Intestinal parasitic infections and the level of immunosuppression in HIV seropositive individuals with diarrhoea in Kilimanjaro, Tanzania: A cross-sectional study

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Background: Opportunistic and non-opportunistic intestinal parasites play a significant role in the morbidity and mortality of HIV/AIDS-infected patients. The frequency of their occurrence strongly correlates with the patient's level of immunity. The most common clinical manifestation of these intestinal parasites is diarrhoea. Prevalence of intestinal parasites among HIV-infected patients has been found to be as high as 95%.

Objective: To determine the prevalence of intestinal parasites among HIV-infected participants presenting with diarrhoea and association with CD4 cell counts, ART and cotrimoxazole prophylaxis.

Methods: A prospective cross-sectional study was conducted in four HIV clinics in Moshi district, Kilimanjaro Region, Tanzania. Stool samples were collected and analyzed from participants presenting with three or more episodes of loose stool per day or a single bloody bowel movement. The identification of parasites was done using direct microscopy and staining techniques. Demographic data, CD4 counts and stool results were recorded. Data analysis was done using STATA IC/11.1.

Results: The study included 83 adult HIV positive patients. There were 36 males (43.4%) and 47 females (56.6%), with a median age of 36 years (range 30-43). The baseline CD4 count was 150 cells/ul (range 72-295 cells/ul). Of our participants, 47 (56.6%) had a baseline CD4 cell count < 200 cell/uL. Only 6(7.2%) had CD4 counts above 500cells/uL. Of the whole group, 62(74.7%) were on ARV therapy and 33(39.8%) were on cotrimoxazole prophylaxis. Intestinal parasites were detected in 25 of our participants. Among these 25 participants, *Ascaris lumbricoides* was found in 52%, *Giardia lamblia* in 32% and *Entamoeba histolytica* in 16%. The frequency of intestinal parasites was significantly associated with CD4 cell counts <200 cells/ul (p=0.02). There was no significant difference in parasitic infections associated with ART status or cotrimoxazole use.

Conclusion: The prevalence of parasitic infection is high in HIV-infected patients presenting with diarrhoea despite the use of ART and other prophylactic medications. Intestinal parasites should not be overlooked in HIV-infected patients presenting with diarrhoea.

Keywords: HIV, intestinal parasites, diarrhoea, immunity, Tanzania

Introduction

HIV/AIDS remains a major public health concern in sub-Saharan Africa [1]. The prevalence in Tanzania is 5.1% in the 15-49 year age group 30 years into the epidemic [2]. Intestinal parasites are endemic in many regions of high HIV/AIDS prevalence [3] including in sub-Saharan Africa [4, 5]. Diarrhoea is reported to occur in 30-60% of HIV/AIDS patients in developed countries, and in up to 90% of patients in developing countries [6, 7].

Intestinal parasitic infections in HIV-infected patients in developing countries is estimated to be as high as 95%. These protozoal and helminthic infections often present with diarrhoea as their main feature [8]. Clinically these intestinal parasitic infections present as both acute and chronic diarrhoea contributing to significant morbidity and mortality in HIV-infected individuals. The epidemiology and outcome of intestinal parasitic infections has been greatly affected by HIV infection [9].

Tanzania has high rates of HIV and parasitic co-infections. However, there are few reports on the association of intestinal parasites in HIV-infected individuals with the level of immunosuppression. The aim of this study is to assess the prevalence of opportunistic and non-opportunistic intestinal parasitic infections among HIV-infected patients presenting with diarrhoea, including the effect of ART and immunosuppression.

Methods

Study design, setting and participants

Table 1. Socio-demographic characteristics of 83 HIV-infected patients with diarrhoea attending HIV clinics in Moshi district, Kilimanjaro region

Characteristic	Number/Median (Percentage/IQR)
Age (years)	36 (30-43)
Sex	
Male	36 (43.4%)
Female	47 (56.6%)
Education	
No formal education	5 (6.0%)
Primary	51 (61.4%)
Secondary	25 (30.1%)
Higher learning	2 (2.4%)
ART Use	
Yes	62 (74.7%)
No	21 (25.3%)
Baseline CD4 (cells/ul)	150 (72-295)
Cotrimoxazole prophylaxis:	
Yes	33 (39.8%)
No	50 (60.2%)

This prospective cross-sectional study was conducted at four HIV clinics in the Kilimanjaro Region of Tanzania from August to October 2013. The study included 83 adult HIV positive patients aged 18 years and above presenting with three or more episodes of loose stools per day or a single episode of bloody bowel movement. Patients were excluded if they had received any antiprotozoal or antihelminthic medications within the past one-month prior to screening for the study.

