Is proteinuria an indicator of the extent of *Schistosoma haematobium* infection in Malawi?

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Abstract

The relationship between proteinuria levels (determined by a colorimetric method and a rapid dipstick method) and the extent of *Schistosoma haematobium* egg meturia (determined by direct microscopic egg counts) from school children in an area around Lake Chilwa in Zomba district, Malawi has been investigated. Results show differential relationships depending on the method of urine protein determination. The colorimetric method showed significant correlation with egg counts ($r=0.404$; $p=<0.001$). The rapid dipstick method showed a lower correlation which was also significant ($r=0.248$; $p=0.013$). The results also suggest that total urine proteins alone cannot be used to successfully determine urinary schistosomiasis infection. The possibility of exploiting schistosomiasis-specific proteins for diagnosis in developing countries is discussed.

Introduction

The association between *Schistosoma haematobium* infection and proteinuria has been widely reported (Ezzart et al., 1974; Mott et al., 1983; Chugh and Harries, 1983; Wilkins et al., 1979). These studies showed close relationships between urinary bilharziasis infection and proteinuria. This then led to the use of proteinuria as a means of diagnosing the infection, especially in cases of selected population chemotherapy. However, no such studies are known to have been carried out in Malawi.

In Malawi, as in many other developing countries, diagnosis of *S. haematobium* infection at the hospitals and health centre laboratories is by directly counting the eggs extruded urine. Filtration techniques (using Nytrel and Nucleopore filters), which offer

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a standard means of counting the eggs, are to a greater extent used only in research. Egg counts, however, have the disadvantage that there tend to be large daily fluctuations in the total number of egg outputs (Warren et al., 1979; Savioli et al., 1990). This is because only a small percentage of the eggs are extruded with the urine while most of the eggs will remain lodged in the tissues. Moreover, microscopy can be carried out only in the laboratory and, to a greater extent, the results are highly dependent on the competence of the person doing the diagnosis. The implication is that egg counts might not be an accurate indicator of the extent of *S. haematobium* infection.

The proteinuria observed in *S. haematobium* infection could be coming from various sources, such as active genito-urinary lesions resulting from eggs lodged in the tissues or soluble antigens and toxins continuously being released by the parasites in the tissues (Deelder et al., 1996) or renal track pathology (Wilkins et al., 1979).

This paper presents results from work on a project aimed at differentiating the urine proteins and exploring the possibility of using urine proteins specifically associated with *S. haematobium* infection as a means of effectively diagnosing the infection. Results of an investigation of the relationship between egg counts and urine protein levels determined by two methods are reported.

Materials and methods

Urine samples used in this study were collected from 131 school children aged from six to 16 years. The children were randomly chosen from three primary schools (Namasalima, Kachulu and St Peters) from an area around Lake Chilwa, about 35km from the municipality of Zomba, Malawi. This area has been classified by the Malawi National Schistosomiasis Control Programme as endemic for *S. haematobium* infection (Kumwenda et al., 1991). The urine samples were collected around midday when egg micturia was at its maximum.

Egg counts

S. haematobium infection was quantitatively determined by egg counts. The conventional direct urine microscopic examination method that is used in the hospital and health centre laboratories in Malawi was used in this study.

Freshly collected urine was centrifuged (3,000 rpm, 5 mins, 27°C). The pellet was then used for microscopy. All eggs that were within the area of the cover glass were counted and recorded at low power magnification (x100).

Protein estimations

Two methods were used to determine total urine protein concentrations from fresh urine samples: a colorimetric and a rapid dipstick method.

Colorimetric method

The Sigma microprotein technique for estimation of total proteins (Sigma, St Louis, MO, USA) was used. Absorbances at 595nm were read using the Spectronic 20 spectrophotometer. Protein concentrations were determined according to the supplier's instructions. Three determinations were performed per sample. Protein levels are recorded as proteinuria I.

Dipstick method

A semiquantitative technique for estimating protein concentrations was performed using the AMES Multistix 10SG method (Bayer Diagnostic, UK). Protein concentration readings were arbitrarily graded as reported by Ezzart et al. (1974): 0 = Negative, 1 = Trace, 2 = 30mg/dl, 3 = 100mg/dl, 4 = 300mg/dl, 5 = >2000mg/dl. Protein levels are recorded as proteinuria II.

For both protein estimation methods used, urine protein levels of 30mg/dl or more were considered as positive infection (Ezzart et al., 1974; Wilkins et al., 1979). The relationships between the two proteinuria levels and egg counts were assessed by regression analyses using the MSTATC statistical package. Proteinuria levels obtained from the two methods were each plotted against the number of eggs excreted.

Results

Table 1 shows comparative *S. haematobium* infection rates as determined by three methods: egg counts and proteinuria by colorimetry (proteinuria I) and proteinuria by a rapid dipstick method (proteinuria II). The infection rates are shown to be different in the same school children population for the three diagnostic methods; 64%, 55% and 57% for egg counts, proteinuria I and proteinuria II respectively.

Table 1. Schistosoma haematobium infection rates in school children as determined by egg counts and proteinuria (n=131)

Diagnostic method	Infection rate (%)
Egg counts	64
Proteinuria I (colorimetry)	55
Proteinuria II (dipstick)	57

Regression analysis also show that there are significant correlations between egg counts and proteinuria as a means of diagnosing the extent of *S. haematobium* infection. Egg counts and proteinuria I showed a higher correlation ($r = 0.404$; $p = >0.001$) than egg count and proteinuria II ($r = 0.248$; $p = 0.013$). The relationships between the two diagnostic methods, based on proteinuria and egg counts are also represented by the scatter plots shown in Figures 1 and 2. The overall low correlations that were observed between egg counts and proteinuria may mean that some of the diagnostic methods were not very specific for *S. haematobium* infection.

