

Comparison of HIV-1 viral loads, CD4-Th₂-lymphocytes and effects of praziquantel treatment among adults infected or uninfected with *Schistosoma mansoni* in fishing villages of north-western Tanzania

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Abstract

Background: It is hypothesised that Th₂ immunological environment associated with *Schistosoma mansoni* infection might favour replication of HIV-1 in co-infected individuals, results in increased viral loads. On the other hand, deworming using praziquantel might result in reduction of HIV-1 viral loads and increased CD4⁺ cell counts. This study was therefore, carried out to compare HIV-1 plasma loads, CD4-Th₂-lymphocytes and the effects of praziquantel treatment on HIV-1 plasma loads and CD4⁺ cell counts among HIV-1 seropositive individuals infected or uninfected with *S. mansoni*.

Methodology: A 9-month prospective longitudinal study was conducted among HIV-1 infected individuals aged 21-55 years with CD4⁺ cell counts ≥ 350 cells/ μ L in fishing villages of North-Western Tanzania. Single stool samples were examined for *S. mansoni* eggs using Kato Katz technique at 6-month follow-up and 12 weeks after treatment. Venous blood samples were collected at baseline, at three and six-month follow-up and 12 weeks after praziquantel treatment for HIV-1 plasma viral loads and CD4⁺ cell quantification.

Results: Of the 50 HIV-1 infected participants at baseline, 44% (22/50, 95%CI: 30.58-58.35) were found to be co-infected with *S. mansoni* at 6-month follow-up with a mean of 93.26 GM-epg (95%CI: 60.42-143.95). The median CD4⁺ cell counts did not differ significantly between individuals infected with HIV-1 and those co-infected with HIV-1 and *S. mansoni* at baseline ($P=0.62$), 3-month ($P=0.64$) and 6-month ($P=0.41$) follow-up. Monthly decrease in CD4⁺ cells did not differ significantly between the two groups at all follow-up points (-30.39 cell/ μ L versus -31.35 cells/ μ L, $P=0.89$). Those infected with *S. mansoni* had a significantly higher mean log₁₀ HIV-1 plasma viral load at baseline (5.98 ± 3.06 versus 9.21 ± 1.91 copies/ml, $P<0.0001$) and 3-month follow-up (8.19 ± 2.17 versus 9.44 ± 1.99 copies/ml, $P<0.042$) compared to those infected with HIV-1 only. This difference was not evident at the time of *S. mansoni* diagnosis at 6-month time point. Praziquantel treatment in co-infected individuals ($n=12$) did not result in any change in CD4⁺ cell counts and mean HIV-1 plasma viral loads ($t=-0.9156$, $P=0.38$), comparing baseline and 3-month follow-up after treatment. No correlation was observed between log *S. mansoni* egg counts and log₁₀ HIV-1 RNA viral loads ($r=-0.066$, $P=0.77$) at six-month follow-up in co-infected individuals ($n=22$).

Conclusion: HIV-1 plasma viral loads varied significantly among mono and co-infected individuals at baseline and 3-month follow-up. However, CD4⁺ cell counts did not vary between the two groups at all follow-up time points. Praziquantel treatment of co-infected individuals did not result in changes in CD4⁺ cell counts and HIV-1 plasma viral loads.

Keywords: HIV-1, *Schistosoma mansoni*, co-infection, HIV loads, praziquantel, Tanzania

Introduction

Immunological evidence from laboratory animal models and human host(s) (Secor, 2005, Secor, 2006, Secor, 2012) indicate that helminth infections may cause immune modulation which may increase the susceptibility of infected individuals to other infections (Mwinzi et al., 2001, Secor et al., 2003, Secor et al., 2004). Specifically, interest has focused on type 1 (CD8⁺-Th₁)/type 2 (CD4⁺-Th₂) arms of the immune system, in which, type 1 (Th₁) immune response, which secretes interferon- γ , interleukin-2 (IL-2), promotes phagocytosis and intracellular killing of viral particles, including HIV-1 (Clerici & Shearer, 1993; Bentwich et al., 1995). Helminths parasites such as

