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Low Prevalence of Helminth Infection among HIV Patients in Cameroon

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ABSTRACT

Helminth infections present one of the most common parasitic infections worldwide with a greater proportion occurring in the less developed areas of the world like Sub-Saharan Africa. Sub-Saharan Africa has the highest regional prevalence of HIV in the world. Due to the overlapping geographical distribution of these infections, coinfection between helminths and HIV are likely to be common. This study was therefore designed to determine the prevalence of helminth infection among HIV patients in Cameroon and to determine the effect on helminth infection in HIV by measuring the $CD4^+$ T cell count. Helminth infection was determined in faecal specimen by the formol ether concentration technique and quantifies with the Kato Katz technique. $CD4^+$ T cell count were determined with a BD FASCount™. Among the 241 HIV-positive patients that were enrolled into the study, 6(2.5%) were coinfected with helminths. Among those coinfected with helminths, 2(33.3%) were infected with *Ascaris lumbricoides*, 2(33.3%) with *Trichuris trichiura*, 1(16.7%) with *Strongyloides stercoralis* and 1(16.7%) with *Schistosoma mansoni*. Dual infection with more than one helminth was not observed in the study. The mean egg intensity were 1944, 420, 144 for the specimen which contained *Ascaris lumbricoides*, *Trichuris trichiura* and *Schistosoma mansoni* ova respectively. The mean $CD4^+$ T cell count among helminth coinfected patients was 627.93 cells/µl. no significant association between $CD4^+$ T cell count and helminth infection was observed ($P = 0.4815$), neither was there any significant association between $CD4^+$ T cell count in co-infected patients and patients without helminth infection ($P = 0.1110$). We came to the conclusion that the prevalence of helminth infection among HIV patients in study population was low and the influence of helminth infection on the outcome of HIV may be limited.

INTRODUCTION

Helminth infections caused by a diverse species of worms are very common with an estimated 2 billion people infected worldwide [1,2]. The majority of cases occur in resource-limited settings like Sub-Saharan Africa [3,4]. Sub-Saharan Africa has also been shown to have the highest regional prevalence (5.0%) of HIV in the world [5]. Due to this overlapping geographical distribution of helminth and HIV infection, it is therefore likely that concomitant infection between one or more helminth and HIV is common in the region [6,7]. Studies to determine the relationship between helminth coinfection with HIV have been performed amid great difficulties. It has been suggested that the effect of helminth infection and HIV are bi-directional: both helminths and HIV have a profound effect on the immune system and one may eventually influence the outcome of the other. Brown et al. [8] argue that helminths on the one hand may be in a

sense normal to the mammalian immune system since they may have evolved together; the effect on the immune system may be minimal. But HIV on the other hand is relatively a new disease with deleterious effects on the immune system [8]. Helminths have been implicated in the modulation [9] of the immune system in ways that may be beneficial or detrimental to the HIV virus. Helminths have been shown to suppress the production of $CD4^+$ and $CD8^+$- derived cytokines and proliferation of lymphocytes, suggesting they play a role in suppressing antiviral immune responses [10-15]. On the other hand, studies have shown that helminths are able to suppress viral replication [16] through suppression of transcription factors [17]. Studies to determine the effect of helminths on the severity of HIV as measured by CD4+ T cell count has been performed with mixed results [8].

Whatever the outcome may be of helminth and HIV infection on the host immune responses, it is evident that coinfection between helminths and HIV is common and widespread [18] and may be one of the causes of anaemia in these patients. Only a few studies that have been published today have exploited the theme of helminth and HIV coinfection. In Cameroon, general prevalence surveys have targeted mostly children of school age and none to the best of our knowledge has been performed on the HIV at risk group. This cross sectional study was therefore designed to determine the prevalence of helminth infection among HIV patients in the country and also attempt at exploiting the effect that helminth infection may have on HIV patients through the determination of the $CD4^+$ T cell count.

MATERIALS AND METHODS

Study Area

This study was approved by the Faculty of Health Sciences Institutional Review Board, University of Buea, Cameroon. The study was carried out in two selected HIV/AIDS treatment centers in Yaounde (political capital of Cameroon) between March and June 2011. Participants were recruited from the Yaounde Military Hospital and Efoulan District Hospital upon giving their signed informed consent. Stool samples were collected and transported to the University Teaching Hospital Centre (CHU) Yaounde where analyses were performed.

Study Population

The study participants were people living with Human Immunodeficiency Virus (HIV) of both sexes, 14 years of age and above, on antiretroviral therapy (ART) or not, and were selected randomly. Socio-demographic and Clinical information including diarrhoea and medication history were obtained by interview from the study participants using a structural questionnaire. Those excluded from the study included patients on specific antihelminthics, including those who had any treatment for intestinal parasitism and those who had had any gastrointestinal contract medication in the past 2 weeks before specimen collection.

