PREDISPOSITION TO URINARY TRACT EPITHELIAL METAPLASIA IN SCHISTOSOMA HAEMATOBIUM INFECTION

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Abstract. Although there is strong epidemiologic evidence linking Schistosoma haematobium infection with carcinoma of the bladder, the utility of cytologic screening for urinary tract cancer has not been critically evaluated in S. haematobium-endemic populations. The present cross-sectional study examined urine cytology findings among 1,014 residents (ages 1 to 91) of the S. haematobium-endemic Msambweni area of Coast Province, Kenya. Among 705 evaluable cytology specimens, prevalence of inflammation (39%), hyperkeratosis (30%), and frank atypia (0.4%) was notably higher than in previously studied, non-endemic populations. Overall, S. haematobium infection was strongly associated with increased risk for cytologic abnormality (>2.8-fold relative risk of metaplasia or hyperkeratosis; P < 0.001). Age-group analysis confirmed parallel increases in metaplasia and S. haematobium infection prevalence early in life (from age 1 to 15 for both boys and girls). However, above age 20, metaplasia prevalence persisted at 33–45% prevalence despite a decline in infection prevalence and intensity. Prevalence of advanced (moderate or severe) metaplasia showed two age-related peaks: the first at 10–14 years of age (at the time of peak infection), and the second among subjects ≥60 years old. No cancers were detected in the study population either on cytology or on follow-up ultrasound examination. These data suggest an age-dependent progression of cellular abnormalities in the urinary epithelium that is associated with chronic S. haematobium infection, which becomes independent of concurrent infection intensity as subjects grow older. Implications for cancer screening are discussed.

INTRODUCTION

Squamous cell carcinoma of the urinary bladder has been associated with Schistosoma haematobium infection in many parts of Africa.1–4 The epidemiologic association is based both on case-control studies,1,2,5,10 and on the close correlation of bladder cancer incidence with prevalence of S. haematobium infection within different geographic areas.5–8,11 A parasite-tumor linkage is further suggested by the predominance of squamous cell carcinomas (as opposed to transitional cell) morphology of bladder carcinomas seen in S. haematobium-endemic areas, and by the frequent association of tumors with parasite eggs and egg-induced granulomatous pathology involved bladder tissues.5,12 In non-endemic countries, urinary sediment cytology has proven to be a sensitive and specific technique for detecting more aggressive carcinomas of the urinary tract in patients with symptomatic urinary tract disease.13,14 Despite the linkage between S. haematobium and bladder cancer, only limited data are available on cytopathologic findings in schistosomiasis-related tumors. While sensitivity for tumor detection appears to be good,14–16 little is known about the specificity of cytodiagnosis for tumor in the presence of the inflammation associated with S. haematobium infection. In particular, a prospective, cross-sectional survey of the cytopathology of urinary epithelium has not been reported in a S. haematobium-endemic area. The present study surveys the cytologic abnormalities of urinary sediments of a population living in a S. haematobium-endemic area of Kwale District in the southern portion of Coast Province, Kenya, an area with a high incidence of squamous cell bladder carcinoma.3 The significant associations of urinary cytologic abnormalities with S. haematobium infection and with increasing patient age are detailed.

Study population. Study subjects were recruited from the villages of Bomani (population = 2,624) and Kingwede (estimated population = 1,500), 50 km southwest of Mombasa in Kwale District of Coast Province, Kenya during the summer of 1985. Bomani Village was selected for primary study because it is located within a designated Schistosomiasis Control Area, and its demography and patterns of water contact had been previously evaluated.17,19 In the present cross-sectional study, all available Bomani residents were evaluated by physical and parasitologic examinations, as well as by urine cytological examination, prior to treatment for S. haematobium infection. Because some school-age children from Bomani had been previously treated in an ongoing S. haematobium control program, they were excluded from analysis in the present cytologic study, which aimed to evaluate abnormalities in untreated individuals. For that reason, school-age children from the adjacent village, Kingwede, which had not been included in previous S. haematobium control programs, were also surveyed to ensure adequate representation of untreated school-age children.

