# PROTEIN EXCRETION IN URINE DURING *Schistosoma haematobium* INFECTION

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**ABSTRACT**

***An epidemiological survey of protein excretion in the urine of people living in Schistosoma haematobium endemic area around Atavu stream in Amagunze, Enugu State, Nigeria was conducted between 1994 and 1995. A verification of the endemicity of the diseases revealed differences in prevalence rate ranged from 46.66 % to 80.00 %. The proteins levels in urine were within normal limits for all pupils and working class studied. The presence of proteinuria and haematuria either singly or in combination signify disease condition. Proteinura was present in 31 (54.38 %) of school children, 17 (29.82 %) of working class and 4 (7.02 %) of aged individual. Four dominant bands were revealed with bands from uninfected persons darker than infected individuals using the cellulose acetate strip method. There were significant differences (P < 0.05) in prevalence and intensity of urinary schistosomiasis due to age. The incidence of infection in these villages shows that Schistosoma haematobium was high in Umunevo than in Isienu.***

**Keywords:** Schistotosomiasis, *Schistosoma haematobium,* Protein excretion, Proteinuria, Amagunze

## INTRODUCTION

*Schistosomiasis* is an ancient disease caused by blood flukes of the genus, *Schistosoma*. Among the several helminthic infections of man in the world. *Schistosomiasis* is ranked the highest in terms of public health importance (Van der Warf *et al.,* 2003). Among infected, 20 million are believed to suffer from serious clinical disease and 120 are symptomatic (Ajasin 1986). In Nigeria, several reports of the prevalence of *Schistosoma heamatobium* have been documented. (Okpala, 1961; Adekolu-John and Abolarin, 1986; Anosike *et al.,* 1992; Emejulu *et al.,* 1994; Savioli *et al.,* 1997). Furthermore the focal nature of the disease transmission and heterogeneity of risk within countries diluted its importance in public health priority setting at the national level (Engels *et al.,* 2002). Proteinuria implies a urinary excretion of up to 150/mg protein in 24 hours. Excretion of more than 6/mg/$m^2$/hour on a timed urine collection is significant proteinuria (Aladekoma, *et al.,* 2007). Intermittent proteinuria indicates that protein is only detectable in some of the patients' urine sample. Glomerular and tabular proteinuria may be distinguished by electrophoresis of the urine when the tubular proteins migrate primarily in the alpha and beta regions with little or no albumen detected (Aladekoma *et al.,* 2007).

In the immunochemical characteristics of proteins in cattle urine and their complexes with silicac acid, Biec (1978) noted that electrophoresis of total non-dialyzable solids (TNDS) from cattle urine showed the presence of components with mobilities covering the same range as reported for the serum. Although most of the components had mobilities similar to those of albumin, the $a_1$-globulin region appeared to contain more glycroprotein than serum components with similar mobilities.

The quantitative assessments of albuminuria in urine have been carried out by rocket immunoelectrophoresis of urine supernatant (Reimert *et al.,* 1993). The serodiagnosis of human schistosomiasis has been evaluated using western blot kits and the results compared with immunoflorescence. Six immunodorminant bands were observed using sera from patients with schistosomiasis (Annie *et al.,* 2005). This work was designated to identify the proteins in urine sample from *Schistosoma haematobium* infected persons and compare with uninfected persons. It was also designed to use albumin as a standard to compare the mobility of albumin with other mobilities in order to detect the proteins present.

## MATERIALS AND METHODS

The study was carried out in the Atavu stream area in Amagunze town, Nkanu Local Government. The villages surveyed were Umunevo and Isienu where schistosomiasis is known to be hyperendemic. Atavu stream is located in the lowlands of Umunevo village. The basin lies within the rainforest zone of Nigeria. Amagunze is made up of nine villages. Two of these villages use the stream very intensely both as source of drinking water and for other domestic chores. Water tankers are used to collect water from the stream and distribute it to villages further away and whose members do not have easy access to the stream.

Annual changes in the water level occur during the dry season when the water level drops to about 1.0m depth. The stream reaches the highest peak by the end of October after the second rainfall in September. Human water contact is higher during the dry season than the wet season. Ecologically, the stream has fairly rich flora and fauna composition.

**Sampling:** The examination of urine was made on random by sampled persons from the two villages. The urine samples were screened using both visible haematuria and urine sedimentation indices. House to house survey was carried out between 10.00 and 14.00/h for urine collection. Each member of the selected households was given a 250/ml plastic wide mouthed screw cap bottles for collection of urine samples irrespective of sex and age. The age and sex of each person was written at the back of the bottle containing the person's urine. The presence of visible haematuria was noted and recorded. In the laboratory about 10/ml of urine were processed using the sedimentation technique (Mcllough and Magendantz, 1974). 10/ml of urine sample was centrifuged for 5/min at 500rpm. The sediments were poured into a Petri dish and examined microscopically for the egg of *Schistosoma haematobium*. The sample containing ova were recorded and egg counts done using Mc-master counting chambers.