Methods

Formol Ether Concentration Technique

Using an applicator stick, about one gram of stool was emulsified in about 7ml of 10% formol water in a screw-cap tube. The proceeding was done as described by Cheesbrough [19].

Kato Katz Technique

The template with hole in the centre was placed on a microscope slide. The screen was pressed on top so that some of the faeces filtered through. This was scraped with the flat spatula across the upper surface and the filtered faeces collected. The collected faeces were then added in the hole of the template so that it is completely filled. The template was carefully removed so that a cylinder of faeces was left on the slide. The faecal material was then covered with the pre-soaked cellophane strip. The microscope slide was inverted and the faecal sample firmly pressed against the cellophane strip on a smooth hard surface (a tile). The material was evenly spread. The slide was carefully removed by gently sliding it sideways to avoid separating the cellophane strip. The slide with the cellophane was then placed upwards. The smear examined in a systematic manner and the eggs of each species reported and multiplied by 24 as recommended by the manufacturer to give the number of the eggs per gram of faeces (epg).

Determination of CD_{4+} T Cell Count

5ml of blood was collected into EDTA test tubes for every participant and the CD4 cell counts were determined using BD FASCount™.

Data Analysis

Data from study participants were entered directly into Microsoft excel, 2007 (Microsoft Corporation Inc. USA) and imported to the statistic software package, MINITAB version 15 for windows, for analyses. $P < 0.05$ was considered to be statistically significant and represent 95% of the population.

RESULTS

Characteristic of Study Population

A total of 241 HIV sero-positive individuals consented to take part in this study. Among these, 67 (27.8%) were males and 174 (72.2%) were females. The mean (±SD) age of the study participants was 39.5years (range: 14-68years). The majority, 180 (74.7%), were from the Military hospital HIV treatment centre while the minority 61(25.3%) were patients attending the Efoulan District Hospital. 217 (90%) of the 241 participants were already on ART.

Helminth Prevalence and Infection Intensity

Using the Formol ether concentration technique, intestinal helminthes was identified in 6(2.5%) of the 241 specimen collected. The detected parasites included: *Trichuris trichiura* 2 (33.3%), *Strongyloides stercoralis* 1 (16.7%), *Ascaris lumbricoides* 2 (33.3%) and *Schistosoma mansoni* 1(16.7%). Dual infection with more than one Helminth was not observed in positive stool samples. Helminth infection was observed to be more common in males than in females where 2 (2.99%) of the 67 men and 4 (2.30%) of the 174 women were infected. However the difference in the prevalence of the infection between males and females was not found to be significant statistically ($X^2 = 0.0240$, $P = 0.8768$). Quantitative ova counts using the Kato Katz thick smear methods were determined for all the study participants regardless of the evidence of helminth infection by the Formol ether concentration technique. Out of the 6 helminth positive samples, 5 were positive by the Kato Katz technique giving a sensitivity of 83.3%. 4 (80%) of the 5 Kato Katz positive individuals had light intensity infection for *Ascaris lumbricoides* (<5000epg), *Trichuris trichiura* (<500epg) while 1 (20%) had moderate infection for *Schistosoma mansoni* (100-299epg), as specified by the World Health Organization classification scheme (Table 1) [20].

Table 1. Quantitative Ova Counts Using the Kato Katz Thick Smear Methods for Study Participants.

Parasites	Egg intensity (epg)	Mean egg intensity (epg)
A. lumbricoides.	288	1944
T. trichuris.	360	420
S. mansoni.	144	144

epg=eggs per gram of stool

CD_4 Cell Count

The mean $CD4_+$ T cell count of the study participants was 627.93. From the 6 helminth infected participants, 3 (50%) had $CD4^+$ T cell count > 500cells/µl, 1 (16.7%) had $CD4^+$ T cell count between 200 – 499cells/µl, and 2 (33.3%) had $CD4^+$ T cell count < 200cells/µl. There was no significant association between range of $CD4^+$ T cell count and the presence of parasite ($\chi^2 = 5.5$, $P = 0.4815$) (Table 2). A comparison between the $CD4^+$ T cell count among the participants infected with helminth and those not infected with helminth revealed no significant difference between the two groups ($\chi^2 = 4.396$, $P = 0.1110$) (Table 3).

Table 2. Distribution of Helminth Infection and CD_{4+} T Cells Count in Coinfected Individuals (N=6).