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Schistosoma mansoni induce type 2 immune responses characterized by the release of interleukin (IL)-4, IL-5, IL-10 and IL-13, which stimulate the proliferation of B-lymphocytes cells, antibodies and eosinophils production (Clerici & Shearer, 1993; Mwinzi *et al.*, 2001; Secor *et al.*, 2004; Borkow & Bentwich, 2004). The ability of helminth parasites to modulate immune response, which shifts from T-helper-1 to predominant T-helper-2 cytokines, is thought to impair the type-1 response (Sher & Coffman, 1992; Maizels *et al.*, 1993; Kalinkovich *et al.*, 1998; Maizels & Yazdanbakhsh, 2003), which, in turn (Clerici & Shearer, 1993), increases the susceptibility of Th₀ and Th₂ cells to HIV-1 infection (Sher & Coffman, 1992; Clerici & Shearer, 1993; Secor, 2005). This observation on immune dysregulation has led to the hypothesis that immune modulations resulting from chronic *S. mansoni* infection are associated with HIV-1 disease progression, through increased HIV-1 replication and decrease CD4⁺ levels (Bentwich *et al.*, 2000; Secor *et al.*, 2003; Borkow & Bentwich, 2004; Secor, 2006, 2012; Mazigo *et al.*, 2013).

In addition, *in vitro* studies have shown that CD4⁺-Th₂ lymphocytes cells from *S. mansoni* infected individuals express chemokines receptors (CCR5 and CXCR4) which render these cells more susceptible to HIV-1 infections, as compared to the cells of helminth free individuals (Kalinkovich *et al.*, 2001; Secor *et al.*, 2003; Secor, 2012). Alternatively, in *S. mansoni* endemic areas, where individuals are infected by the parasite at an early age, their CD4⁺ T-lymphocytes are stimulated by the parasites' antigens and, by the time they are infected by HIV-1 at older ages, they may end up with higher viral loads and progress very fast to AIDS stages (as measured in term of CD4⁺ cell counts) (Bentwich *et al.*, 1999; Borkow & Bentwich, 2004; Secor, 2012). If these assumptions are correct, it is expected that individuals co-infected with *S. mansoni*+HIV-1 with a heavy intensity of *S. mansoni* infection will have higher HIV-1 viral loads as compared to HIV-1 mono-infected individuals. However, up to date, inconsistent results have emerged (Lawn *et al.*, 2000, Brown *et al.*, 2004, Kallestrup *et al.*, 2005a). Thus, the role of immune modulation of Th₁/Th₂ responses associated with *S. mansoni* on the HIV-1 pathogenesis remains a subject for further studies, especially in sub-Saharan Africa.

The observations on immune dysregulation as well as chronic immune activation associated with HIV-1+ *S. mansoni* in co-infected individuals, it has been argued that early treatment of *S. mansoni* using praziquantel (PZQ) could reduce the rate of viral replications (Erikstrup *et al.*, 2008), improve prognosis and delay the initiation of antiretroviral treatment (ART) (Walson & John-Stewart, 2008; Modjarrad & Vermund, 2010). In fact, PZQ treatment reduces the density of chemokines receptors (CCR5 and CXCR4), which, in turn, reduce/control the replication of HIV-1 (Secor *et al.*, 2003). The reported evidence has remained inconsistent, increased (Lawn *et al.*, 2000; Brown *et al.*, 2005) or decreased (Brown *et al.*, 2005) HIV-1 RNA viral loads have been documented after PZQ treatment in *S. mansoni*+HIV-1 co-infected individuals. In addition, few community-based studies have addressed this issue in HIV-1 cohorts constantly exposed to a high risk of *S. mansoni* transmission and not qualifying for initiation of ART (Lawn *et al.*, 2000; Kallestrup *et al.*, 2005a).

In the current study, we hypothesized that, if Th₂ immunological environment associated with *S. mansoni* favours the replication of HIV-1 in co-infected individuals, then individuals who are infected with *S. mansoni* prior to be infected by HIV-1, will, by the time they acquire HIV-1 infection the disease, progress faster (as measured by the decrease in CD4⁺ cell counts and the increase in HIV-1 viral load) overtime than those mono-infected with HIV-1. Furthermore, PZQ treatment in co-infected individuals will result into a decrease in plasma HIV-1 viral loads. Thus, the present study was therefore conducted to compare (a) Human Immunodeficiency Virus-1 plasma loads, CD4-Th₂-lymphocytes and (b) effects of praziquantel treatment on HIV-1 plasma loads and CD4⁺ cell counts among HIV-1 seropositive individuals infected or uninfected with *S. mansoni*.

