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Short Report: Association Between CD4+ T-Lymphocyte Counts and Fecal Excretion of *Schistosoma mansoni* Eggs in Patients Coinfected with *S. mansoni* and Human Immunodeficiency Virus Before and After Initiation of Antiretroviral Therapy

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Abstract.

Previously, we have shown that persons with human immunodeficiency virus 1 (HIV-1) infection and reduced CD4+ T-lymphocyte counts excrete significantly fewer *Schistosoma mansoni* eggs than HIV-1–negative persons with similar intensities of schistosome infections. To determine how antiretroviral therapy (ART) might affect egg excretion, we conducted a study of HIV+ adults living in an area highly endemic for *S. mansoni* as they began an ART program. Fecal egg excretion and CD4+ T-lymphocyte counts were evaluated at enrollment as well as 2 and 4 weeks after initiation of ART. Fourteen individuals who were Kato–Katz-negative at enrollment subsequently started excreting *S. mansoni* eggs accompanied by a significant increase in CD4+ T lymphocytes ($P = 0.004$). Study participants who were *S. mansoni* egg-positive at enrollment and received both praziquantel and ART also showed significantly increased CD4+ T-lymphocyte counts compared with baseline ($P < 0.0001$). Our data support a role for CD4+ T lymphocytes in *S. mansoni* egg excretion.

Coinfections with helminths and human immunodeficiency virus (HIV) are widespread in sub-Saharan Africa and result in unique immunologic and pathologic outcomes compared with single-agent infections.[1] Our previous studies in western Kenya indicated that *Schistosoma mansoni* and HIV-1 coinfected car washers excrete fewer *S. mansoni* eggs than persons infected with similar levels of mature adult worms measured by circulating antigen concentrations.[2] Other studies have similarly reported that egg excretion by *S. mansoni* or *S. haematobium* patients may be decreased if they have HIV-1 coinfections.[3–5] However, these studies did not measure CD4+ T lymphocytes, and circulating antigen concentrations were not determined for either of the *S. haematobium* reports. The only investigation that ascertained both CD4+ T-lymphocyte counts and circulating antigen concentration for both *S. mansoni* and *S. haematobium* patients did not find an effect of HIV-1–related immunodeficiency on the ability to excrete eggs in low-intensity schistosome infections.[6] To date, no study has explored the possible effects of antiretroviral therapy (ART) on schistosome egg excretion in individuals coinfected with schistosomiasis and HIV. The current study was performed to examine the relationship between *S. mansoni* egg excretion and CD4+ T-lymphocyte counts in HIV+ individuals who are receiving ART and examine whether an increase in CD4+ T

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lymphocytes during ART is associated with increased egg excretion from the human host.

The study was approved by the Institutional Review Board of the Kenya Medical Research Institute. Participants were drawn from three government HIV treatment centers (Naya, Madiany, and Ragengni) that were located within 5 km of Lake Victoria. This area is highly endemic for *S. mansoni*; thus, participants had a high likelihood of infection.[7] The study participants were antiretroviral treatment-naive individuals ages 18 years and older at the time of enrollment. Informed written consent, study activities, and counseling were conducted confidentially in the native language of the participants (Luo) by qualified counselors. HIV treatment was given according to Kenya national guidelines for ART, which includes HIV+ persons with < 350 $CD4^+$ cells/mm^3, World Health Organization (WHO) clinical stage 3 or 4, or coinfection with tuberculosis or hepatitis B virus. Study participants provided stool for detection of *S. mansoni* or other helminth ova and blood for determination of $CD4^+$ T-lymphocyte counts and HIV-1 viral load. They were also asked to answer a demographic and water contact questionnaire. Parasitological evaluations were carried out at baseline and 2 and 4 weeks after initiation of ART. Standard Kato–Katz thick smears were prepared for stool examinations for schistosome and other helminth eggs using two slides on the one stool sample collected at each time point.[8] Slides were quantitatively evaluated for eggs of *S. mansoni*, *Ascaris lumbricoides*, hookworm, and *Trichuris trichuria* by well-trained laboratory technicians. Quality control was assured by randomized selection and examination of 25% of the samples by another technician who was blinded to the first technician's results. The study participants were treated with praziquantel and/or albendazole for schistosomiasis and other helminth infections, respectively, under the observation by a Kenyan ministry of health nurse.

