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## CHAPTER 3

# ASSOCIATION OF SCHISTOSOMIASIS AND HIV INFECTION IN TANZANIA

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## Abstract

Animal and human studies suggest that *Schistosoma mansoni* infection may increase risk of human immunodeficiency virus (HIV) acquisition. Therefore, we tested 345 reproductive age women in rural Tanzanian villages near Lake Victoria, where *S. mansoni* is hyperendemic, for sexually transmitted infections (STIs) and schistosomiasis by circulating anodic antigen (CAA) serum assay. Over one-half (54%) had an active schistosome infection; 6% were HIV-seropositive. By univariate analysis, only schistosome infection predicted HIV infection (odds ratio [OR] = 3.9, 95% confidence interval = [1.3–12.0],  $p = 0.015$ ) and remained significant using multivariate analysis to control for age, STIs, and distance from the lake (OR = 6.2 [1.7–22.9],  $p = 0.006$ ). HIV prevalence was higher among women with more intense schistosome infections ( $p = 0.005$ ), and the median schistosome intensity was higher in HIV-infected than –uninfected women (400 versus 15 pg CAA/mL,  $p = 0.01$ ). This finding suggests that *S. mansoni* infection may be a modifiable HIV risk factor that places millions of people worldwide at increased risk of HIV acquisition.

## Introduction

Schistosomiasis is caused by a parasitic infection that affects over 200 million people worldwide, with approximately 85% of cases in Africa (1,2). Previously, in a cross-sectional study of Tanzanian women, we found that the odds of being infected with human immunodeficiency virus (HIV) were fourfold higher for subjects with *Schistosoma haematobium* infection than subjects without *S. haematobium* (3), and we postulated that the chronic genital inflammation caused by *S. haematobium* eggs pre-disposes to HIV infection. We observed a similar trend in women with *S. mansoni* infection, although the numbers were small, and the association did not reach statistical significance (unpublished data).

Primate models support the hypothesis that *S. mansoni* infection predisposes to HIV infection. Rhesus macaques with active *S. mansoni* infection were 17 times more susceptible to simian HIV (SHIV) acquisition after rectal inoculation than macaques without *S. mansoni* (4). Although a variety of interactions between *S. mansoni* and HIV infection in humans have been described (5–10), a direct association between active *S. mansoni* and HIV has not been documented in humans. If *S. mansoni* is a risk factor for HIV acquisition, this finding could have major implications for HIV prevention work in much of the world. We, therefore, performed a cross-sectional study to explore the relationship between *S. mansoni* and HIV infections in women living on the shores of Lake Victoria, including screening for other genital tract infections, which are well-known HIV risk factors, to adjust our analysis for any possible confounders.

The screening of large numbers of women in *S. mansoni*-endemic villages was facilitated by the use of a sensitive and specific serum test for the diagnosis of *Schistosoma* infection. The circulating anodic antigen (CAA) test detects a *Schistosoma* worm antigen in the serum, and it is 80–95% sensitive and 98–100% specific for diagnosis of schistosomiasis (11–13). CAA is a highly glycosylated excretory antigen originating from the parasite gut and released into the host blood circulation when the worm regurgitates the undigested compounds of the gut (14). Schistosome circulating antigen detection performs well in both HIV-negative and -positive individuals (9), and it is recognized by the World Health Organization as an alternative diagnostic method to parasitologic examination of multiple stool or urine samples (15,16).

## Materials and methods

**Study sites and subjects.** This study was conducted in seven rural villages with high rates of both *S. mansoni* and HIV, chosen for their locations at a distance

of 1–25 km from Lake Victoria in western Tanzania (**Figure 1**). Lake Victoria is infested with the *Biomphalaria* snail, the intermediate host of *S. mansoni*, and inhabitants of the study sites have among the highest prevalence of *S. mansoni* infection in the world.