Materials and Methods

Study area, population and inclusion criteria

Descriptions of the study area, inclusion and exclusion criteria and sampling procedures of the study participants are described elsewhere (Mazigo *et al.*, 2014). Briefly, the study was conducted at Ilemela district, located on the southern shore of the Lake Victoria. The study included inhabitants living at Kayenze, Igalagala, Sangabuye and Igombe villages. The villages were chosen for the study because of their close proximity to the lake and high prevalence of *Schistosoma mansoni* (Mazigo *et al.*, 2014). The inhabitants of the study villages depend on the Lake for domestic and economic activities such as washing, bathing, cooking, drinking and recreation (Malenganisho *et al.*, 2008).

All people who had lived in the study villages for more than two years (referred as permanent residence) and were aged 21 to 55 years were eligible for enrolment. Individuals with a history of treatment for schistosomiasis (praziquantel) in the past six months and those who were on antiretroviral treatment (ART) were excluded from the study at the baseline. A two-step sampling procedure was used to select households and household members to participate in the study (Mazigo *et al.*, 2014).

Study design and follow-up

This was a prospective longitudinal study in which a cohort of HIV-1 infected individuals with CD4⁺ cell counts ≥ 350 cells/ μ L was followed for nine (9) months. Study participants were followed at three and six months to collect venous blood samples for monitoring of CD4⁺ T-lymphocytes and HIV-1 plasma viral loads. Fifty study participants completed a 6-month follow-up and were screened for *S. mansoni* infection. Those who had *S. mansoni* eggs in their stool samples were treated using Praziquantel (40mg/kg) and followed up at 12 weeks after treatment to assess the efficacy of treatment and its effects on HIV-1 plasma viral loads. Twelve weeks after treatment, only 12 study participants who had detectable *S. mansoni* eggs in their stool samples were available for assessment. The main reasons for loss to follow-up were migration, refusal to continue to participate and absence from home.

HIV-1 viral loads and CD4⁺ cell counts were compared in individuals who were co-infected with *S. mansoni*+HIV-1 and those who were HIV-1 mono-infected at six-month follow-up. The same outcomes were compared among the co-infected individuals before and after praziquantel treatment. Furthermore, we assessed if the intensity of *S. mansoni* infection correlated with HIV-1 RNA viral loads.

Screening for HIV-1 infection, CD4⁺ analysis and plasma viral load quantification

Demographic information on sex, age, marital status and other socioeconomic information were collected by questionnaire. Human Immunodeficiency Virus-1 testing was conducted according to the Tanzanian National HIV algorithms which recommend the use of a rapid test qualitative immunoassay (Lyamuya *et al.*, 2009). The quantification of CD4⁺ cells was carried out using FACSCalibur machine (Becton Dickinson-BD Biosciences, San Jose, CA, USA) following standard procedures (Borato *et al.*, 2012).

Plasma samples for HIV-1 RNA viral load quantification were collected and stored at -80°C within 24 hours of blood collection. Plasma HIV-1 viral loads in all samples were determined by Artus[®] HI Virus-1 RG TR-PCR kit (Qiagen GmbH, Qiagen strasse 1, D-40724 Hilden) following the manufacturer's instructions. The diagnostic test kit utilizes the reverse transcription polymerase chain reaction (RT-PCR). The lowest analytical detection limit in consideration of the purification of the artus HI Virus-1 RG RT-PCR Kit in combination with the Rotor-Gene Machine is 35.8 log RNA copies/ml.

Parasitological screening for *Schistosoma mansoni* and treatment

A single stool sample was collected from all study participants at six and 12 weeks after treatment, from which, four (4) Kato Katz thick smears were prepared from different parts of the single stool sample using a template of 41.7 mg (Vestergaard Frandsen, Lausanne, Switzerland), following a

standard protocol (Katz *et al.*, 1972). For quality assurance, a random sample of 10% of the negative and positive Kato Katz thick smears were re-examined by a third technician.

After examination at six months of follow-up, all individuals found infected with *S. mansoni* were treated using a single dose of praziquantel (40mg/kg). Treatment was performed under the direct observation (DOT) of a qualified a nurse. Study participants received a cup of tea and half a bread before treatment to relieve any potential side effects of praziquantel. In addition, after treatment, participants were requested to remain at the treatment point for two hours for observation by a medical doctor.

