CHAPTER 4

DETECTABLE UROGENITAL SCHISTOSOME DNA AND CERVICAL ABNORMALITIES SIX MONTHS AFTER SINGLE-DOSE PRAZIQUANTEL IN WOMEN WITH *SCHISTOSOMA HAEMATOBIUM* INFECTION

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Abstract

We explored the response to single-dose praziquantel therapy in a cohort of 33 women with *Schistosoma haematobium* infection in rural Mwanza, Tanzania. Women with *S. haematobium* infection confirmed both by eggs in urine and by polymerase-chain reaction (PCR) received single-dose praziquantel and treatment for concomitant sexually-transmitted infections. Macroscopic cervical abnormalities were also quantified. After six months, microscopically-detectable egg excretion was eliminated but 8 of 33 women (24%) were persistently positive for *S. haematobium* by PCR and 11 (33%) had cervical abnormalities potentially attributable to schistosomiasis. This suggests that praziquantel treatment more frequently than every six months may be necessary for complete elimination of the parasite and prevention of genital tissue pathology. This aggressive therapy may in turn play a key role decreasing HIV susceptibility in millions of people living in regions in which *S. haematobium* is endemic.

Introduction

Schistosoma haematobium is a parasitic worm infection acquired through contact with infested fresh water that affects an estimated 112 million people living in sub-Saharan Africa (1). Adult *S. haematobium* worms live for years in host pelvic venous plexi and lay hundreds of eggs per day (2). The schistosome miracidium within the egg secretes proteolytic enzymes through the egg's shell that facilitate the migration of the egg through the vessel wall and into the surrounding tissues toward the mucosal tissue and lumen of the genitourinary tract (2). Some eggs reach the lumen and are excreted in urine or genital secretions (3), but a significant fraction become trapped in the tissues of urogenital organs, where they cause acute and chronic inflammation. Histopathological and autopsy studies of women with *S. haematobium* infection demonstrate eggs throughout the upper and lower genital tract (4–6). *S. haematobium* infection in women has been associated with HIV infection in cross-sectional studies (7–9) and is postulated to increase HIV susceptibility both by damaging the mucosal integrity of the female genital tract and by causing local mucosal and systemic immune responses (8,10–14).

The World Health Organization (WHO) recommends empiric anti-schistosome treatment with praziquantel for at-risk individuals in sub-Saharan Africa to reduce disease morbidity and with the possibility of preventing HIV (15,16). In highest-prevalence areas (>50%), annual single-dose praziquantel is recommended for both children and adults (17). Self-report of even one anti-schistosome treatment before the age of 20 years in girls has been associated with significantly lower rates of genital manifestations of schistosome infection such as sandy patches and contact bleeding (18).

Two longitudinal studies have suggested that single-dose praziquantel significantly reduces urinary egg excretion but does not completely cure *S. haematobium* infection, particularly its gynecological manifestations. The smaller study reported regression of genital sandy patches in 2/4 women re-examined nine weeks after treatment but persistence in the other two (19). In the larger study of 338 women seen after 12 months, praziquantel treatment was not associated with improvements in gynecological abnormalities or contact bleeding (20). In order to explore and extend these findings, we added molecular diagnostics to investigate response to single-dose praziquantel in a cohort of women with *S. haematobium* infection in rural Tanzania.

Methods

Study design. We conducted a six-month cohort study in which women with *Schistosoma haematobium* infection were recruited from the *S. haematobium*-endemic villages of Lubiri and Nyamilama, treated with praziquantel, and retested after six months. This was a proof-of-concept study to explore the utility of PCR for monitoring treatment response in urogenital schistosomiasis. We enrolled women only for this preliminary study because our work was incorporated into an ongoing women's health program administered by the Tanzanian Ministry of Health.

As previously-described, women aged 18-50 had been screened for cervical cancer using visual inspection with acetic acid (the standard of care in Tanzania) in partnership with a governmental outreach program (7). We enrolled women who screened negative for cervical cancer and who had *S. haematobium* infection confirmed by visualization of eggs in urine plus were PCR-positive for schistosome DNA in urine. We additionally examined cervical smears for eggs and tested cervicovaginal lavages for schistosome DNA. A nurse collected socio-demographic data using a structured questionnaire in Kiswahili, the local language.

Women received free observed treatment with single-dose praziquantel (40mg/kg) at enrollment. We included pregnant and lactating women as praziquantel is recommended for these groups by the WHO (17). Women and their sexual partners also received free treatment for the sexually-transmitted infections (STIs) for which testing can be routinely done in Tanzania, including syphilis, trichomoniasis, chlamydia, gonorrhea, candidiasis, and bacterial vaginosis if indicated. Women participated in an educational seminar about schistosomiasis, STIs, and possible associations with HIV infection on the day of study enrollment. Study participants were re-evaluated for *S. haematobium* and gynecological infections at the six-month follow-up visit and again received indicated treatment without cost.