Clinical and parasitologic investigations. Prior to participation, informed consent was obtained from study participants (or if minors, from their parents or legal guardians) under a protocol approved by the Human Investigations Review Board of University Hospitals of Cleveland, and by the Ministry of Health, Kenya. Each participant received an intake physical examination to assess general health and hepatosplenomegaly.18 Following instruction in midstream urine collection, urine samples were then collected from each individual. Urines were semi-quantitatively scored for hematuria and proteinuria using Chemstrip 5 dipsticks (Fisher Scientific, Fair Lawn, NJ),19 and S. haematobium infection was detected and quantified by Nuclepore (Nuclepore, Pleasanton, CA) filtration of two 10-ml aliquots of stirred urine,
as previously described. Portable ultrasound examination of the urinary tract was performed on a 1:12 random subset of the study population. The resulting images were scored for hydronephrosis and bladder abnormalities as previously described.

Cytologic evaluation. Forty-five milliliters of urine from each subject was placed in a plastic container containing 5 ml of fixative (2.5% Carbowax in ethyl alcohol). Within 24 hr of collection, samples were centrifuged and smears of each sediment were prepared. The dried smears were transported to a central laboratory, where Papanicolaou staining was performed. Smears were graded, without knowledge of the subject’s clinical or infection status, on a 0 to 3 scale (0 = none, 1 = mild, 2 = moderate, 3 = marked) for squamous cell metaplasia, and for inflammation.

Statistical analysis. The association of cytologic abnormalities with categorical patient parameters was determined by chi-square analysis or Fisher’s exact test, as appropriate. Ranked outcomes or continuous parameters not following a normal distribution in the study population (age) were evaluated using the Mann-Whitney U test or the Kruskal-Wallis test.

RESULTS

Study participation. A total of 875 individuals (ages 1 to 91), or approximately one-third of Bomani village residents participated in the present study. The subjects came from 319 of the 399 village households identified by household-to-house census performed the previous year. The most common reason for non-participation, particularly among young male adults, was travel away from the area for purposes of employment. Because 48 of the school-age participants were known to have received treatment for schistosomiasis in the preceding 12 months, the survey sample was supplemented by inclusion of 139 previously untreated schoolchildren (ages 6 to 15) from Kingwede, an adjoining village. Table 1 summarizes the demographics of all 1,014 participants, and of the subsets of evaluable patients analyzed in this study. Overall, the Bomani village participants in this study comprised 43% of the previously untreated population of that village.

Of the total 1,014 participants, 112 had poorly-preserved samples or no urine cytology data were obtained, leading to exclusion from the study. Cytology samples from 197 women showed vaginal contamination, also leading to exclusion. Thirty-five of subjects with evaluable cytology were known to have received therapy before the study, and an additional 39 individuals had missing or incomplete information on sex, birth date, or infection status, and were not included in later analyses according to age and sex.

Prevalence of abnormal findings on urine cytology examination. Table 2 details the prevalence of abnormal cytology findings among subjects with evaluable specimens (n = 705), among those without previous therapy for S. haematobium (n = 650), and among those school-age individuals known to have recently received therapy (n = 34).

Overall, community prevalence of cellular inflammation, hyperkeratosis, and squamous cell metaplasia was quite high (≥30%) compared to rates previously reported in S. haematobium-endemic areas in Egypt (8%) and from non-endemic areas (<1%). Frank cellular atypia was noted in three subjects. With the exception of atypia, cytologic abnormalities were all significantly more common among the segment of the population (24%) that had documented S. haematobium infection (Table 2). The prevalence of cytologic abnormalities among previously infected children who had been treated one year earlier was found to be similar to levels seen among uninfected, untreated individuals.

Metaplasia was significantly more common among women than men (40% versus 23%; P < 0.01). However, there was no sex difference in the prevalence of the higher grades of moderate and severe metaplasia (6% for males and 7% for females). Likewise, there were no significant sex differences in the prevalence of inflammation, hyperkeratosis, or atypia. Metaplasia was significantly more common among older age groups (P < 0.03 by Kruskal-Wallis test) and the three subjects found to have atypia were all more than 35 years of age.