Ethical Considerations

Ethical approval was obtained from the Higher Degrees Research and Ethics Committee of the School of Public Health, Makerere University (Institutional Review Board, Ref: 00005856/2011) and from the Bugando University College of Health and Allied Sciences-Institutional Review Board, (BREC/001/32/2011). Ethical clearance was granted by the National Ethical Review Committee, National Institute for Medical Research, Tanzania and the study was registered in the clinical trial network, Clinical Trial (Number: NCT-01541631).

Data analysis

The data were double-entered using CPro and the final data set was stored in a MySQL database. Data analysis was performed using Stata version 13 (Stata Corp, College station, Texas, USA). The Mann-Whitney test was used to compare median CD4⁺ cell counts between those with and without *S. mansoni* infection at baseline, three and six month's follow-up. Plasma HIV-1 viral loads were log (log₁₀) transformed to approximate a normal distribution. The mean log₁₀ plasma HIV-1 RNA viral loads were compared between individuals who were co-infected and mono-infected using t-test for continuous outcomes.

At baseline and 3-month follow-up, samples from 19 individuals were detected to be below the detectable range of the machine and were classified as having 34copies/ml for the purpose of analysis. Monthly changes in CD4⁺ cell counts and HIV-1 viral loads were analysed using a mixed linear model for repeated measures. The effect of PZQ treatment was evaluated by comparing change in log₁₀ plasma HIV-1 RNA viral load between baseline and 12 weeks (three months) after treatment. The analysis of follow-up viral loads after treatment mainly focused on comparing those remained with detectable *S. mansoni* eggs and those who had no detectable eggs at 12 weeks after treatment.

The arithmetic mean of *S. mansoni* egg counts for each participant was calculated from the counts of four Kato Katz thick smears and multiplied by 24 to obtain individuals eggs per gram of faeces. *S. mansoni* egg counts were over-dispersed so were logarithmically transformed and used to calculate the geometric mean egg per gram of faeces (GM-epg) that was obtained as the antilog of the mean of the transformed egg counts. Pearson's and Spearman's correlation coefficient for individuals co-infected with *S. mansoni* and HIV-1 was used to determine any correlation between HIV plasma loads and *S. mansoni* intensity of infection. Statistical significance was set at $P \leq 0.05$.

Results

Characteristics of study participants

At baseline, a total of 2,138 individuals were recruited and enrolled for HIV-1 screening. Of these individuals, 167 were identified as HIV-1 positive at baseline, 77 had CD4⁺ T-lymphocytes counts <350 cells/ μ L and 90 had CD4⁺ T-lymphocytes counts \geq 350 cells/ μ L. The fifty individuals who completed a six-month follow-up were aged 21-55 years, the median age was 34.5years (IQR= 28-43 years). The two groups of the study participants, those mono-infected with HIV-1 and those co-infected with HIV-1 and *S. mansoni*, did not differ by sex distribution ($\chi^2=0.1523$, $P=0.69$) and

age ($t=0.9193$, $P=0.36$). The majority of the study participants were from Sangabuye (44%) and Kayenze villages. Baseline CD4⁺ cell counts and demographic information were compared for the study participants who were present and absent at 6 months and 9 months of follow-up. There was no significant difference on age ($t=-1.6990$, $P=0.09$). There was a difference in sex distribution ($\chi^2=5.9533$, $P<0.015$), village of residence ($\chi^2=7.4772$, $P<0.001$), mean CD4⁺ cell counts ($t=-6.5360$, $P<0.0001$), whereby those who were present had a higher mean CD4⁺ than those who were absent (716.14 ± 320.25 versus 395.97 ± 273.36 cells/ μ L) (Table 1).