Field investigations. Gynecological examinations were performed at enrollment and six months to document any macroscopic cervical abnormalities including sandy patches, irregular blood vessels, contact bleeding, condylomata, and abnormal discharge. Prior to any sample collection, the cervix was visually inspected using a flashlight and magnifying glass. Macroscopic abnormalities were identified by the physician and recorded and drawn to scale on a standardized data collection form. Abnormal discharge, defined as discharge of any color other than white or white discharge that was thick and/or copious, was also noted and described on the form. The physician was blinded to the prior examination findings at the time of follow-up data collection.

The first sample collected was a wet preparation for candidiasis, trichomoniasis, and bacterial vaginosis, followed by an endocervical swab for chlamydia and gonorrhea as previously described (7,21). Next, cervical smears were taken from abnormal-appearing regions if present and otherwise from the transformation zone. Lastly, cervicovaginal lavage samples were prepared using four milliliters of normal saline to wash the face of the cervix three times.

Single urine samples at enrollment and follow-up were collected between 10am and 2pm, filtered, and examined microscopically for schistosome ova by a trained microscopist. Serum was tested by Rapid Plasma Reagin (RPR), with *Treponema pallidum* particle agglutination (TPPA) testing performed on positive samples for diagnosis of syphilis.

Real-Time PCR. DNA isolation and PCR was performed as described previously (12). Briefly, DNA was isolated from well-mixed pre- and post-treatment urine and cervicovaginal lavages using QIAamp Tissue Kit spin columns (QIAgen, Hilden, Germany). Quantitative real-time PCR testing was performed using *Schistosoma* genus-specific primers and detection probe. PhocineHerpes Virus 1 (PhHV-1) was used as an internal control in each sample along with PhHV-1-specific primers and detection probe to detect any inhibition. Amplification, detection, and analysis were performed with the CFX96 real-time detection system (Bio-Rad laboratories). The PCR output from this system consists of a cycle-threshold (Ct) value, representing the amplification cycle in which the level of fluorescent signal exceeds the background fluorescence, and reflecting the parasite-specific DNA load in the sample tested. Negative and positive control samples are included in each amplification run.

Statistics. Continuous variables were summarized by median and interquartile range (IQR) and categorical variables were summarized by frequency and percentage. Proportions were compared using Fisher's Exact test and continuous variables by the Wilcoxon rank-sum test. Pre-post treatment values were compared with the Wilcoxon matched-pairs signed-rank test. Two-sided hypotheses/tests were assumed for all confidence intervals and p-values. Data were analyzed using Stata Version 11 (College Station, Texas).

Ethics. Written informed consent was obtained by a trained study nurse fluent in the local language who read the consent form to all study participants. Women who decided to participate signed their names or made their mark/thumbprint (if illiterate) on the consent form. Ethical permission was granted by Bugando Medical Centre, the Tanzanian National Institute for Medical Research, and Weill Cornell Medical College.

Results

Patient characteristics. Between November 2009 and May 2010, we enrolled 39 women with *S. haematobium* infection detected by both microscopy and PCR. Of these, 33 (85%) were re-examined a median of 188 (IQR, 183-188) days after treatment and included in the analysis. There were no significant differences between age or number of urinary ova between women who were lost to follow-up (n=6) and those who were not.

Socio-demographic information is detailed in Table 1. Patients were young, with a median age of 22 (20-28) years, and 6 patients were aged 18-19. The large majority (90%) were married. Sexual risk factors were assessed indirectly. Nearly one-third of women had children with at least two different men, and more than half of women stated that their husbands had children with another woman during the marriage. Ten women (30%) reported going to bed hungry in the past month

Table 1. Demographic Characteristics of 33 Adult Women (>18 years) Treated for Urogenital Schistosomaisis and Followed Up After Six Months.

CHARACTERISTIC	VALUE
Age in years—median (interquartile range)	22 (20—28)
Married—number (percent)	28 (90%)
Number of children	
0	3 (9%)
1--2	16 (49%)
3--4	9 (27%)
≥ 5	5 (15%)
Number of fathers of a woman's children	
1	23 (70%)
2	8 (24%)
3	2 (6%)
Husband had children outside of marriage during the marriage—number (percent)	18 (55%)
Went to bed hungry in past month—number (percent)	10 (30%)
Reported no prior treatment for schistosomiasis—number (percent)	8 (24%)

Non-missing data were included for each calculation.