**Urinary Protein Analysis:** Protein in urine samples were analyzed using electrophoresis on cellulose acetate strip. The method used was horizontal separation of urine proteins. This was done by placing a strip of cellulose acetate (10 x 25cm) in a flat dish and allowing the baribitone buffer ($P_H$/8.6, 0.07m) to soak up from below. The tank was filled with buffer. The strips of cellulose acetate were highly blotted and placed horizontally across the tank on the chromatographic paper wick connected to the buffer compartment from where the buffer was drain to reach the cellulose strip during the separation. Capillary tube was used to apply a streak of the urine sample on the cellulose strip after concentrating the urine. The spotting on the strip was done separately for uninfected and infected. On each strip bovine albumin was equally spotted to serve as a standard. Those excreting 300, 200 etc eggs were separately spotted. Current was allowed to pass through for a period of 2hours. At the end different bands were obtained. The strips were removed and analyzed by staining with ponceas in acetic acid (0.5%). The migration of the sample was marked with pencil. The movement of protein in the sample was then measured with a ruler and recorded. The electrophoretic mobility was calculated as: Electrophoretic mobility = Distance moved by sample / distance moved by albumin.

## RESULTS

A series of two surveys provided data on prevalence intensity of infection incidence on urinary schistosomiasis and the electrophoretic mobility of urine protein on cellulose acetate strip by those infected with *S. haematobium* and those uninfected (Table 1). Prevalence of infection: The prevalence rates of urinary schistosomiasis in the area were variable as shown in the demographic details of the studied population (Table 2). The prevalence rate lowered as the months progressed. The prevalence of the infection was high in Umunevo than in Isienu.

**Table 1: The protein mobility pattern in infected individuals**

| S/NO | Number of eggs | Age | Sex | Distance move by protein (cm) | Electrophoretic mobility |
|---|---|---|---|---|---|
| **1** | **Bovine serum albumin** | NH | NA | 4.8 | 0.00 |
| **2** | **200** | 20 | F | 4.7 | 0.98 |
| **3** | **100** | 9 | M | 2.9 | 0.60 |
| **4** | **50** | 35 | F | 3.6 | 0.75 |
| **5** | **4** | 10 | F | 3.6 | 0.75 |
| **6** | **3** | 21 | F | 3.5 | 0.72 |
| **7** | **2** | 62 | M | 3.7 | 0.77 |

The prevalence rates by school, working class and aged has been presented (Table 3). The prevalence of infection by age showed that children and adolescents were more susceptible to the infection (Table 3). The electrophoretic pattern of protein in urine of people infected with haematobium shows that urinary protein varied with intensity of infection and were usually lower than in normal person (Table 4).Comparison between the infected and uninfected showed that the distance moved by urine protein of healthy persons were higher than for unhealthy ones (Table 5). In addition the specific bands for *Schistosoma* free individuals were darker than those positive for schistosomiasis. The electrophoretic mobilities for infected people decrease with increase in egg output though there were exceptions between those excreting 200 and 300 eggs / 10 ml urine (Table 6).

## DISCUSSION

Umunevo and Isienu are settlements on the banks of Atavu stream. The occurrence of schistosomiasis in these villages and the transmission sites show that the area is an established endemic area. The prevalence rates and incidence estimates of these villages can be interpreted to mean that transmission is higher at Umunevo than Isienu. Furthermore, a look at the prevalence rates on the communities in progressive years indicates that the rates in 1994 were higher than those of 1995. This may be as a result of recent control package by the state Government health Education which is part of disease eradication programme. However the figures are high but lower than figures reported by Udonsi (1990), but higher than the report of Aladekoma *et al* (2007) and Abayomi *et al.* (1971) in other parts of Nigeria.

In our study excretion of protein in urine was used as a marker. The result of investigation provided useful information for determining the differences in electrophoretic mobility of protein in urine for subjects infected with varying intensity of the disease and those uninfected. Protein mobility in the urine of 13 patients with different egg output indicates general disease which may be as a result of quantitative albumin variant and is characterized by a marked deficiency in albumin synthesis. Thus, anaemic patients might not be able to resist *Schistosoma* disease when contracted because of

**Table 2: Prevalence of *Schistosoma haematobium* by villages around Atavu stream in Amagunze**

| Survey | villages | Number examined | | Total | Number infected | | Total | % Infected | |
|---|---|---|---|---|---|---|---|---|---|
| | | M | F | | M | F | | M | F |
| **1994** | **Umunevo** | 20 | 30 | 50 | 16 | 14 | 30 | 80.00 | 46.66 |
| | **Isienu** | 25 | 30 | 55 | 19 | 21 | 40 | 76.00 | 70.00 |
| **1995** | **Umunevo** | 34 | 33 | 67 | 22 | 16 | 36 | 64.70 | 48.48 |
| | **Isienu** | 30 | 33 | 63 | 18 | 17 | 35 | 60.00 | 51.51 |