Table 1: Comparison of CD4⁺ levels and HIV-1 plasma viral loads among participants infected with HIV-1 only and those co-infected with HIV-1 and *S. mansoni* at the 6 month follow up time point

Variables	HIV-1 only (n=22)	HIV-1 + <i>S. mansoni</i> co-infected (n=28)	F-test	P-values
CD4⁺ - T-lymphocytes (Median and Interquartile range)				
Baseline	574 (IQR: 484.5 – 801.0)	654.5 (IQR: 546.0-773.0)	----	0.62
3 month	514.5 (IQR: 430.5 – 723.0)	615 (IQR:462.0-718.0)	----	0.64
6 month	470.5 (IQR:385.5 – 593.0)	567 (IQR:412.0 – 614.0)	----	0.41
HIV-1 viral load (Mean log₁₀ RNA copies/ml of plasma)				
Baseline	5.98 ± 3.06 (95%CI:4.79 – 7.17)	9.21 ± 1.91 (95%CI: 8.36 – 10.06)	t = -4.72553	0.0001
3 month	8.19 ± 2.17 (95%CI: 7.35-9.03)	9.44 ± 1.99 (95%CI: 8.55 – 10.32)	t = - 2.0892	0.042
6 month	10.49 ± 1.29 (95%CI: 9.99 – 10.99)	10.59 ± 1.44 (95%CI: 9.96 – 11.24)	t = -0.2714	0.79

Prevalence and intensity of *Schistosoma mansoni*

Of all the 50 study participants who were infected with HIV-1 and had CD4⁺ \geq 350cells/ μ L, 44% (95%CI: 30.58-58.35) were co-infected with *S. mansoni*. Of these, 46.43% (n=13/28) were females and 40.91% (n=9/22) were males. The overall geometrical mean eggs per gram (GM-epg) of faeces of *S. mansoni* were 93.26GM-epg (95%CI: 60.42-143.95). By sex, males had a higher GM-epg, 148.41(95%CI: 45.19-487.28) than females, 68.41GM-epg (95%CI: 35.08-133.43) ($P<0.003$).

CD4⁺ T-lymphocytes and HIV-1 plasma viral loads at baseline and follow-up

At baseline and at the subsequent follow-up at three and six-month time point, the median CD4⁺ cell counts did not differ significantly between the two groups (Table 1). There was a trend in the decrease of CD4⁺ cell counts with time, baseline to three months ($P<0.0001$, paired t-test), baseline to six months ($P<0.0001$) and baseline to 12 weeks after treatment ($P<0.0147$), for all the study participants. The mean monthly decrease in CD4⁺ cell counts from baseline to the six-month follow-up time point for all study participants was -30.81cells/ μ L (95% CI: -38.06 to -23.55). In relation to the *S. mansoni* infection status, those infected with HIV-1 only had a monthly decrease in CD4⁺ cell counts of -30.39cells/ μ L (95% CI: -40.08 to -20.69) and those co-infected with HIV-1+S.*mansoni* had a monthly decrease of -31.35cells/ μ L (-42.29 to -20.41). The difference in monthly decrease of CD4⁺ counts between the two groups was not statistically significant ($P=0.89$).

For the HIV loads, at baseline ($P<0.0001$) and at three months ($P<0.042$), the mean log₁₀ HIV-1 plasma viral load was higher in the *S. mansoni* infected groups than the *S. mansoni* non-infected group (Table 1). However, at six-month follow-up time point, the difference in mean log₁₀ HIV-1 viral load did not differ between the *S. mansoni* infected groups and the HIV-1 mono-

infected ($P=0.79$). There was a trend of increase in mean \log_{10} HIV-1 plasma viral loads with time for all study participants from baseline to three months ($P<0.01$, paired t-test), baseline to six months ($P<0.0001$, paired t-test) follow-up time points and from baseline to 12 weeks after treatment ($P<0.001$, paired t-test). The monthly increase in log plasma HIV load for the whole study population was 0.52copies/ml (95%CI: 0.39 – 0.65) for the six-month follow-up point. In relation to *S. mansoni* infection, those participants who were mono-infected with HIV-1 had a significantly higher monthly increase in plasma HIV-1 load of 0.75copies/ml (95%CI: 0.59 – 0.91) than those co-infected with HIV-1+*S.mansoni*, 0.23copies/ml (95%CI: 0.049 – 0.41). The difference observed between the two groups in monthly increase in plasma HIV-1 loads was significant ($P<0.0001$).

