Squamous Cell Abnormalities in Exfoliated Cells from the Urine of *Schistosoma haematobium*–Infected Adults in a Rural Fishing Community in Nigeria

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Abstract

Schistosoma haematobium infection is endemic in Nigeria, with substantial transmissions in all the states of the federation and a high prevalence rate in schools. Literature has linked bladder cancer, mostly squamous cell type, with long-term S. haematobium infections. The objective of this descriptive study was to screen exfoliated cells in the urine of *S. haematobium*–infected patients for squamous cell abnormalities through cytopathological examinations. Study participants were drawn from Imala Odo, a community near Oyan Dam in Abeokuta North Local Government Area, Ogun state, Southwest Nigeria. Due to a considerable day-to-day variation of *S. haematobium* eggs in urine, 3 rounds of 200

ml of urine samples were collected on 3 different days from 32 infected patients and 10 uninfected controls and examined. Cytological preparations of the infected 15 males and 8 females and 10 controls (5 males and 5 females) were screened for squamous cell abnormalities. Severely dysplastic to frankly malignant squamous cells were observed in 1 (3.1%) male and 2 (6.3%) females, while no abnormality was observed in the controls.

Introduction

Schistosomiasis ranks second to malaria among parasitic diseases of socio-economic and public-health importance. It afflicts more than 200 million people living in developing countries, with at least 600 million people at risk of infection and 1.7 million Disability Adjusted Life Years (DALYs) lost annually (Chitsulo et al. 2000). In many African countries, there occur both urinary and intestinal schistosomiases caused by *Schistosoma haematobium* and *Schistosoma mansoni*, respectively. Schistosomiasis is hyper-endemic in Nigeria, with substantial transmissions occurring in all the states of the federation and a high prevalence rate in school children (Mafe et al. 2000). Transmission is largely confined to water development project areas and along the main rivers and streams. Haematuria and egg counts are the only indicators of morbidity currently being used for surveillance, but Poggensee et al. (1998) reported urinary tract morbidity in some infected Tanzanian women with negative haematuria and scant or no egg output in their urine.

Bladder cancer, mostly squamous cell–type, has been associated with long-term infection with *S. haematobium* (Muscheck et al. 2000). The epidemiologic association is based both on case-control studies and on the close correlation of bladder cancer incidence with prevalence of *S. haematobium* infection within different geographic areas (Bedwani et al. 1998). A parasite–tumour linkage is further suggested by the predominance of squamous cell morphology of bladder carcinomas seen in *S. haematobium*–endemic areas, and by the frequent association of tumours with parasite eggs and egg-induced granulomatous pathology in involved bladder tissues (Christie et al. 1986). Bladder cancer caused by *S. haematobium* is difficult to diagnose without invasive measures such as cystoscopy; consequently there is little information on its epidemiology.

The objective of this descriptive study was to screen exfoliated cells in the urine of some *S. haematobium*–infected patients for the presence of squamous cell abnormalities. Our work focused on using evaluation of Papanicolaou-stained urine sediment cytology to show an association between squamous cell abnormalities and *S. haematobium* infections in a group of infected adults from a rural fishing community in Nigeria, where the infection is endemic. This study was part of a just-concluded pilot project designed to characterize exfoliated cells in the urine of *S. haematobium*–infected patients in order to generate specific genetic markers that could be used to detect bladder cancer in *S. haematobium* through a noninvasive diagnostic method.

Methodology

Study Site

Study participants were drawn from Imala Odo, a community with a population of about 780 located in Abeokuta North Local Government Area of Ogun state, Southwest Nigeria. The community lacks basic infrastructure such as pipe-borne water, safe waste disposal, electricity and a health centre, although it has a primary school. It is about 10 km from a major asphalt road, while the road approaching and within it is a laterite bush road. The community is inhabited by migrant fishing families, mainly from the middle belt area of Nigeria, who depend largely on fishing in the Oyan River, located within the community. The river also meets all their water needs and the needs of their domestic animals. The nearest health centre is about 12 km away and serves the population of about 4895 people of Imala, a predominantly rural farming community that is also hyper-endemic for urinary schistosomiasis (Mafe et al. 2005). The community was selected for this work based on the previous urinary schistosomiasis survey, which showed that the disease was hyper-endemic (Sulyman et al. 1998; Mafe et al. 2005). Previous treatment with praziquantel about 4 years ago (Mafe et al. 2005) targeted only school children aged 5 to 19 years.

Study Subjects, Selection Criteria and Ethical Considerations

The baseline examination included adult males and females aged between 40 and 70 years, with a mean age of 47.5 years. This age group was chosen for the study based on the observation of Muscheck et al. (2000) that bladder cancer caused by *S. haematobium* infection occurs especially in the fifth decade of life. Other selection criteria used for this study were haematuria and presence of *S. haematobium* eggs in all the three rounds of urine collected from each patient. Thirty-two of a total of 73 infected patients, made up of 21 (65.6%) males and 11 (34.4%) females, met the selection criteria. Ten controls – five males and five females – within the same age group took part in the study. The study was approved by the Institutional Review Board of the Nigerian Institute of Medical Research, while permission to carry out the study in the village was granted by the Ogun State Ministry of Health. Informed consent was obtained from each participant under a protocol approved by the Ethical Review Committee of the World Health Organization.

