Original Research Article

Prevalence of leukocyturia among *Schistosoma haematobium* infected school children in Cameroon

ABSTRACT

Background: Urinary schistosomiasis is endemic in many areas in Cameroon and for effective control measures, an efficient, quick and cheap diagnosis should be integrated. This will ensure the proper management of infection due to *Schistosoma haematobium* in low resource communities.
Aim: This study aim to evaluate the use of leukocyturia as an indicator for the indirect diagnosis of urinary schistosomiasis in endemic areas in Cameroon.
Methodology: 266 school children were recruited in this cross-sectional study. Urine samples were collected, examined macroscopically for haematuria and subsequently screened for microhaematuria and leukocyturia using urine reagent strips. The microscopic examination of urine samples for schistosome eggs was carried out using filtration technique.
Results: The mean ± SD age of school children was 8.55 ± 2.03 years (range, 5 to 15 years). The prevalence of *S. haematobium* and geometric mean intensity of infection were 6.4% (17/266) and 4.1 ± 6.2 eggs/10 mL of urine respectively. Infection with *S. haematobium* showed significant association with age ($P < 0.05$). The prevalence of microhaematuria, and leukocyturia among infected children were 64.7 % (11/17) and 70.6 % (12/17) respectively. Microhaematuria and leukocyturia was high in girls compared to boys with a significant difference observed for leukocyturia ($P = 0.005$) and among participants aged 9-11 years.
Conclusion: Our findings suggest that the prevalence of leukocyturia was higher among the infected children. Future studies are needed to elucidate the possible use of urine leukocytes as an indicator for rapid diagnosis of urinary schistosomiasis in endemic areas of Cameroon.

Keywords: Schistosoma haematobium, leukocytes, prevalence, child, Cameroon.

1. INTRODUCTION

Schistosomiasis remains one of the most devastating tropical parasitic diseases [1,2], with an estimated 249 million people infected (90% live in Africa), and about 779 million people at risk in 78 countries (WHO, 2012) [3]. Urinary schistosomiasis is caused by *Schistosoma haematobium*. The helminth eggs may remain trapped within the bladder or the ureter walls, causing major pathological disorders in the urogenital system [4-8]. It causes approximately two thirds of the schistosomiasis cases in Sub-Saharan Africa [9,10].
In Cameroon, records of urinary schistosomiasis are mostly from rural areas [11-15]. Despite the efforts of the national program against schistosomiasis and intestinal helminths to eradicate the disease in these areas, problems are still encountered. One problem is the choice of the rapid and effective diagnostic method to detect infection. Microscopy, which is the method used for the detection of schistosome eggs is considered one of the most successful for urinary schistosomiasis diagnostic criteria. Unfortunately, this process is cumbersome, long, too technical and costly for the rural population. This limitation therefore dictates the need to identify new indicator that can be used for simple, rapid, cheap and reliable diagnosis method for the detection of infected people in these areas. Screening

using rapid indirect tests has been proposed as a procedure to simplify the mapping surveys [16].

Indirect diagnosis of urinary schistosomiasis through the use of chemical reagent strips has been proven highly sensitive and specific in many endemic areas of the world [17,18]. Proteinuria and hematuria are recognized as the clinical signs of infection with *S. haematobium* and often used as indicators of infection in endemic areas. A limitation of hematuria is that it can be affected by factors such as menstruation [19]. This cross-sectional pilot study was to evaluate the use of leukocyturia as an indicator for the indirect diagnosis of urinary schistosomiasis in a rural area of South West region in Cameroon.

2. MATERIAL AND METHODS

2.1 Study setting

A cross-sectional school based study was carried out in Munyenge health area (4° 23' 57.9'' N, 9° 16' 17.6'' E), a small village located in Muyuka subdivision (South West, Cameroon). This village is located about 27 km from the city Muyuka in the Muyuka subdivision. It lies at the foot (behind) of Mount Cameroon with a population of about 15,000 inhabitants.Two major seasons exist in the area, the rainy (March to October) and the dry seasons (November to February). Primary and secondary forests with savannah vegetation dominate flora species of the village. Agriculture is the major economic activity and source of livelihood for majority of individuals, families and households, and most residents are involved in the cultivation of cash crops (cocoa, palm oil) and food crops (plantain, cocoyams, bananas, yams, maize). Access to potable water within this village is inadequate; and inhabitants depend on streams and rivers for their sources of water for domestic purposes. The snail intermediate hosts (*Bulinus sp*) of *S. haematobium* have been discovered in the river bodies in the area [15].