Repeat surveys conducted by our group between 1999 and 2011 have documented a high prevalence of *S. mansoni* in these villages; in contrast, the prevalence of

**Figure 1. Lake Victoria, Surrounding Countries, and the Study Area.**



*S. haematobium* is < 3% in adults in the same villages (3,17–20)95% confidence interval [CI] = 1.2–13.5. HIV is also prevalent in these villages; a 2004 study showed that 8% of adults greater than 15 years of age were HIV-infected (21). The major mode of HIV transmission is heterosexual intercourse, and there are more females than males infected, with a female to male ratio of HIV infection of 1.2:1.0 (21).

We recruited women ages 18–50 years who were seeking routine pre-natal, post-natal, or pediatric care

for themselves or their newborn children in the seven study villages. The study team visited each of the health posts for 1 week between January of 2010 and August of 2011 and invited consecutive women at the posts to participate in the study.

**Study procedures and sample collection.** The study included an oral questionnaire, a gynecologic exam, and phlebotomy. Gynecologic examinations included wet preparations, which were examined on-site for diagnoses of *Trichomonas vaginalis*, *Candida* species, and bacterial vaginosis by the criteria in the work by Amsel and others (22). Endocervical swabs were collected for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* testing at the laboratory of the National Institute for Medical Research (NIMR) in Mwanza, Tanzania. Venous blood was also collected, and serum was separated and stored at  $-20^{\circ}\text{C}$  at the NIMR laboratory. Testing for syphilis and preparation of a portion of CAA test strips

were performed at NIMR. Additional test strip preparation and all test strip reading were performed at the Leiden University Medical Center.

Women were offered on-site voluntary HIV counseling and testing in Kiswahili by a trained nurse counselor. Rapid tests (SD Bioline; Standard Diagnostics, Inc., Kyonggi-do, South Korea) were used with confirmatory testing by a second test (Alere Determine; Inverness Medical, Princeton, NJ) as per the national testing algorithm, and patients received their results and post-test counseling on the same day.

Patients diagnosed with HIV were referred to the local HIV clinic for free care and treatment. Women with trichomoniasis, candidiasis, or bacterial vaginosis were provided treatment on the same day. Women who tested positive for gonorrhea, chlamydia, syphilis, or schistosomiasis were treated at a followup visit as soon as laboratory results were available.

**Laboratory analyses.** *Schistosoma*. CAA is a glycoprotein that is produced by gut epithelial cells of schistosomal worms (14) and secreted in large quantities into the host bloodstream during active infection (23). The CAA test does not distinguish *S. haematobium* from *S. mansoni* infection. The test usually becomes negative within 1 week of successful antischistosomal therapy (24). CAA testing was performed using the upconverting phosphor (UCP) technology lateral flow assay as previously described (12). Serum was treated with 4% (wt/vol) trichloroacetic acid to remove proteins and antibody complexes.

After centrifugation, the supernatant was mixed with an assay buffer containing an anti-CAA mouse monoclonal antibody conjugated to UCP reporter particles and incubated for 1 hour at 37°C. The mixture was applied to a lateral flow test strip with a capture line of the same antibody, and chromatography was permitted to continue until strips were dry. Strips were read using a modified Packard Fluorocount meter, and testline results were normalized to the control line results for each test strip. A CAA value  $\geq 10$  pg/mL was considered positive based on a series of negative controls (highest value plus 2 SDs). CAA values were stratified by intensity as greater or less than 3,000 pg/mL, which represents approximately 100 eggs per 1 gram of stool (25).

*C. trachomatis* and *N. gonorrhoeae*. DNA was extracted from endocervical swab specimens and tested for *C. trachomatis* and *N. gonorrhoeae* using Amplicor CT/NG specimen preparation, amplification, internal control, and detection kits (Roche Molecular Systems, Branchburg, NJ). Gonorrhea results were confirmed using 16S rRNA PCR testing.

*Syphilis serology.* Serum was tested for syphilis using the Rapid Plasma Reagin (RPR) test, with confirmation of positive RPR results by the *Treponema pallidum* Particle Agglutination assay (TPPA).