Blood was collected in a vacutainer tube containing ethylenediaminetetraacetic acid (EDTA) anticoagulant at each visit. $CD4^+$ T-lymphocyte counts were determined using flow cytometry (FacsCalibur; Becton Dickinson, San Jose, CA). Viral loads were determined by the Amplicor version 1.5 monitor test (Roche Diagnostics Systems, Branchburg, NJ). Comparisons between time points were performed using paired *t* tests analyzed by GraphPad Prism 5.03 (GraphPad Software, Inc., La Jolla, CA). P values < 0.05 were considered significant. An enzyme-linked immunosorbent assay (ELISA) to detect immunoglobulin G (IgG) to schistosome adult worm antigens (SWAPs) was performed on baseline sera according to previously described methods.[9]

Study participants were categorized into three groups: patients negative for schistosome ova at baseline (at the start of ART) but found to excrete eggs later ($N = 14$); patients excreting schistosome ova at baseline ($N = 53$); and patients who were schistosome egg-negative throughout the study period ($N = 23$). The three groups were similar in terms of age, approximate number of years spent in proximity to the lake, and baseline $CD4^+$ T-lymphocyte counts (Table 1).

S. mansoni eggs per gram (EPG) of feces in study participants who were shedding eggs at enrollment are shown in Figure 1A. Although a few persons were still excreting eggs 2 weeks after initial drug treatment, these individuals had an average 96% decrease in their *S. mansoni* EPG ($P < 0.0001$) compared with baseline. Fourteen individuals who were *S. mansoni* egg-negative at baseline began excreting eggs after 2–4 weeks of ART

(Figure 1B). For individuals excreting eggs at enrollment, 95.7% (45/47) were positive for anti-SWAP antibodies, whereas 69.2% (9/13) of the individuals who began excreting eggs after the start of ART were positive. Of the study participants who remained egg-negative for the entire follow-up period, 64.3% (9/14) were anti-SWAP antibody-positive.

We compared the $CD4^+$ T-lymphocyte counts at baseline and 1 month after the start of ART in the three groups (Figure 2). There was a significant increase in $CD4^+$ T-lymphocyte counts after 1 month of ART in patients who excreted schistosome eggs at baseline ($P < 0.0001$). $CD4^+$ T-lymphocyte counts also significantly increased in the group of patients who were negative at baseline but started egg excretion later ($P = 0.004$). The group that was negative for schistosome eggs throughout the study period showed no change in $CD4^+$ T-lymphocyte counts after 1 month of ART ($P = 0.3$). The proportion of participants that increased $CD4^+$ T-lymphocyte counts on ART was significantly higher (χ^2 $P = 0.0025$) for those individuals who were egg-positive at baseline (39/53, 73.5%) or became egg-positive (11/14, 78.6%) than for individuals who remained egg-negative throughout the study (8/23, 37.8%). We do not know why ART did not increase $CD4^+$ T-lymphocyte counts in most patients who remained egg-negative.

Results from this study are consistent with our earlier observations and data from experimental models of schistosomiasis that suggest that $CD4^+$ T lymphocytes are among the immune cells involved in the excretion of *S. mansoni* eggs from the infected host.[2,10] Some schistosome and HIV coinfected study participants did not excrete eggs at baseline but started excreting eggs within 1 month after initiation of ART, corresponding with a significant increase in their $CD4^+$ T-lymphocyte numbers. Thus, restoration of immune capacity was temporally associated with the increase of egg excretion. In contrast, the group that did not begin egg excretion after the initiation of ART did not show increased $CD4^+$ T lymphocytes within the 1-month timeframe (Figure 2C). It may be possible that these individuals simply did not have active schistosome infections at the time of the study. However, the high rate of positivity in the SWAP ELISA and their proximity to Lake Victoria suggest that most of them would have been infected with schistosomes at some point in their lives. In the absence of previous mass praziquantel administration programs in this area, it is likely that at least some of these individuals were still actively infected.