**Table 3: The prevalence of infection was analyzed for distribution by age and screened for proteinuria**

| S/NO | Age groups | Age | Infected | Uninfected | Total | % |
|---|---|---|---|---|---|---|
| **1** | **School children** | 4 - 20 | 31 | 45 | 76 | 54.38 |
| **2** | **Working class** | 21 - 37 | 17 | 9 | 26 | 29.82 |
| **3** | **Working Class** | 38 - 54 | 5 | 9 | 14 | 8.77 |
| **4** | **Aged** | 55 - 71 | 4 | 10 | 14 | 7.02 |
| **Total** | | | **57** | **75** | **130** | |

**Table 4: Electrophoretic mobility of serum proteins in urine of people free from *Schistosoma haematobium* infection**

| S. No | Age | sex | Distance moved by protein sample (cm) | Electophoretic mobility |
|---|---|---|---|---|
| **1** | 61 | F | 4.7 | 0.98 |
| **2** | 20 | M | 4.0 | 0.82 |
| **3** | 8 | M | 3.8 | 0.79 |
| **4** | 22 | F | 3.6 | 0.75 |
| **5** | 15 | F | 3.8 | 0.79 |
| **6** | 20 | F | 4.8 | 1.00 |

**Table 5: Comparison between the electrophoretic mobility of serum protein in urine of uninfected and infected people**

| S/NO | Age | Sex | Distance moved by protein sample (cm) | Electrophoretic mobility | Disease state |
|---|---|---|---|---|---|
| **1** | 16 | M | 4.7 | 0.98 | |
| **2** | 11 | M | 4.5 | 0.94 | Uninfected |
| **3** | 45 | F | 4.5 | 0.94 | |
| **4** | 22 | F | 4.0 | 0.83 | |
| **5** | 40 | M | 3.9 | 0.81 | Infected |
| **6** | 61 | F | 3.5 | 0.73 | |

**Table 6: The mobility of infected individuals according to intensity of infections**

| S. No | Egg output | Distance moved by sample (cm) | Electrophoretic mobility |
|---|---|---|---|
| **1** | **300** | 3.0 | 0.63 |
| **2** | **200** | 4.7 | 0.98 |
| **3** | **100** | 2.9 | 0.60 |

reduction on immune system and stunting in protein metabolism.

Protein depletion therefore predisposes infection, and is a key in diagnosis (Waterloo and Bras, 1967). Albumin catabolism therefore decreases with malnutrition. A look at those free from *Schistosoma* infection shows that electrophoretic mobility of serum protein was high, the dominant band was darker and the distance moved by the protein was higher. Thus, protein rich food on the other hand, produces an increase in the concentration capacity of the infantile and adult kidney and therefore easily destroys any incoming disease, thereby maintaining its normal

serum albumin concentration. However, Jawetz *et al.* (1989) illustrated that the ability of the cercariae to penetrate depends on the ease with which the acellular glycoprotein can be altered by cercariae enzymes.

The electophoretic mobility of infected people decreased with increase in egg output though sometimes there are exceptions as seen between those exerting 200 and 300 eggs/10m urine.

The association of schistosomasis and proteinuria in endemic area is a common though not a universal finding. Most patients with urinary tract infections excrete less than 2 grams of protein in 24 hours, excretion of 3 grams or more suggest glomerular disease (Aladekoma *et al.,* (2007). This is probably because of low hydrostatic pressure in the glomeruli and so hindered the system from filtering its substances properly. Inconclusive evidence suggests that *Schistosoma haematobium* affects the glomeruli, the unit of the kidney that function to separate out wastes and extra fluid from the blood. When the glomeruli are damaged, the protein and often red blood cells leak into the urine. Albumin is the protein with higher concentration in plasma and acts as a sponge that draws extra fluid from the body into the blood stream for the kidney to remove.

In this study sex was not significant but age was significant in the distribution of infection. The ages 4 -20 show the highest prevalence rate. Thus, due to equal exposure to the risk factors as there are no restrictions on movement due to individuals' sex category in the study area. In general, those who have greater contact with the river had high prevalence, irrespective of the sex of the individual. Among the various shades of urine colouration, there is a wide range of egg output. In general deeper colours (brown) contain higher number of eggs. The study show that the disease is that of the young and that with advancing age there is a decreased passage of eggs which is believed to be due, at least in part, to the development of acquired resistance.

The implication of this finding to the epidemiology of the disease is not clear but what is clear in epidemiology studies here is that, like the studies of Waterloo and Bras (1967), liver being the site for the synthesis of almost all plasma protein, any disease of the liver will affect, the concentration of plasma protein which will manifest in the serum protein; thus lead to fall in electrophoretic mobility as a result of cellular damage and injury.

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