*Ethics.* The study was approved by the Institutional Review Boards at Bugando Medical Center and Weill Cornell Medical College and the National Institute for Medical Research in Tanzania. All women were informed about the study by a nurse fluent in Kiswahili and provided written informed consent before participation.

*Statistical methods.* Data were entered into a REDCap database (Vanderbilt University, Nashville, TN) and analyzed using Stata version 11 (Stata Corporation, College Station, TX). Continuous variables were summarized by median and interquartile range (IQR), and categorical variables were summarized by frequency and percentage. Simple logistic regression (for univariate analysis) followed by multiple logistic regression (for multivariate analysis) were used to examine factors associated with HIV. In multiple logistic regression models, comprehensive adjustment was not pursued, because it yielded failure in convergence. Associations between factors and the endpoint were summarized using odds ratios (ORs) with 95% confidence intervals (CIs) and associated P values. In regression analyses, age and distance from the lake were analyzed as continuous variables, whereas other variables were analyzed as binary variables.

We also compared the intensity of schistosome infection between the HIV-positive and -negative groups using the non-parametric Wilcoxon test (because of severe non-normality of this data) and the non-parametric Jonckheere–Terpstra (JT) trend test (26). Two-sided hypotheses/tests were assumed for calculation of all CIs and P values.

## Results

**Patient characteristics.** We invited 432 eligible women living in seven villages within 25 km of Lake Victoria to participate. Of these women, 345 (80%) women provided informed consent and completed all study procedures. The median age in this population was 30 years (IQR = 24–36) (Table 1). All but one woman reported contact with potentially contaminated water at least daily. Over three-fourths of women had not been treated for schistosomiasis in the past 5 years, and more than one-half of women had never been treated.

**Prevalence of schistosomiasis, genital tract infections, and HIV.** We diagnosed active schistosomiasis in 185 (54%) women in this study population (Table 2).

**Table 1. Baseline Characteristics of 345 Women in *Schistosoma mansoni*-endemic Villages near Lake Victoria in Tanzania.**

Characteristic	Value
Age in years	
Median	30
Interquartile range	24-36
Number of children	
Median	4
Interquartile range	2-6
Reports more than one sexual partner in past six months	12 (5%)
Reports no current sexual partner	23 (9%)
History of miscarriage	116 (39%)
Distance of village from Lake Victoria	
0-8 kilometers	123 (36%)
9-17 kilometers	125 (36%)
18-25 kilometers	97 (28%)
Daily contact with potentially-infectious water	344 (99%)
Never previously treated for schistosomiasis	178 (52%)
Never previously tested for HIV	114 (42%)

Non-missing data were included in each calculation.

**Table 2. Prevalence of Infections in 345 Rural Tanzanian Women.**

Infection	Prevalence— Number (Percent)
Schistosomiasis	185 (54%)
Human immunodeficiency virus-1	21 (6%)
Syphilis	26 (8%)
Gonorrhea	1 (0.3%)
Chlamydia	15 (4%)
Trichomoniasis	4 (1%)
Bacterial vaginosis	19 (6%)
Candidiasis	15 (4%)



**Table 3. Associations of Potential Risk Factors with Schistosome Infection (Univariable Analysis).**

Potential Risk Factor	Schistosomiasis-Positive (n=185)	Schistosomiasis-Negative (n=160)	Odds Ratio for Association with Schistosomiasis [95%CI]	p-value
Median age (IQR)	30 (25-36)	29 (23-37)	1.02 [0.99-1.05]	0.15
Received praziquantel in past 5 years	21 (19%)	16 (15%)	1.4 [0.7-2.9]	0.35
Gonorrhoea and/or chlamydia cervicitis	7 (4%)	9 (6%)	0.7 [0.2-1.8]	0.42
Syphilis	17 (10%)	9 (6%)	1.8 [0.8-4.0]	0.19
Median kilometers from Lake Victoria (IQR)	10 (1-14)	12 (8-22)	0.95 [0.92-0.97]	<0.001

Non-missing data were included in each calculation.