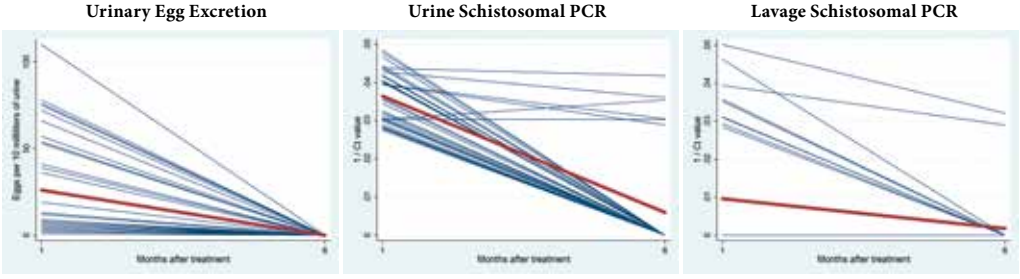
because their household did not have enough food, a measure of food insecurity (22,23).

Microscopy and PCR pre- and post-treatment. At enrollment, all women had urinary ova with a median of 8 (4-53) per 10 milliliters. Three women additionally had viable *S. haematobium* ova in cervical smears. All 33 women had positive urine PCR results. Urine Ct values ranged from 20.6-36.3 (median 27.8). Cervicovaginal lavage PCRs were positive in 9 women (27%), ranging from 19.9-35.1 (median 28.3). Two of the three women with eggs in cervical smears had PCR-positive lavages.

Six months after treatment, ova were no longer detected in any urine or cervical sample. Eight women (24%) still had detectable schistosome DNA by PCR with Ct values <35 (six urines and two lavages, **Figure 1**). In women with persistently positive PCR findings, the DNA quantity remained approximately unchanged. Urine PCRs became zero in the two women with persistently-positive lavages.

Gynecological abnormalities pre- and post-treatment. STIs diagnosed included: four chlamydia (12%) and one each (3%) of syphilis, gonorrhea, trichomoniasis, and multiple cervical condylomata. At enrollment, 9/33 women (27%) were negative for STIs but had at least one macroscopic gynecological abnormality

Figure 1. Pre- and Post-Treatment Urine Egg, Urine Schistosome PCR, and Cervico-vaginal Lavage Schistosome PCR.



Results for each patient are shown at the time of praziquantel treatment (Month 0) and follow-up examination six months later (Month 6). PCR, Real-Time Polymerase Chain Reaction to detect schistosome DNA. Bold red line indicates the mean value of all women tested (n=33).

that, in the absence of STIs or malignancy, could be consistent with *Schistosoma* involvement of the genital tract (Table 2). These included sandy patches, contact bleeding, convoluted blood vessels, and abnormal discharge.

After treatment, the same macroscopic abnormalities persisted in 7 of these 9 women, and four others had new abnormalities that could be consistent with cervical *Schistosoma* infection in the absence of STIs and malignancy. Thus we observed, in total, 11 women (33%) with gynecological abnormalities that were possibly consistent with schistosomiasis six months after praziquantel treatment. Four of these 11 (36%) were persistently PCR-positive (3 urines and one lavage), compared with 4 (18%) of the 22 who had no gynecological abnormalities (p=0.25). Ages were not significantly different between groups of women who remained positive by PCR or who had persistent gynecological abnormalities and those who did not.

In total, 10 women had evidence of *S. haematobium* involvement of the genital tract by eggs or PCR at the time of enrollment, and 6 months after treatment, 2 (20%) remained PCR-positive in cervicovaginal lavages. Schistosome DNA additionally was detectable in urine of 6 other women at 6 months. Of the 25 women who were negative for both schistosome eggs and DNA at 6 months, 7 (28%) had gynecological abnormalities in the absence of STIs or cervical cancer, suggestive of *S. haematobium*-induced pathology.

Discussion

In this cohort of 33 young adult women with *Schistosoma haematobium* infection who received single-dose praziquantel therapy, *Schistosoma* DNA was detectable 6 months later in the genital tract of 2/10 women with initial genital tract involvement and in urine of 6 additional women. Moreover, nearly one-third had ongoing cervical pathology potentially attributable to schistosome infection. Our findings suggest that, after single-dose praziquantel, which is the WHO's current recommendation for schistosomiasis (24), parasite remnants and genital tract tissue pathology persist in a substantial minority of adult women. Other groups have advocated higher and/or repeated praziquantel doses (25–27). Our work supports this concept and raises serious concern given recent findings that schistosomiasis may be a risk factor for HIV acquisition (7,8,21).