Because study participants were treated with praziquantel whenever they were found to be egg-positive, we do not know whether ART would have increased egg excretion in the group of study participants who had positive stools at baseline and increased their $CD4^+$ T-cell counts within 1 month. Nevertheless, egg counts in this group were reduced by 96% after praziquantel treatment, consistent with our previous results showing good drug efficacy in HIV-1 coinfected individuals.[11]

We have previously found that schistosome infection itself may be associated with reduced levels of circulating $CD4^+$ T lymphocytes independent of HIV-1 infection.[12] The stage of HIV infection may also have an influence on these results. Although it is not possible from the design of the current study to know whether praziquantel treatment of schistosomiasis in coinfected individuals promotes increases in $CD4^+$ T cells greater than ART alone, attention to this question may provide advantages to HIV-1^+ individuals living in schistosomiasis-endemic areas beyond the recognized benefits of clearing a

person's schistosome infection. Furthermore, failure to attend to infections in persons starting on ART may put them at greater risk for immune reconstitution inflammatory syndrome (IRIS).[13]

The primary limitation of this study is that it relied on two slides from one stool sample for microscopy diagnosis at each time point. Because Kato–Katz stool examination can miss low-intensity infections and egg excretion can fluctuate daily, it is possible that we did not detect eggs at baseline of the participants who eventually became stool-positive or missed eggs at follow-up time points in the group that remained negative throughout the study. However, these initial results warrant additional study with more rigorous stool examinations in a larger sample size. The role that schistosomiasis may play in determining the pathology of HIV coinfected individuals at the initiation and during ART is an area of ongoing study.

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FIGURE 1. Comparison of EPG of feces at baseline and 2 and 4 weeks after treatment and the start of ART in patients who enrolled when (**A**) positive ($N = 53$) or (**B**) negative at baseline ($N = 14$) for *S. mansoni* eggs. Participants were offered praziquantel treatment (**A**) at baseline or (**B**) when they became egg-positive. Each line represents an individual study participant.

FIGURE 2. Comparison of $CD4^+$ T-cell counts on initial study enrollment and 1 month after the start of ART in individuals who were (**A**) egg-positive for *S. mansoni* ($N = 53$) at baseline, (**B**) egg-negative at baseline but became egg-positive after ART initiation ($N = 14$), or (**C**) remained egg-negative throughout the study ($N = 23$). Each line represents an individual participant.

TABLE 1

Characteristics of the study population categorized by *S. mansoni* egg excretion

	S. mansoni egg-positive at	*S. mansoni* egg-negative at baseline	*S. mansoni* egg-negative

	baseline ($N = 53$)	and became egg-positive ($N = 14$)	throughout ($N = 23$)
Age (in years; mean ± SD)	36.8 ± 10.1	34.3 ± 13.3	35 ± 9.1
Years spent in proximity to the lake (mean ± SD)	21 ± 2.2	21 ± 2.7	23 ± 3.1
$CD4^+$ at baseline (median, range)	201, 22–546	234, 69–507	189, 15–393