Among those women harboring a schistosome infection, the median CAA value was 446 pg/mL (IQR = 86–2,338); 21 of 345 (6%) women were HIV-infected. The prevalence of other gynecologic infections ranged from gonorrhoea in 1 (< 1%) woman to reactive syphilis serology in 26 (8%) women (Table 2).

**Factors associated with schistosome infection.** In both univariate and multivariate analysis, distance from Lake Victoria was the only factor that was significantly associated with schistosome infection, with an OR of 0.95 (0.92–0.97) for each increasing kilometer away from the lake ( $p < 0.001$ ) (Table 3). The highest prevalence was 81% in a village situated < 1 km from the shore of Lake Victoria, whereas the lowest prevalence (38–42%) was observed in villages that were 12–22 km inland. Age, prior receipt of praziquantel, and genital tract infections did not significantly predict whether women were currently infected with schistosomes.

**Factors associated with HIV infection.** Schistosome infection was significantly associated with HIV infection, whereas other factors were not. Of 185 women with positive CAA levels, 17 (9%) women were HIV-infected, whereas of 160 women without detectable CAA, 4 (3%) women were HIV-infected (OR = 3.9 [95% CI = 1.3–12.0],  $p = 0.015$ ) (Table 4). The ORs for chlamydia and syphilis

**Table 4. Associations of Potential Risk Factors with HIV Infection (Univariable Analysis\*).**

Potential Risk Factor	HIV-Positive (n=21)	HIV-Negative (n=324)	Odds Ratio for Association with HIV Infection [95%CI]	p-value for Odds Ratio
Median age (IQR)	32 (25-36)	30 (24-36)	1.00 [0.95-1.06]	0.90
Median kilometers from Lake Victoria (IQR)	12 (10-22)	10 (8-22)	1.01 [0.95-1.07]	0.71
Chlamydia infection	2 (10%)	13 (4%)	2.4 [0.5-11.4]	0.27
Gonorrhoea infection	0	1 (0.3%)	---	---
Positive syphilis serology	3 (16%)	23 (7%)	2.4 [0.6-8.7]	0.19
Trichomonas infection	0	4 (1%)	---	---
Bacterial vaginosis	0	19 (6%)	---	---
Candidal infection	0	15 (5%)	---	---
Positive CAA test	17 (81%)	168 (52%)	3.9 [1.3-12.0]	0.015

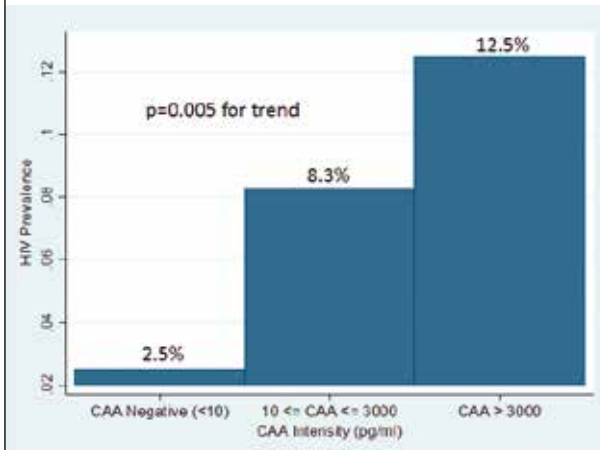
\*Non-missing data were included in each calculation.

For age and kilometers from the lake, the odds ratio corresponds to the odds ratio estimate for one unit increase in the exposure (i.e., per one year increase in age and one kilometer increase in distance).

were both 2.4, although neither was statistically significant. The OR for each increasing 1 year of age was 1.00 (0.95–1.06;  $p = 0.90$ ). The OR for each increasing 1 km away from Lake Victoria, which correlates with living closer to major roads, was 1.01 (0.95–1.07;  $p = 0.71$ ). In multivariate analysis, schistosome infection was the single best predictor of HIV infection. Other variables were not significant and did not affect the relationship between *Schistosoma* and HIV infection. Specifically, when multiple regression models were used to control for age, sexually transmitted infections (STIs), and distance from the lake, the association between *Schistosoma* and HIV remained statistically significant with an OR of 6.2 (1.7–22.9;  $p = 0.006$ ).