Effects of treatment of *S. mansoni* on HIV-1 plasma viral load

The HIV-1 plasma viral load results, both at baseline and 12 weeks after praziquantel treatment were available for 12 individuals who were co-infected at the six-month time point. Of the 22 study participants screened for *S. mansoni* and treated with PZQ at six-month time point, 10 were lost to follow-up due to migration from the study villages. The remaining 12 individuals, at baseline, had a mean HIV-1 plasma viral load of 8.54 ± 1.64 RNA copies/ml which insignificantly increased to 9.41 ± 2.98 RNA copies/ml ($t=-0.9156$, $P=0.38$) 12 weeks after PZQ treatment. Of the 12 individuals screened for *S. mansoni* infection at 12 weeks after PZQ, 8 had no detectable *S. mansoni* eggs in their stool samples and 4 remained with detectable *S. mansoni* eggs. At baseline, the 8 participants had a median CD4⁺ cell counts of 663.5 (IQR= 570–831) cells/ μ L and, at 12 weeks after treatment, had a median CD4⁺ cell count of 418.5 (393–599) cells/ μ L. For HIV-1 loads, at baseline, the group had a mean \log_{10} HIV-1 plasma viral load of 8.28 ± 1.45 (95%CI: 7.06- 9.49) copies/ml and at 12 weeks after treatment, they had a mean \log_{10} HIV-1 plasma viral load of 10.37 ± 1.35 (95%CI: 9.24–11.50) copies/ml.

For the study participants who remained with detectable *S. mansoni* eggs in their stool they had, at baseline, a median CD4⁺ cell count of 472.5(IQR: 368.5 – 922) cells/ μ L and, at 12 weeks after treatment, had a median CD4⁺ count of 387(348-426) cells/ μ L. For the HIV-1 viral load, the group had a mean \log_{10} HIV-1 plasma of 9.06 ± 2.08 (95%CI; 5.74– 12.37) copies/ml and, 12 weeks after treatment, had a mean \log_{10} HIV-1 plasma viral load of 11.46 ± 0.68 (95%CI; 5.74 - 12.37) copies/ml. In general, PZQ treatment did not result in changes of either CD4⁺ cell counts or plasma HIV-1 loads, between baseline and 12 weeks after treatment. No correlation was observed between log *S. mansoni* egg counts and HIV-1 RNA viral loads ($r=-0.0689$, $P=0.77$) at 6-month follow-up point in 22 individuals infected with *S. mansoni*.

Discussion

The present study highlighted the fact that the six-month follow-up on CD4⁺ T-lymphocytes counts did not differ significantly between individuals who were mono-infected with HIV-1 and those who were co-infected with *S. mansoni* +HIV-1 in a HIV-1 cohort with CD4⁺ cells counts ≥ 350 cells/ μ L. There was a trend in the monthly decrease in CD4⁺ cell counts with increasing follow-up time points. However, the decrease in CD4⁺ counts did not differ significantly between those mono-infected with HIV-1 and those co-infected with HIV-1+*S.mansoni*. In relation to plasma HIV-1 loads, at baseline and three-month follow-up, individuals who were identified to be co-infected with HIV-1+*S.mansoni* at six-month follow-up time point had a significantly higher viral load in comparison to those who were mono-infected with HIV-1. HIV-1 plasma viral loads increased with follow-up time from baseline to six months. Furthermore, praziquantel treatment, evaluated at 12 weeks after treatment, did not result into changes in neither \log_{10} HIV-1 plasma loads nor CD4⁺ cell counts in participants who were co-infected with HIV-1+*S.mansoni* infection. In the present study, we found no correlation between the intensity of *S. mansoni* infection and \log_{10} HIV-1 plasma viral loads among the co-infected study participants at six-month follow-up time point.

The relatively small sample size of study participants and a short follow-up time of 6 - 9 months may in part require a cautious interpretation of the findings of the present study. However, these results are notable and, to a degree, support the hypothesis that individuals co-infected with *S. mansoni* and HIV-1 may have significantly higher \log_{10} HIV-1 plasma viral loads.