Figure 1

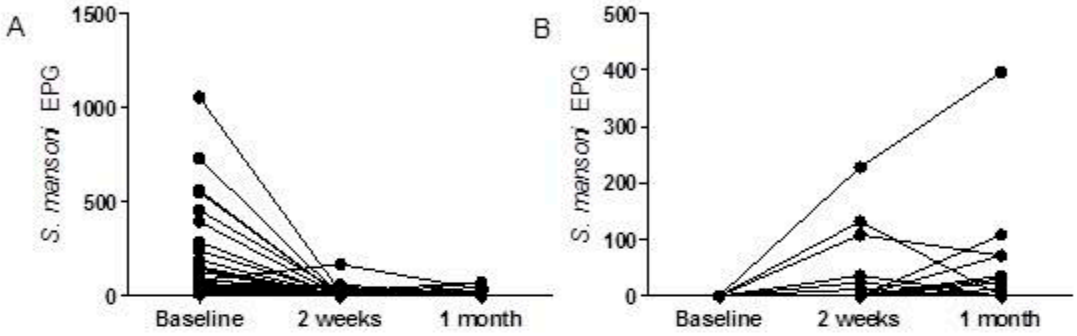
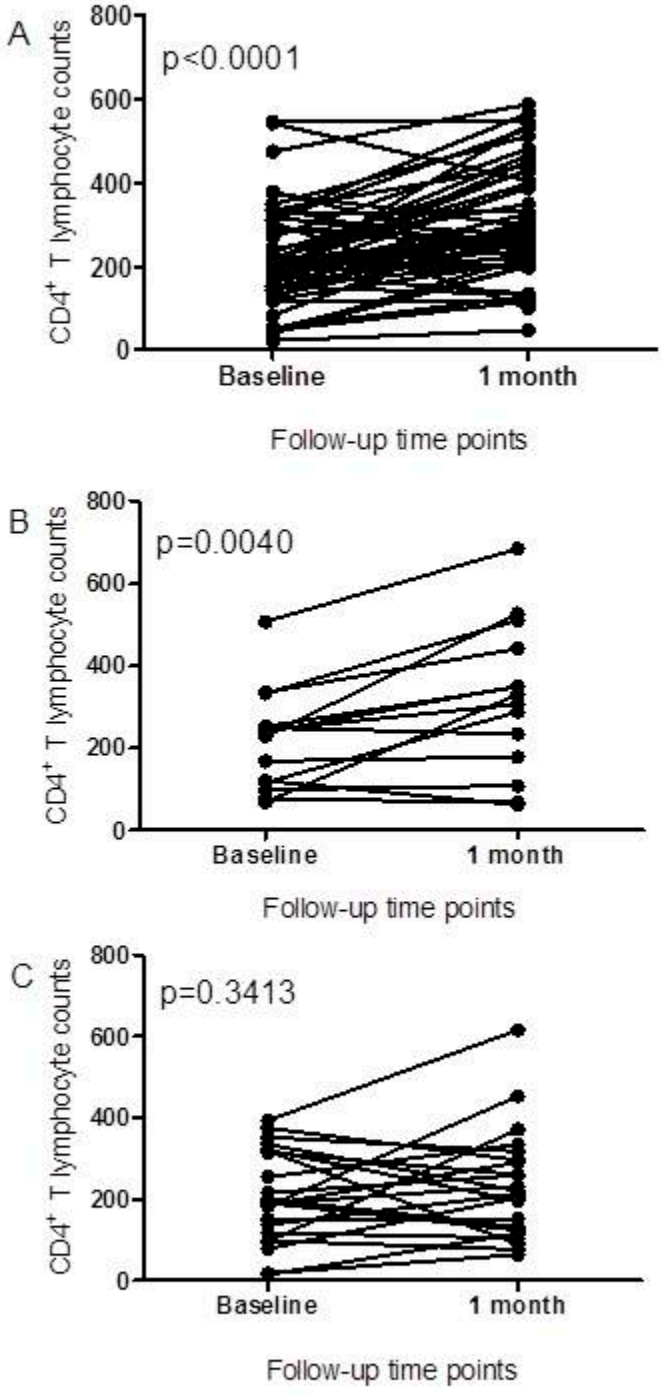

A
1500
1000
500
0
S. mansoni EPG
Baseline
2 weeks
1 month
B
500
400
300
200
100
0
S. mansoni EPG
Baseline
2 weeks
1 month

Figure 2


A
p<0.0001
CD4+ T lymphocyte counts
800
600
400
200
0
Baseline
1 month
Follow-up time points
B
p=0.0040
CD4+ T lymphocyte counts
800
600
400
200
0
Baseline
1 month
Follow-up time points
C
p=0.3413
CD4+ T lymphocyte counts
800
600
400
200
0
Baseline
1 month
Follow-up time points