Schistosoma real-time PCR has been evaluated as an indicator of genital *S. haematobium* infection (28) but not as a marker of effectiveness of praziquantel treatment. Our work documents that *Schistosoma* DNA was detectable six months after treatment in 8/33 women whose negative egg counts appeared to indicate

Table 2. Findings in 33 Adult Women (>18 years) Treated for *Schistosoma haematobium* Infection and Re-tested after Six Months.

Measurement	Pre-Treatment	Six Months Post-Treatment	p-value
Urine PCR (Ct value)			
Positive urine PCR—number (percent)	33 (100%)	6 (18%)	<0.001
Median	27.8	0	<0.001
Interquartile range (IQR)	23.8-32.9	0-0	
Range	20.6-36.3	0-34.6	
Urinary ova (per 10 ml)			
Positive urinary ova—number (percent)	33 (100%)	0	<0.001
Median	8	0	<0.001
IQR	4-53	0	
Range	1-110	0	
Lavage PCR (Ct value)			
Positive lavage PCR—number (percent)	9 (27%)	2 (6%)	0.044
Median	0	0	0.069
IQR	0-19.9	0-0	
Range	0-35.08	0-34.4	
Positive cervical smear ova—number (percent)	3 (9%)	0	n.s.
Convoluted blood vessels—number (percent)	1 (3%)	2 (6%)	n.s.
Sandy patches—number (percent)	7 (21%)	9 (27%)	n.s.
Contact bleeding—number (percent)	1 (3%)	1 (3%)	n.s.
Abnormal discharge—number (percent)	2 (6%)	1 (3%)	n.s.
Any gynecological abnormality potentially consistent with genital *S. haematobium* infection—number (percent)	9 (27%)	11 (33%)	n.s.

n.s. = not significant

resolved infection. Previous studies in Zimbabwe and Malawi have similarly reported unresolved macroscopic gynecological pathology (sandy patches, blood vessel abnormalities, and/or contact bleeding) in women treated for genital *S. haematobium* infection and any STIs and re-examined up to a year later (19,20). Our work now substantiates these clinical reports by showing that, after praziquantel treatment, a sizeable percentage of women have not only ongoing cervical pathology but also detectable parasite DNA in urine and cervicovaginal lavage fluid.

The persistence of gynecological lesions potentially caused by *S. haematobium* suggests that *S. haematobium*-induced pathology may be irreversible. Liver fibrosis due to *S. mansoni* infection is often permanent even after parasite death (29). Similarly, gynecological lesions are believed to begin as inflammatory tissue reactions to migrating ova (30,31) which, over time, become fibrotic "sandy patches" (18,30) that may not be curable. In support of this hypothesis, women from an *S. haematobium*-endemic region who reported receiving praziquantel before age 20 were less likely to have gynecological contact bleeding and sandy patches than women treated after age 20 (18). Thus regulartreatment beginning in childhood appears optimal to prevent potentially-irreversible genital lesions that may predispose to HIV infection (11,16,18). Establishment of the most effective treatments for genital *S. haematobium* infection remains an urgent research priority.

A limitation of this study was our inability to determine whether *Schistosoma* DNA detected at six months was due to re-infection, sample contamination by semen of infected sexual partners or by stool, partial treatment failure, post-treatment maturation of juvenile worms which are less susceptible to praziquantel (32), or lingering ova and parasite products in tissue and blood (33). We believe that the latter three explanations are the most likely sources of DNA in urine samples, which were clean-catch and showed no ova. Cervicovaginal lavages are inherently not sterile and could more easily have been contaminated. Second, neither testing for Herpes simplex virus and Human papillomavirus infections, nor exclusion of premalignant lesions, was possible. Lastly, the sensitivities of cervical smears (34), PCR of cervicovaginal lavages (particularly in women older than age 25) (28), and single rather than double urine samples are all suboptimal for detection of *S. haematobium* infection. Lower sensitivity would bias our study towards finding less residual infection in patients with confirmed *S. haematobium* at the study outset. Thus our detection of persistent schistosome DNA in 8/33 women may be low. Either way, our findings suggest that treatment may need to be repeated more frequently than the WHO recommends (annually) and more frequently than even the six-month duration of this study in order to maximize parasite elimination and minimize tissue pathology.

In conclusion, our results suggest that single-dose praziquantel does not completely eliminate *S. haematobium* DNA in a substantial portion of women. Moreover, one dose of praziquantel appears not to reverse cervical pathology. Our data supports using praziquantel early and often in girls and women living in endemic areas. Given that *S. haematobium* may be a risk factor for HIV acquisition, this low-cost ($0.32) (11) intervention may have the potential not only to lessen schistosome-related morbidity, but also to prevent new HIV infections among millions of at-risk women in Africa.

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