2.2 Study population and data collection

The cross-sectional survey which employed a systematic random sampling technique, using the class register was conducted in two primary school (Full gospel primary school and Catholic primary school) in Munyenge, South West Cameroon. Clean and sterile universal urine containers were given to each of the pupils to collect urine samples. Urine was collected between 10 am and 2 pm under the proper guide of the teachers and the research team. The urine containers were clearly labelled with the sample number assigned to each participant. School children were eligible for the study if they were aged between 5-15 years and if we obtained the consent of his parent or legal guardian. Thereby, a total of 319 pupils were identified to participate in the study using class register. 53 of them were excluded for incomplete information and 266 pupils were finally included in the study.

The freshly passed urine samples were inspected macroscopically for haematuria and then screened for microhaematuria and leukocyturia using commercially available urine reagent strips (URIT 11V, URIT Medical Electronic Co., Ltd., China). The strip testing was performed in accordance with the manufacturer's instructions. The urinary leukocytes content was recorded as negative (number of cells < 15 cells/µL), trace (number of cells < 70 cells/µL), 1+, 2+ or 3+. The symbols are intended to correlate as follows: 1+ with 70 cells/µL, 2+ with 125 cells/µL and 3+ with 500 cells/µL. The samples were transported to the Parasitology laboratory of Medical research Center of the Institute of Medical Research and Medicinal Plants studies in Yaoundé where the eggs load were determined using the filtration technique [20]. Briefly, the urine sample was filtered; then the filter was transferred to a clean slide on which a drop of 1% Lugol's solution was added and examined under a light microscope for the eggs of *S. haematobium* using the x10 objective lens. Intensity of infection was classified using the classification of Briand et al. (2005) [21]. Intensity of infection was thus categorized as no infection, light infection (1-9 egg/10mL of urine),

moderate infection (10-49 eggs/10 mL of urine) and heavy infections (≥50 eggs/10 mL of urine).

2.3 Statistical analysis

Data were entered into an Excel spreadsheet, checked for entry errors and transferred to the Statistical Package for the Social Sciences software version 16.0 (SPSS Inc., Chicago, IL, USA), where appropriate statistical calculation were performed. Results are presented as proportions and means with standard error. The Pearson's Chi-square test was applied to assess the potential statistical relationships between infection and morbidities indicators of urinary schistosomiasis of the pupils. Student's t-test was used to compare means in the study groups. Differences were considered statistically significant at $P < 0.05$.

2.4 Ethical considerations

Ethical approval was obtained from Hospital Ethical Review Board of Munyenge. The community leaders and the school administrators were duly informed of the objectives and benefits of the study. The children parents were also informed one week before sample collection of the objectives and benefits of the study. Experiments have been examined and approved by the appropriate ethics committee. Participant's personal information was treated privately and was not divulged to third party. All infected children were referred to the munyenge hospital for free treatment with praziquantel.

3. RESULTS

Two hundred and sixty-six school children were examined, comprising 48.49 % males (n = 129, 8.53 ± 2.22 years) and 51.50 % females (n = 137, 8.55 ± 1.87 years). Children 5-8 years of age were 129 (48.49 %) while those 9-11 and 12-15 years were 115 (43.23 %) and 22 (8.27 %) respectively. Overall prevalence of urinary schistosomiasis was 6.4 % (17/266). Females were infected more than males (6.5 % versus 6.2 %), but the difference was not statistically significant ($\chi^2 = 0.015$, $P = 0.9$). The prevalence of *S. haematobium* infection in the age group 5-8 years, 9-11 and 12-15 years were 3.9 % (5/129), 6.1 % (7/115) and 22.7 % (5/22) respectively. The difference in prevalence in age groups was significant ($\chi^2 = 11.196$, $P = 0.004$) (Table 1). The global mean ± SD egg count of school children was 4.1 ± 6.2 eggs / 10 mL of urine (range, 1 to 26 eggs / 10 mL of urine). The difference between the mean ± SD egg count/ 10 mL of urine between males (3.8 ± 3.5 eggs / 10 mL of urine) and females (4.4 ± 8.1 eggs / 10 mL of urine) was not statistically significant ($P = 0.83$). Table 2 presents age and sex prevalence of urinary schistosomiasis morbidities indicators among positive cases detected by microscopy. The overall prevalence of microhaematuria, proteinuria and leukocyturia among infected children was 64.7 % (11/17), 58.8 % (10/17) and 70.6 % (12/17) respectively. Female were more affected with microhaematuria (8/11), proteinuria (6/10) and leukocyturia (9 /12) than male. Leukocyturia was observed among all infected girls and among all school children age between 9 – 11 years, leukocyturia varied significantly with sex ($\chi^2=7.969$, $P = 0.005$).