Age prevalence of S. haematobium infection and metaplasia. The above findings, coupled with observed age-specific variation in S. haematobium infection intensity, and the known association of chronic forms of urinary tract morbidity with age, prompted further detailed evaluation of age-specific changes in cytologic abnormalities. Results
were further analyzed separately by sex to allow for the fact that squamous metaplasia of the trigone is reported to be common (up to 50% prevalence) in some normal adult female populations.25

Figure 1 illustrates the prevalence and mean intensity of urinary schistosomiasis by age group for all study subjects. It also demonstrates the age-prevalence profile for metaplasia (all categories) and for higher grades of metaplasia (moderate and severe). Of note is the typical increase in metaplasia (all categories) and for higher grades of metaplasia (moderate/severe) during this period of life. Among adult age groups, both sexes demonstrated a late increase in the prevalence in moderate/severe metaplasia: for women, prevalence of moderate/severe metaplasia decreased to 5% in the 20–25-year-old age group, then increased to a plateau between 7% and 9% among age groups more than 25 years old. For men, these higher grades of metaplasia were rare (< 5%) between ages 25 and 40, but their prevalence increased to > 10% above the age of 40, and was even more common (16%) among males more than 59 years old (P < 0.03, compared to 5% for males less than 60 years old).

Table 3 details the association of metaplasia with S. haematobium infection and inflammation among different male and female age groups. There was a significant, positive association between metaplasia and S. haematobium infection in younger (< 20 years old), but not older (≥ 20 years old) age groups. This difference was attributable to a higher metaplasia prevalence among “egg-negative” adults (36%, compared to 18% among egg-negative children; P < 0.001). In contrast, prevalence of metaplasia among egg-positive individuals remained fairly high (i.e., > 35%) across all age groups.

Other sources of inflammation beside S. haematobium infection could have contributed to the high prevalence of metaplasia, particularly among adult women, who in other series have shown higher levels of bladder metaplasia, presumably related to intercurrent bacterial infections. In the present study population, 38% of the males and 40% of the females had evidence of inflammation on urine cytology (difference was not significant). We noted a positive association, but not complete concordance, between metaplasia and cytologic findings of inflammation: 146 (65%) of 225 subjects with metaplasia had inflammation, while 146 (56%) of 262 subjects with inflammation had metaplasia (P < 0.001). For both males and females, metaplasia was more

### Table 3

| Age Group | Infection | | Inflammation | |
|-----------|-----------| |-----------| |
| Males (age in years) | Present | Absent | Present | Absent |
| < 5 | 0/5 (0%) | 9/6 (14%) | 5/15 (33%) | 4/53 (8%) |
| 5–19 | 28/59 (48%) | 12/84 (14%) | 33/74 (45%) | 7/71 (10%) |
| 20–39 | 4/4 (100%) | 18/50 (36%) | 12/18 (67%) | 10/38 (26%) |
| 40–59 | 6/12 (50%) | 15/48 (31%) | 12/22 (54%) | 9/39 (23%) |
| ≥60 | 4/6 (67%) | 7/24 (29%) | 8/11 (73%) | 4/20 (20%) |
| Females (age in years) | Present | Absent | Present | Absent |
| < 5 | 2/3 (67%) | 9/35 (26%) | 5/10 (50%) | 8/32 (32%) |
| 5–19 | 23/34 (68%) | 15/58 (26%) | 30/49 (61%) | 9/46 (20%) |
| 20–39 | 15/22 (68%) | 25/66 (38%) | 26/36 (72%) | 15/55 (27%) |
| 40–59 | 2/9 (22%) | 13/37 (40%) | 9/18 (50%) | 9/31 (31%) |
| ≥60 | 1/3 (33%) | 4/10 (40%) | 2/4 (50%) | 3/9 (33%) |
common in subjects with inflammation (metaplasia prevalence: males = 50%, females = 62%) then those without inflammation (males = 15%, females = 25%; \( P < 0.001 \) for both sexes). In age group analysis, metaplasia was more common among older males (\( \geq 20 \) years old) either with or without inflammation; with inflammation, males \( \geq 20 \) years old with inflammation had a 63% prevalence of metaplasia compared to 43% in younger males with inflammation (\( P < 0.03 \)); without inflammation, metaplasia prevalence among older males was also significantly higher (24%) than among younger males (9%; \( P < 0.005 \)). This age effect was not noted in females, either with or without inflammation. However, in females without inflammation, moderate and severe metaplasia were significantly more common among those more than 40 years old (10% prevalence) than among those less than 40 years old (2% prevalence; \( P = 0.05 \)).