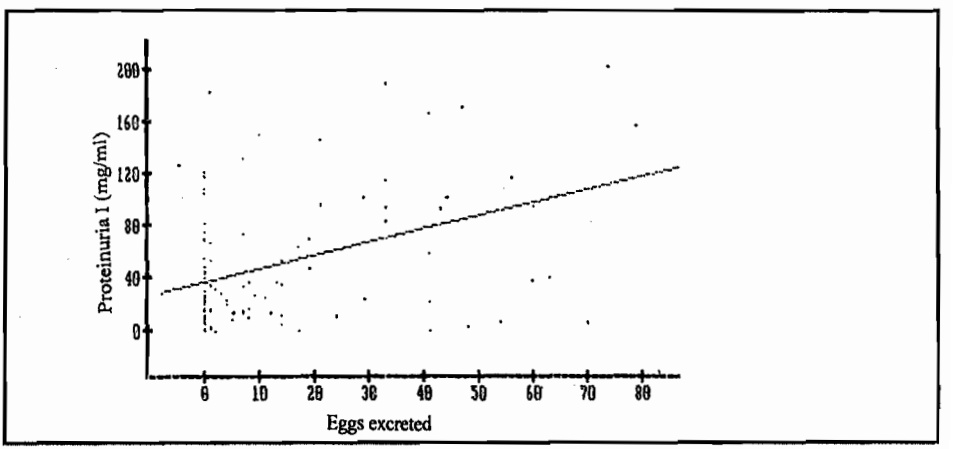

Figure 1. Scatter plot showing the relationship between proteinuria I levels (determined by a colorimetric method) and the extent of egg excretion. The line is represented by the regression model: $Y = 37.2 + 1.025X$, where Y is the proteinuria levels and X is the egg counts

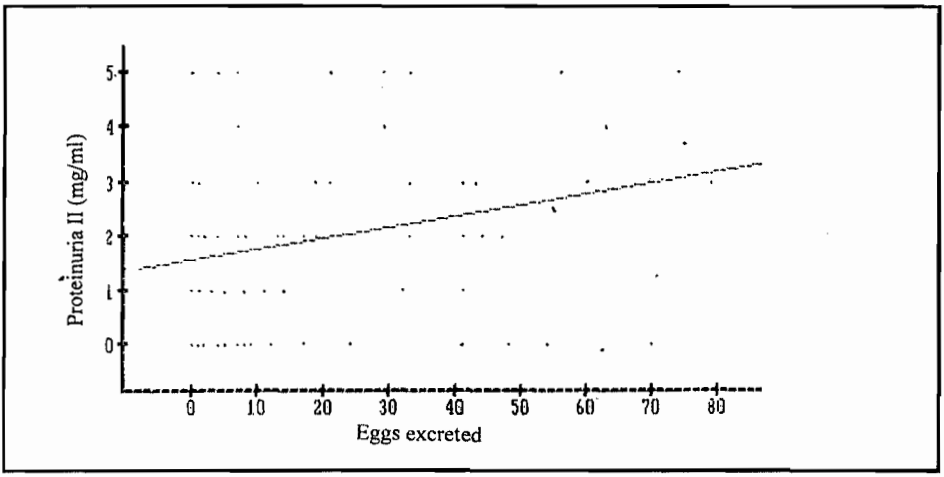

Figure 2. Scatter plot showing the relationship between proteinuria II levels (determined by a dipstick method) and the extent of egg excretion. The line is represented by the regression model: Y = 1.56 + 0.02X, where Y is the proteinuria levels and X is the egg counts

Discussion

Studies carried out in Egypt (Ezzart et al., 1974) and in the Gambia (Wilkins et al., 1979) indicated close relationships between the extent of infection and proteinuria levels. Proteinuria, therefore, has been suggested to offer an attractive diagnostic alternative in developing countries where access to suitable laboratory facilities is difficult for most rural communities (Taylor, 1982; Mott et al., 1983). Proteinuria was also shown by Ofori-Adjei et al. (1986) in Ghana as a diagnostic method with a sensitivity of 65% and specificity of 75.9% compared to direct microscopy which has 81.3% sensitivity and 98.1% specificity. It can therefore be deduced that not all the proteinuria detected in school children was due to *S. haematobium* infection. This, therefore, means that total proteinuria alone cannot be used to effectively diagnose urinary schistosomiasis.

When proteinuria is used as a means of diagnosing the infection for purposes of schistosomiasis control, there is a possibility that a considerable number of healthy individuals can be treated for the infection, while some infected individuals will not be treated due to the relative proteinuria levels. In developing countries, where this infection occurs, such a control strategy would not be cost effective considering the fact that the need for effective community health control programmes is paramount.

It is suggested here that the significant correlation that has been shown to exist between *S. haematobium* infection and proteinuria might mean that only some of the urine proteins are due to the infection. Identifying these *S. haematobium* infection-specific proteins and exploiting them for diagnostic purposes might provide a more reliable and effective way of diagnosing the infection. This would consequently lead to effective schistosomiasis chemotherapy control.

Diagnosis by detecting circulating anodic antigens (CAA) and circulating cathodic antigens (CCA) using enzyme-linked immunoabsorbent assays (ELISA) as shown by Jonge et al. (1989) and Lieshout et al. (1991) might not be practical for use in a normally equipped developing country public health laboratory. This is due to the sophistication of the method and the expenses involved (De Clercq et al., 1995). In developing countries, for mass screening, especially in cases of selected population chemotherapy, further simplification of such as assay is therefore necessary.

The identification of functional groups on *S. haematobium* infection-specific proteins would provide a key to designing simple and more specific semiquantitative diagnosis of the infection.

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