4. DISCUSSION

The results obtained in this pilot study confirm the active transmission of urinary schistosomiasis in the study area. Our overall prevalence (6.4%) of infection is far lower than earlier prevalence obtained by Ntonifor et al. (2015) (40.3%) in the same locatity**,** Tchuem Tchuente et al. (2003) (62.8%) and Nkengazong et al. (2009) (50.8%) in school children in others rural localities in Cameroon [11,14,15]. This low prevalence of infection observed in this study may be due to the effect of the mass distribution of praziquantel in some areas by the national program against schistosomiasis in recent years. Our data show that the

prevalence of *S. haematobium* infection increases with age. In our population, school children of the age group of 12-15 years were the most infected. This result is comparable to that obtained by Poda et al. (2001) and Ogbonna et al. (2012) which had shown a significant statistical difference between the infestation and age groups [22,23]. This can be attributed to the fact that it is the children of this age group who are most often responsible for supplying water in house for domestic use such as dishes and laundry; which increases their contact with water sources contaminated by snails intermediate host of schistosomiasis. Our observations also suggest that infection with *S. haematobium* seems not to be associated with sex factor. This finding is different from those obtained by other authors. Indeed, Seck et al. (2007) showed in their work that the prevalence of infection was significantly higher in boys than girls. They showed that the reason for this difference was related to the types of activities that boys and girls were doing in the water points. In their study, it appeared that only boys were responsible to drive herd into the water points to give them water [24]. Our study area is not an area where there are herds, the activity level of boys and girls in the water points is probably the same. This observation is consistent with that observed by Nkengazong et al. (2009) [14] who obtained similar results to ours, and may explain why boys are sometimes more infected than girls in some endemic areas.

The use of blood detected by reagent strip has been considered as an alternative in the diagnosis of *S. haematobium* infection [25,26]. In this study, we noted that most of the infected school children presented microhaematuria, proteinuria and leukocyturia. This high prevalence of these morbidities indicators is similar to a study conducted by Chidozie and Daniyan in 2008 [27] and are recognized as clinical features of *S. haematobium* infection [28,29]. These symptoms are indicators of damage in the urinary tract and kidney [27]. Inconclusive evidence has suggested *S. haematobium* associated glomeruli pathology. When the glomeruli are damaged, protein and often red blood cells leak into the urine. Although, at the present, the precise origin and clinical significance of the proteinuria and leukocyturia observed in *S. haematobium* infection remains unknown [30].

5. CONCLUSION

The present study revealed that leukocytes excretion in urine is frequent in school children with *S. haematobium* infection. It was observed among all infected girls and among all school children aged between 9 – 11 years. These results suggest the need of future studies in order to elucidate the possible use of urine leukocytes as an indicator for indirect diagnosis of urinary schistosomiasis in low resource communities in Cameroon.

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TABLES

Table 1: Prevalence and number of *S. haematobium* eggs / 10 mL of urine by age and sex among school children.

	Number examined	Number Positive (%)	Intensity n (%)		
Sex			**Mean (SD) Egg/10 mL urine**	**Egg load < 9 eggs/10 mL urine**	**10 < egg load < 50 eggs/10 mL urine**
Male	129	8 (6.2)	3.8 (3.5)	7 (5.4)	1 (0.8)
Female	137	9 (6.5)	4.4 (8.1)	8 (5.8)	1 (0.7)
Age group (year)					
5 – 8	129	5 (3.9)	2.8 (2.9)	5 (3.9)	0 (0.0)
9 – 11	115	7 (6.1)	5.6 (9.1)	6 (5.2)	1 (0.9)
12 – 15	22	5 (22.7)	3.4 (3.9)	4 (18.2)	1 (4.5)
Total	**266**	**17 (6.4)**	**4.1 (6.2)**	**15 (5.6)**	**2 (0.8)**

Mean (SD) = Mean values (Standard deviation)

Table 2: Age and sex prevalence of urinary schistosomiasis morbidities indicators among infected school children.

	Haematuria		Proteinuria		Leukocyturia		Total
	No (%)	Yes (%)	No (%)	Yes (%)	No (%)	Yes (%)	
Sex							
Male	5 (29.4)	3 (17.6)	4 (23.5)	4 (23.5)	5 (29.4)	3 (17.6)	8 (47.1)
Female	1 (5.9)	8 (47.0)	3 (17.6)	6 (35.3)	0 (0.0)	9 (52.9)	9 (52.9)
Age group (year)							
5 – 8	3 (17.6)	2 (11.8)	2 (11.8)	3 (17.6)	2 (11.8)	3 (17.6)	5 (29.4)
9 – 11	1 (5.9)	6 (35.3)	3 (17.6)	4 (23.5)	0 (0.0)	7 (41.2)	7 (41.2)
12 – 15	2 (11.8)	3 (17.6)	2 (11.8)	3 (17.6)	3 (17.6)	2 (11.8)	5 (29.4)
Total	**6 (35.3)**	**11 (64.7)**	**7 (41.2)**	**10 (58.8)**	**5 (29.4)**	**12 (70.6)**	**17 (100)**