Our observations on the lack of difference in CD4⁺ cell counts between those who were co-infected with HIV-1+*S.mansoni* and mono-infected with HIV-1 have also been described in a HIV-1 semi-urban cohort in Uganda (Brown *et al.*, 2004). In that cohort, being infected with *S. mansoni* was not associated with lower CD4⁺ T-lymphocytes (Brown *et al.*, 2004). Higher and lower CD4⁺ cell counts in HIV-1 infected individuals co-infected with different helminths species, including *S. mansoni*, in sub-Saharan Africa have been reported in other studies (Wolday *et al.*, 2002; Brown *et al.*, 2004; Walson & John-Stewart, 2008). In comparison to other helminth species endemic in sub-Saharan Africa, in particular gastro-intestinal nematodes, the comparison of CD4⁺ cell counts between HIV-1 mono-infected and co-infected individuals at different follow-up time points has revealed no significant difference in lymphocytes counts (Wolday *et al.*, 2002, Walson and John-Stewart, 2008) and profiles (Mkhize-Kwitshana *et al.*, 2011). In the present study, we observed a CD4⁺ cell counts decrease with increased follow-up times for both groups. These observations are expected for individuals infected with HIV-1 and not getting ART, as the pathogenesis of HIV-1 targets the CD4⁺ T-cells (Clerici and Shearer, 1993).

Conversely, comparisons of mean \log_{10} HIV-1 plasma viral loads between individuals co-infected with HIV-1+*S.mansoni* and those mono-infected with HIV-1 revealed a significant difference in mean viral load at baseline and at three-month follow-up time. However, this difference was not evident at the time of *S. mansoni* diagnosis at the 6-month time point. As hypothesized elsewhere, chronic immune activation resulting from *S. mansoni* infection may partly explain the difference of mean HIV loads in the two groups (Bentwich *et al.*, 1995; Secor, 2006; Secor *et al.*, 2003; Kamal & El Sayed Khalifa, 2006). *In vitro* studies have shown that Th₂ cells from *S. mansoni* infected individuals express a high density of CCR5 and CXCR4 receptors which increases their susceptibility to HIV-1 and HIV-1 replication (Secor *et al.*, 2003). A higher baseline mean HIV-1 viral load has been reported in HIV-1+*S. mansoni* cohorts in Uganda (Brown *et al.*, 2004); however, this association disappeared after adjusting for other factors such as age, sex and socioeconomic factors, leading to a conclusion that demographic and socio-economic differences are more closely associated with higher HIV-1 viral loads than helminth infections (Brown *et al.*, 2004).

In the present study, after multiple linear regressions analysis, none of the demographic factors (age, sex, village of residence, *S. mansoni* status and baseline CD4⁺ counts) remained significantly associated with HIV-1 viral loads at baseline. In comparison to other endemic helminths infections in sub-Saharan Africa, such as soil-transmitted helminths, a non-significant difference was observed on mean \log_{10} HIV-1 viral loads among HIV-1+STH co-infected individuals and those mono-infected with HIV-1 in Ethiopia (Wolday *et al.*, 2002). However, in South Africa, HIV-1+STH co-infected individuals who presented with a higher eosinophilia with STH eggs in stool, had almost three-fold higher viral loads and lower absolute CD4⁺ counts compared to other sub-groups (Mkhize-Kwitshana *et al.*, 2011). Perhaps, there is a variation in the ability of helminths species to activate and modulate the immune system in response to various helminths antigens, which also plays a pivotal role in HIV-1 pathogenesis in sub-Saharan Africa (Mkhize-Kwitshana *et al.*, 2011). When comparing gastrointestinal nematodes and blood trematodes, one expects that *S. mansoni*, a blood trematode residing in the intravascular system, would locally and systematically activate the immune system more remarkably than the gastro-intestinal nematodes and that, therefore, individuals co-infected with HIV-1+*S.mansoni* would have higher HIV-1 viral loads than those mono-infected with HIV-1 (Mkhize-Kwitshana *et al.*, 2011).

In the present study, no significant correlation was observed between the intensity of *S. mansoni* infection. A lack of correlation between HIV-1 plasma viral load and changes in *S. mansoni* burden before and after PZQ treatment have been reported from Western Kenya (Lawn *et al.*,

2000). Evidence from other helminth species such as STH indicates a strong correlation between the intensity (egg loads) of STH infections and high HIV-1 plasma viral loads (Lawn *et al.*, 2000). In earlier studies in Tanzania (Mazigo *et al.*, 2014) and Zimbabwe (Kallestrup *et al.*, 2005b), no correlation was observed between CD4⁺ cell counts and the intensity of *S. mansoni* infection.