The median intensity of schistosome infection was significantly higher among HIV-infected than -uninfected women (400 versus 15 pg/mL,  $p = 0.01$ ). When women were stratified by CAA intensity, the prevalence of HIV infection significantly increased among groups ( $p = 0.005$  by JT test) (**Figure 2**).

**Figure 2. Prevalence of HIV Infection by Intensity of Schistosome Infection.**



**Factors associated with other STIs.** Cervicitis caused by gonorrhea and/or chlamydia was significantly associated with younger age (OR = 0.87 [0.79–0.96] for each increasing year of age,  $p = 0.006$ ). No other significant associations were observed.

## Discussion

In women living in *S. mansoni*-endemic areas near Lake Victoria, more than one-half were schisto-

some-infected, and *Schistosoma* infection was strongly associated with HIV. This finding suggests that *S. mansoni* infection and the chronic inflammation from the gut caused by *S. mansoni* eggs may be a risk factor for HIV acquisition. Schistosomiasis may be placing millions of women throughout sub-Saharan Africa at increased risk of becoming HIV-infected, and mass therapy of women of reproductive age for schistosomiasis may be an effective HIV prevention strategy.

Animal models support the hypothesis that *S. mansoni* infection increases host susceptibility to HIV infection. Studies in macaques showed that the rectal inoculum of SHIV required for SHIV acquisition was 17 times lower in macaques with than without *S. mansoni* infection (4). In contrast, prior *S. mansoni* infection did not significantly change the required infectious dose when SHIV was inoculated intravenously (4,27). Researchers postulated that *S. mansoni* infection increased the number of activated CD4+ T cells in the gut-associated lymphoid tissue (GALT) and thereby increased the optimal targets for SHIV infection. Mouse models of schistosomiasis show that *S. mansoni* eggs induce a Th17 CD4+ T-cell response in the gut mucosa (28,29). A growing body of literature suggests that HIV preferentially infects Th17 CD4+ T cells in gut and genital tissue mucosa and that increased numbers of these cells may increase susceptibility to HIV infection (30,31). Additional human studies are needed to determine if *S. mansoni* eggs induce a Th17 cell immune response in the GALT and if these Th17 cells express HIV susceptibility factors, such as CC Chemokine Receptor 5 (CCR5) and integrin  $\alpha 4\beta 7$ .

Our work is also supported by recent findings from the Rakai region of Uganda, where *S. mansoni* is endemic (32). As noted perceptively in the work by Secor (33), HIV-infected individuals more often had antibodies to schistosome antigens than HIV-uninfected individuals. Unlike in our study, in which CAA positivity indicates live worm infestation, the detection of antibodies to schistosome antigens does not prove active schistosomiasis. Coupled with our findings, this population-based study lends additional clinical support to our finding that *S. mansoni* infection and not only *S. haematobium* infection (as previously reported) is associated with HIV.

In addition, *S. mansoni*-infected individuals displayed higher densities of the HIV chemokine receptors CCR5 and CXCR4 on their CD4+ T cells and monocytes than individuals with schistosomiasis that had been previously treated (34). *S. mansoni* infection was also shown to increase the HIV RNA viral load in HIV-positive patients with untreated *S. mansoni* infection compared with patients with *S. mansoni* infections that had been treated (35). Several earlier non-randomized studies did not find an effect of praziquantel treatment on viral load (5,6,36,37), but this result has been postulated to be caused by transient increases in the schistosomiasis-conducive Th2 environment immediately after treatment (5,38). *S. mansoni*-infected individuals are also reported to excrete fewer ova than individuals without HIV (7,10). Our results support these findings that show complex interactions between *Schistosoma* and HIV infections as well as the growing consensus that schistosome infection, including *S. mansoni*, may be a risk factor for HIV acquisition (1,33).