Parasites	$CD4^+$ T cell count		
	>500 cells/µl n (%)	200-499 cells/µl n (%)	<200cells/µl n (%)
Trichuris trichiura	1(50)	1(50)	0(0)
Schistosoma mansoni	1(100)	0(0)	0(0)
Ascaris lumbricoides	1(50)	0(0)	1(50)
Strongyloides stercoralis	0(0)	0(0)	1(100)
Total	3 (50)	1(16.7)	2 (33.3)

$\chi^2 = 5.5$, $P = 0.4815$

Table 3. Comparison of CD4+ T Cell Count between Helminth Infected and Non Helminth Infected Participants

CD4+ T cell count	Helminth infection	No helminth infection	Total
> 500 cells/µl n (%)	3	92	95
200 – 499 cells/µl n (%)	1	123	124
< 200 cells/µl n (%)	2	25	27
Total	6	235	241

$\chi^2 = 4.396$, P = 0.1110

DISCUSSION

The prevalence of 2.5% for helminth and HIV coinfection observed in this study is very low when compared to the 24.9% reported by Modjarrad et al. [21] in Zambia and also quite low when stacked close to the 42.4% reported by Nkemgazong et al. [22] in children of school age in Cameroon. This discrepancy between the result observed in this study and that reported by Nkemgazong et al. [22] in children of school age can be explained in the different demographic distribution of HIV and helminth infection: helminth infection is more common in children of school age meanwhile HIV is more common among youths. Helminth infections were observed more commonly among men (2.99%) than women (2.30%) in this study, however the difference was not significant statistically (χ^2 =5.5, P = 0.4815). The frequencies of the helminth seen in this study were *Trichuris trichiura* (33.3%), *Strongyloides stercoralis* (16.7%), *Ascaris lumbricoides* (33.3%) and *Schistosoma mansoni* (16.7%). These frequencies were limited to the low prevalence of helminth among HIV patients in this study and will therefore need to be investigated further. Dual infection with one or more helminths was not observed in this study.

Among the 6 samples that were positive with the formol ether concentration technique, 5 (83.3%) were equally positive with the Kato Katz technique suggesting that 1 sample had infection burden below the lower detection limit of the Kato Katz method. This finding suggest that the Kato Katz technique is not very sensitive in the detection of helminth ova but it uses are indispensible in the quantification of ova. The mean egg intensity (epg) were 1944, 420, 144 for *Ascaris lumbricoides*, *Trichuris trichiura* and *Schistosoma mansoni* respectively. The sample that *Strongyloides stercoralis* was observed in appeared to be below the detection limit of Kato Katz method (24 epg). According to the WHO classification scheme [20], light intensity infection was observed for *Ascaris lumbricoides* (<5000epg), *Trichuris trichiura* (<500epg) while a moderate infection was observed for *Schistosoma mansoni* (100–299epg). This is similar to the findings of Modjarrad et al. [21] were almost all infection with *Ascaris lumbricoides* and *Schistosoma mansoni* were of light to moderate intensity.

Helminth infection in HIV patients has been shown to impair the host immune responses by suppressing the production of CD4+ and CD8+–derived cytokines and proliferation of lymphocytes [10–15]. Studies to determine the effect of helminths on the severity of HIV as measured by CD4+ T cell count has been performed with mixed results [8]. This study attempted to determine the effect of helminth on the CD4+ T cell count in the study participants and there was no significant association between range of CD4+ T cell count and the presence of parasite (χ^2 =5.5, P = 0.4815), neither was the a significant difference between the CD4+ T cell count in those who harboured worm and those who did not (χ^2 = 4.396, P = 0.1110). These findings are very similar to that of Kallestrup et al. [23], Feitosa et al. [24], Brown et al. [25], and Wolday et al. [6], but different from the findings reported by Modjarrad et al. [21] and Elliot et al. [26] who observed a significant difference. This suggest that helminths may indeed have a limited influence on the CD4+ T cell count than the HIV counterpart which is in line with the point led down by Brown et al. [8]. This is possible in that 90% of the participants in this study were on antiretroviral therapy including all the patients who were coinfected with helminth, which have kept the virus under control thereby improving upon the immune status of these patients. However our study is limited to the very low prevalence of helminth infection in this study. We therefore recommend that similar studies be performed on a much larger scale in treatment naïve patients to ascertain these findings.

CONCLUSION

The prevalence of helminth infection among HIV patients observed in this study is very low. This low prevalence can be attributed to the different demographic distribution of helminth and HIV infection where HIV is more common among youths and helminths among children of school age. Helminth infection in HIV has a limited role to play in the severity of HIV as measured by the CD4+ T cell count but these findings are limited to the small proportion of helminth coinfected patients observed in this study and will require further investigation on a larger scale.

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