Parasitological Investigations and Treatment

The study population was registered on household forms, and the name, surname, age, sex and weight of all participants were recorded. Every participant was allocated a unique code of six digits representing village, household and individual numbers. For maximum egg yield, mid-stream urine was collected between 10:00 am and 2:00 pm on each collection day, following the observations made by Weber et al. (1967). Due to a considerable day-to-day variation of *S. haematobium* eggs in urine, three rounds of 200 ml of urine samples were collected into sterile containers on 3 different days. Containers were labelled with the study number of each participant and taken immediately to the laboratory in an icebox at 4°C. *S. haematobium* infection was detected by centrifugation of 100 ml of the urine samples and examination of the sediment under the microscope, while haematuria was detected using commercially prepared reagent strips (Hemastix; Boehringer Mannheim, Germany). All infected participants were treated with a single dose of praziquantel at 40 mg/kg body weight at the end of the investigation.

Cytological Evaluation

Within 4 hours of collection, the remaining 100 ml of each urine sample was prepared for cytological analysis as follows. The urine was centrifuged and slides were made from the filtrates from each round of urine samples per patient, fixed in 90% alcohol and stained with Papanicolaou stain. Slides were examined for exfoliated and reactive cellular changes by a consultant pathologist who was not aware of the parasitological results and infection status of participants. The cost of Papanicolaou stain for each specimen was about 40 US dollars.

Results

A total of 32 infected individuals and 10 uninfected controls aged between 40 and 55 years participated in the study. All 32 infected participants were positive for haematuria, and *S. haematobium* eggs were also detected in their urine samples collected on 3 different days. Some severely dysplastic malignant squamous cells were seen amidst a few normal squamous cells in two infected female participants aged 40 and 45 years and one infected male patient aged 48 years. Figure 1 shows a malignant squamous cell as seen in one of the cytological preparations of an infected participant, but in the cytological preparations of the controls, there were seen a few normal squamous cells against light backgrounds (Figure 2).

Discussion

This study was carried out in Imala Odo, a rural, isolated community in Abeokuta North Local Government Area of Ogun state, Southwest Nigeria, with no basic infrastructure. Our results support those from a study in Kenya that showed an association between urinary tract hyperplasia and *S. haematobium* infection (Hodder et al. 2000). Three (9.4%) of the 32 infected patients who participated in this study had malignant squamous cells of the bladder. Koss et al. (1985) noted

that the basis of inflammation in urinary schistosomiasis is chronic inflammation associated with egg deposition in the bladder wall.

Figure 1. Cytology of an infected participant showing (1) a malignant squamous cell, (2) a plasma cell, and (3) a lymphocyte

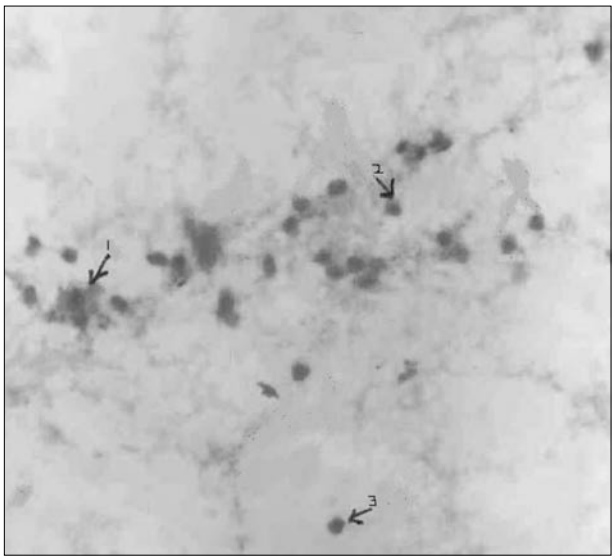
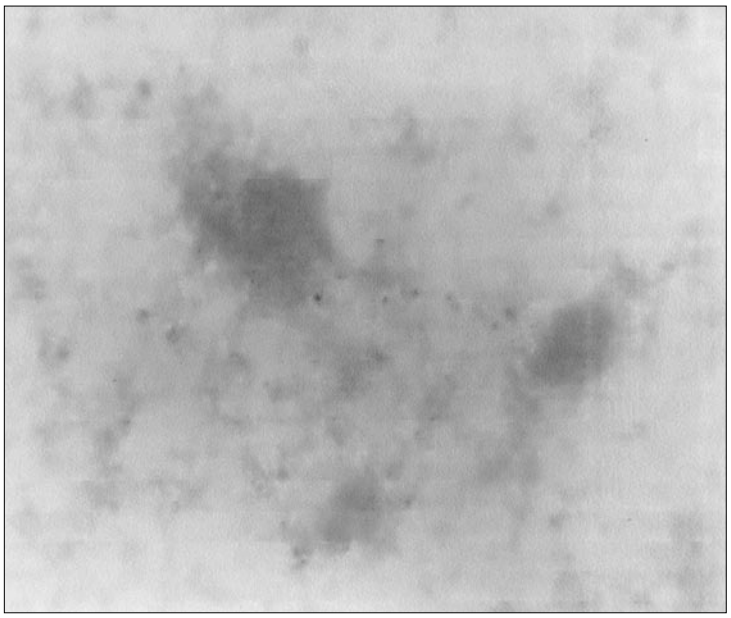


Figure 2. Cytology of one of the controls showing a cluster of normal squamous cells against a clear background



Cytology was used in this pilot study because the study was designed to characterize exfoliated cells in participants' urine. However, cytology can miss up to 50% of tumours, especially low grade and low stage ones, and bladder cancer detection at an early stage is crucial to patients' survival. We

recommend that these patients would benefit from a careful follow-up using a diagnostic method that is more efficient than cytology. We therefore suggest to the state Ministry of Health that efforts be made to follow up these patients using cystoscopy, which is the "gold standard" for diagnosis of bladder cancer, so as to be able to identify cases that might have been missed by cytology, the method used in this descriptive study.

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