Based on the immunological evidence on the interactions of HIV-1+*S. mansoni* in co-infected individuals (Sher & Coffman, 1992; Secor, 2012), it has been hypothesized that an effective PZQ treatment against *S. mansoni* in HIV-1 co-infected individuals would result into the reduction of HIV-1 plasma viral loads (Erikstrup *et al.*, 2008) and improve CD4⁺ cell counts. Although the interpretation of our results is limited by a small sample size, PZQ treatment did not result in any change neither in HIV-1 plasma viral loads nor in CD4⁺ cells counts. Similar findings have been reported from Western Kenya (Lawn *et al.*, 2000). In contrast, in rural Zimbabwe, PZQ treatment resulted in the reduction of HIV-1 plasma viral load in the group which received early treatment compared to the group which received a treatment delayed for three months (Kallestrup *et al.*, 2005a).

We observed an insignificant increase in HIV-1 plasma viral loads 12 weeks after treatment, probably indicating that PZQ treatment had no effect on HIV-1 infection. In Uganda (Brown *et al.*, 2005) and Western Kenya (Lawn *et al.*, 2000), an increase in HIV-1 plasma viral loads and decrease in CD4⁺ cell counts was reported four weeks post PZQ treatment in HIV-1+*S. mansoni* co-infected individuals. Although the mechanism through which HIV-1 plasma viral loads increase following PZQ treatment remains unknown, there are number of explanations. It is possible that the death of adult schistosomes following treatment results into antigenic stimulations that leads to the activation of the Th₂ arm of the immune system and, hence, create a good environment for HIV-1 replication (Joseph *et al.*, 2004; Brown *et al.*, 2005; Chenine *et al.*, 2005; Kamal & El Sayed Khalifa, 2006). Other authors have suggested that, the effects of *S. mansoni* infections is highly prolonged and reverses to normal very slowly after praziquantel treatment or eradication of the adult schistosomes compared to gastrointestinal nematodes. This can maintain inflammatory responses important for replication of HIV-1. Alternatively, praziquantel treatment results into the suppression of the production of inflammatory and anti-inflammatory cytokines that are maintained by chronic helminth infections (Joseph *et al.*, 2004). In part, the increase of HIV-1 viral loads following praziquantel treatment is thought to result into a decline in T-cell activities which in turn suppress antiviral immune responses (Aandahl *et al.*, 2004).

Our study was subjected to some limitations; it was not possible to determine the exact time the study participants were either infected with HIV-1 or *S. mansoni*. Similarly, the use of one Kato Katz technique, to characterized individuals infected or not infected with *S. mansoni* based on egg counts could have resulted into a false negative misclassification of individuals. However, studies using more sensitive techniques, such as circulating cathodic antigen test (CCA) or Circulating Anodic Antigen (CAA) test have produced similar results (Lawn *et al.*, 2000). The small sample size also limited the study. It is worthwhile to note that, it was not possible to measure humoral responses (antibodies responses) and cytokines to verify some of the observations. These factors may have confounded the study. Nevertheless, this was a community prospective longitudinal survey and the results are notable.

In conclusion, we found that, mean HIV-1 plasma viral loads varied significantly, with co-infected individuals having a higher mean at baseline and at three-month follow up time point than the HIV-1 mono-infected individuals. Furthermore, no correlation was observed between mean HIV-1 plasma viral loads and the intensity of *S. mansoni* infection. The treatment of *S. mansoni* infection in co-infected individuals did not result in changes in either HIV-1 plasma viral loads or CD4⁺ cell counts. However, based on the available evidence on the immunological interaction of HIV-1+*S. mansoni* infections, our results do not disqualify the bio-directional effects of each infection on their pathogenesis in co-infected individuals. In addition, although deworming may not have effects on HIV-1 markers in co-infected individuals, at least for the present study population, it should be part of the HIV-1 care to reduce and control the co-

morbidities associated with *S. mansoni* infection in co-infected individuals. Further studies are recommended to understand, in a larger cohort, the biological interactions of the two infections and the effects of deworming on the risk of HIV-1 transmission and its progression in sub-Saharan Africa.

Author's contributions

HDM, DWD, SMK, FN designed the study. HDM, DM and FN participated in data analysis. HDM drafted the first version of the manuscript. All authors read and approved the final manuscript, contributed to the critical review and made substantial contributions to its content.

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