteinuria detected with reagent strips as a practical tool in identifying urinary schistosomiasis in onchocerciasis endemic areas.

The close association between proteinuria and presence of *S. haematobium* eggs in the urine ($\rho=0.71$) is similar to the reports of Klinger [11] who found a significant association ($\rho=0.73$) between proteinuria and *S. haematobium* eggs in an endemic area for urinary schistosomiasis in Tanzania. The close relationship observed between proteinuria and *S. haematobium* in this study could be exploited for the assessment of urinary schistosomiasis in onchocerciasis endemic areas. Its simplicity and cost-effectiveness can make it to be used by primary health centers with limited infrastructures and laboratory facilities.

The considerable number of false positive results (32.6%) observed in this study may be due to the presence of other urinary tract infections that can precipitate kidney damage and lead to protein leakage. Additionally, it is pertinent to consider the potential misdiagnosis of clinical proteinuria (trace protein in urine not exceeding a concentration of 30 mg/100ml) in individuals who do intense physical activity [12]; particularly as a large proportion (87.9%, 982/1124) of the participants' parents are farmers. It is likely that these children are exposed to farm work, requiring great physical strength and thereby leading to excretion of protein in urine. Amuta [4] also reported such cases of clinical proteinuria in onchocerciasis endemic areas of Benue State. Furthermore, a highly proteinaceous diet, the daily variation of *S. haematobium* eggs in infected individuals, or cases of chronic infection where eggs become encapsulated in the tissues could also lead to the excretion of protein in urine. Finally, the limited number of subjects observed as having eggs in their urine in combination with the absence of proteinuria could simply be the result of new infection in which kidneys are yet to be affected.

This study is subject to various limitations. First, the survey was limited to school children and therefore data cannot be inferred to the general population. Second, the results may only be valid in settings with a high prevalence of urinary schistosomiasis. Third, onchocerciasis was not tested in these participants; this could have drawn a conclusion on a negative or positive association. Furthermore, in areas with a lower prevalence of urinary schistosomiasis but high prevalence of onchocerciasis, the diagnostic performance of proteinuria may be less reliable. Additionally, the cross-sectional nature of this study did not permit assessment of the day-to-day variation of egg excretion.

In summary, it is possible that proteinuria could be used as a rapid screening method of urinary schistosomiasis in onchocerciasis endemic areas with limited diagnostic facilities, but further studies are needed in order to confirm its feasibility.

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