Metaplasia was positively associated with hyperkeratosis (\( P < 0.05 \)) in all age groups for both sexes. Dipstick hematuria was highly associated with \textit{S. haematobium} infection in all age groups, but was not significantly associated with metaplasia in either infected or uninfected males or females.

**Ultrasound examination of kidneys and bladder.** Ultrasound examination of a subset of village residents revealed a trend toward higher prevalence of hydronephrosis among adults \( \geq 20 \) years old (23%) than among untreated children (13% [not significant]; \( P = 0.08 \)). In contrast, bladder thickening and bladder irregularity were less common in adults than in children (2% thickening and 19% irregularity for adults compared with 16% thickening and 34% irregularity among children [\( P < 0.05 \) for each abnormality]). Metaplasia was not significantly more common in individuals with bladder abnormalities detected on ultrasound, although there was a trend toward higher prevalence of moderate/severe metaplasia among individuals with bladder irregularity (23% prevalence versus 5% [not significant]; \( P < 0.10 \)). Ultrasound examination of patients with atypia did not show any definite kidney or bladder pathology at the time of study.

**Discussion**

The results of this survey indicate a high level of urinary cytologic abnormalities among residents from a \textit{S. haematobium}-endemic area. No overt carcinomas were detected either by cytology or by ultrasound, but this was not extraordinary given the estimated incidence (2–4/100,000) of bladder carcinoma found in other \textit{S. haematobium}-endemic regions.\textsuperscript{5,7,8} Overall, the presence of urinary epithelial metaplasia was significantly associated with concurrent \textit{S. haematobium} infection and cytologic evidence of inflammation. Cellular abnormalities were particularly linked with concurrent parasite infection among children. Among adults, however, the link between \textit{S. haematobium} infection and metaplasia was less strong, in that older individuals tended to have cytologic abnormalities independent of infection status or evidence of inflammation in the cytology specimen. Of note was the relatively high prevalence of moderate and severe metaplasia among the oldest segment of the population (\( > 60 \) years old).

These findings have several implications for cancer screening in endemic areas. Because of the bladder pathology induced by \textit{S. haematobium} infection, the finding of hematuria and chronic dysuria, which would typically evoke evaluation for bladder cancer in non-endemic areas, cannot be used as reliable screening or diagnostic criteria in areas with a high prevalence of urinary schistosomiasis. Urinary cytology is reported to have good sensitivity (\( \geq 90\% \)) for high-grade cancer detection among symptomatic individuals in the United States and Europe,\textsuperscript{26,27} and among \textit{S. haematobium}-infected populations in Egypt.\textsuperscript{17,18} Specificity for cancer detection in hospitalized patients in non-endemic areas is 100%,\textsuperscript{13,14,27} and the negative predictive value for voided urine cytology is 86–93%.\textsuperscript{26,27}

These performance characteristics have not been confirmed in \textit{S. haematobium}-endemic populations. Because the present study did not detect any patients with tumors, test characteristics cannot be determined. Given the high background prevalence of inflammation and metaplasia detected in our study, we would predict an increased number of equivocal cytology specimens in screening large populations in \textit{S. haematobium}-endemic areas. This effect would degrade the sensitivity and specificity of cytologic screening for bladder cancer, resulting in increased levels of secondary testing (cystoscopy and biopsy) to exclude carcinoma in cytology-equivocal patients. For this reason, further study is needed to determine the actual predictive value of positive and negative cytology testing, alone or in combination with other field-based testing, such as portable ultrasound, in clinical practice in these areas.\textsuperscript{26} It will also be appropriate to evaluate any possible change in cytologic test characteristics after treatment and cure of \textit{S. haematobium} infection.