Our work suggests that, among rural African women in whom the prevalence of genital tract infections is low, schistosome infection may be a major contributor of risk for HIV acquisition. Over one-half of our population was infected with schistosomes, leading to an estimated population-attributable fraction for HIV acquisition caused by schistosome infection of 69% (36–81%) using previously described methods (39). In contrast, the population-attributable fractions for genital tract infections in our population were 7% and below. The direction of our findings supports the well-described association between HIV and STIs, but the inability of our study to show statistical significance may be caused by the low prevalence of STIs in this rural population. We postulate that, in our population and other rural populations with few traditional HIV risk factors, such as multiple sexual partners and high rates of genital tract infections, schistosome infection may play a role as a key driver of HIV transmission. Of note, this study addresses the risk only in women aged 18–50 years who were seen at rural health clinics, and it does not address whether men or younger adolescent girls are also at increased risk.

The CAA test is a valuable diagnostic test for schistosome infections. The antigen becomes detectable in serum approximately 5 weeks after infection (40). CAA levels fall rapidly posttreatment, with a serum half-life of 2 days (24), and they rise with reinfection (41). The test is, therefore, highly time-specific for active schistosome infections. CAA assays have been recommended for serologic screening programs, in which repeated parasitologic examinations of urine and stool are logistically complicated (42). The CAA test has recently been developed into a very sensitive, robust lateral flow strip test, making test performance in rural settings increasingly feasible (12). The test is unable to distinguish between species of schistosomal infections. Therefore, projects relying on CAA testing either will only allow species-specific conclusions when performed in regions with sharp geographic demarcation of schistosome species, like in our study, or will not be able to differentiate between schistosome species.

Schistosomiasis is usually acquired in childhood, infecting 50–90% of children living in endemic areas by early adolescence (2). Thus, acquisition of schistosome worms typically predates sexual activity and concomitant exposure to HIV. The World Health Organization recommends that children in endemic areas receive targeted, periodic school-based praziquantel treatment (1). Women do not often receive mass treatment. In our population, more than one-half of women had never received antischistosomal treatment, despite living in a hyperendemic area and coming into daily contact with unclean water. A policy of routine periodic praziquantel administration for women seeking reproductive healthcare services (including family planning, cervical cancer screening, and pre-natal/post-natal care) would be a safe (1), efficient, and inexpensive way to control schistosomiasis and, moreover, potentially to prevent HIV acquisition in this vulnerable group.

This cross-sectional study shows a strong association but does not prove a causal role of schistosomiasis in HIV acquisition. An interventional trial would be necessary to show causality. However, we feel that the best explanation of our findings is that pre-existing schistosome infection modifies mucosal immunity and predisposes to HIV infection. The highest incidence of schistosome infection typically occurs in childhood between the ages of 5 and 15 years, and in individuals with ongoing exposure, it produces a chronic infection over decades. More than 80% of our study participants denied receiving praziquantel in the past 5 years, and more than one half reported never being treated. Therefore, it seems most likely that the large proportion of our study participants had chronic, untreated schistosome infection that pre-dated their exposure to HIV. Although it is conversely possible that HIV infection increases susceptibility to schistosomiasis (43), the former explanation for the association

most aptly combines both natural history and biological plausibility data. Also, it should be noted that the use of ORs in this study could possibly overestimate the relationship between HIV and schistosome infection compared with relative risk because of the fact that schistosome infection was common in our population (44).

In conclusion, more than one-half of rural Tanzanian women seen at health posts in *S. mansoni*-endemic areas of the Lake Victoria region had evidence of active schistosome infection, and the prevalence of HIV among these women was markedly higher than among those women without schistosome infection. Active *S. mansoni* infection may be a modifiable HIV risk factor that is contributing to high rates of new HIV infections among millions of women living in sub-Saharan Africa.

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