Several factors, alone or in combination, may explain the dissociation between infection/inflammation status and observed metaplasia findings in our older study subjects. First, the sensitivity of urine filtration for parasitologic diagnosis is known to be lower in individuals with light intensity infections.\textsuperscript{23} Such light infections are more common among adults,\textsuperscript{24} making the detection of their active infections less likely. In addition, sensitivity of parasitologic testing may be further limited by chronic scarring of the bladder, (common among older individuals from \textit{S. haematobium}-endemic areas),\textsuperscript{12,28} which may limit the release of eggs into the bladder lumen. Thus, adults who score as “egg-negative” in urine filtration may, in fact, be lightly infected with \textit{S. haematobium}, with ongoing tissue injury and inflammation.\textsuperscript{29} Other forms of inflammation, particularly bacterial infection in adult women, are likely contributing to higher baseline levels of metaplasia from the trigone area.\textsuperscript{25} Finally, as suggested by our data and most relevant to cancer formation, proliferative changes in bladder epithelium may become independent of ongoing infection after long periods of chronic \textit{S. haematobium}-induced inflammation.\textsuperscript{28,30} Similar metaplastic changes have been identified in American and European paraplegics in those with long-term catheter-induced bladder infection and irritation,\textsuperscript{29,31} and these changes become significantly more common with longer duration (\( \geq 10 \) years) of catheterization.

The cellular mechanisms linking \textit{S. haematobium} infection with cancer formation are not yet defined.\textsuperscript{32,33} In some cases, severe metaplasia in bladder urothelium may represent a pre-cancerous transformation, while in others it may merely serve as a marker for the prolonged inflammation that is
associated with high cancer risk.\textsuperscript{11,14,36} Keratinizing metaplasia, \textit{per se}, as observed in our study subjects, has a strong association with cancer formation in patients with chronic irritation due to bladder stones, chronic infection, or prolonged catheterization.\textsuperscript{2,23,31,35,38} In the capuchin monkey model, Cheever and others\textsuperscript{33} have demonstrated that intense \textit{S. haematobium} infection is associated with the development of significant multifocal proliferative lesions that resemble low-grade carcinomas. In these animals, natural loss of infectious burden after several years is associated with regression of these lesions, indicating that they are non-cancerous in nature. However, this sort of proliferative growth, combined with increased excretion and/or local formation of mucin, is likely to contribute significantly to the onset of cancer formation in humans. The risk of cancer formation is greater when chronic inflammation is combined with exposure to urinary carcinogens.\textsuperscript{7,39,41} In particular, chromosomal breakage and modification of p53 and CDKN2 tumor-suppressor genes have been documented in \textit{S. haematobium}-infected bladder cancers in Egypt.\textsuperscript{29,30,42,43}

With this in mind, the increase in moderate and severe metaplastic changes seen in our older adults may reflect a progression to intermediate level proliferative transformation, with significant risk of cancer formation despite the observed age-related reduction in \textit{S. haematobium} infection intensity after the age of 40.\textsuperscript{9,29,30} Given both the high background of urinary-tract symptoms and the high incidence of bladder carcinoma in \textit{S. haematobium}-endemic areas, we conclude that additional diagnostic screening for cancer is appropriate, especially for older adults. While urinary cytology has the advantage of requiring only limited technology, it may not, by itself, be sufficiently sensitive or specific to provide effective screening. Combined testing with ultrasound imaging\textsuperscript{26} and with immunohistochemical staining for tumor cell markers\textsuperscript{27,44,45} is likely to prove necessary.

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