Data collection and analysis

Stool samples were collected using a clean wide mouthed container. The samples were then transferred to labeled, leak proof, clean and sterile plastic containers. All of the collected samples were examined within 2 hours of collection. A direct wet mount of the stool sample was prepared and examined for the presence of motile intestinal parasites and trophozoites under a light microscope. For the detection of cysts, Lugol's iodine staining was used. The microscopic examinations were performed by trained clinical laboratory technicians. Patients' demographic data, baseline and maximum CD4 cell counts and stool results were recorded on a data collection sheet. The CD4 counts were measured using a FACS count system (BD Biosciences, San Jose, CA, US). Data analysis was done using the computer software STATA IC/11.1 (College station, Texas).

Ethical consideration

Approval for the study was obtained from the University of Dodoma Ethics Review Board, Moshi District Medical Officer (DMO) and Mawenzi Regional Hospital Authorities. Also an informed consent was obtained from all patients before their participation in the study. All patients found to have intestinal parasites were managed by clinicians according to available guidelines.

Results

The study included 83 adult HIV positive patients. Of these 36 (43.4%) were male and 47 (56.6%) female, with a combined median age (range) of 36 (30-43) years. Among the study participants, 62 (74.7%) were on ART and 21(25.3%) were not on ART; 33(39.8%) were on co-trimoxazole prophylaxis, while 50 (60.2%) were not on co-trimoxazole. The educational level of the study group was 6.0% (no formal education), 61.4% (primary education) and 30.1% (secondary education). Baseline clinical and socio-demographic characteristics of the study participants are summarized in Table 1.

Stool examination revealed intestinal parasites in 25(30.1%) study participants; out of whom 7(28%) were on cotrimoxazole and 20(80%) were on ART. The parasites found were of three species; *Entamoeba histolytica,*

Table 2. Prevalence of intestinal parasites in 83 HIV-infected patients with diarrhoea attending HIV clinic in Moshi district, Kilimanjaro region by ART and cotrimoxazole status

Parasites	HIV+ with diarrhoea			
	On ART (n=62)	Not on ART (n=21)	On CTX(n=33)	Not on CTX(n=50)
Entamoeba histolytica	2 (3.2%)	2 (9.5%)	2(6.1%)	2(4.0%)
Giardia lamblia	6 (9.7%)	2 (9.5%)	2(6.1%)	6(12%)
Ascaris lumbricoides	12 (19.4%)	1 (4.8%)	3(9.1%)	10(20%)
Stool negative	42 (67.7%)	16 (76.2%)	26(78.8%)	32(64%)

*CTX: Cotrimoxazole

Table 3. Prevalence of intestinal parasites by CD4 cell counts among 83 HIV-infected patients with diarrhoea attending HIV clinics in Moshi district, Kilimanjaro region

Level of CD4 (cells/ul)	Entamoeba histolytica	Giardia lamblia	Ascaris lumbricoides	Total	P-value
<200	4 (21%)	6 (31.6%)	9 (47.4%)	19 (76%)	
>200	0	2 (33.3%)	4 (66.7%)	6 (24%)	0.02

Giardia lamblia and *Ascaris lumbricoides*. *Ascaris lumbricoides* was the most frequent being found in 13(52%) participants of whom 12 were on ART. *Giardia lamblia* was found positive in 8(32%) participants, 6 of whom were on ART. *Entamoeba histolytica* was found in 4(16%) participants, 2 of whom were on ART. Out of the 62 patients who were using ART, 42(67.7%) were not found to have intestinal parasites as summarized in Table 2.

Among the 25 participants with intestinal parasites (see table 3), 19(76%) had CD4 count of <200 cells/ul. Each individual parasite was significantly associated with CD4 counts <200 cells/ul as compared to CD4 counts >200 (p=0.02). Cotrimoxazole use was found to be protective against occurrence of intestinal parasites, although the association was not statistically significant (OR=0.48 [IQR 0.17-1.32], p=0.16).

Discussion

The main finding from this study is the high prevalence of intestinal parasites despite ART use and cotrimoxazole prophylaxis in this group of HIV-infected patients presenting with diarrhoea. In HIV-infected patients, the immune response is significantly impaired resulting in susceptibility to gastrointestinal parasitic infections, a significant cause of morbidity and mortality in this population. A majority of these patients present with diarrhoea which can serve as an indicator of disease progression. In our study, non-opportunistic parasites were found to be common. The prevalence of intestinal parasites in this study was found to be 30%. This finding was consistent with another study done in Jaipur city [7], whereby non-opportunistic parasites were also found to be common with a prevalence of 31%. The prevalence was even higher in another study in Dhule district, India, at 49% [10]. Other studies, assessing both opportunistic and non-opportunistic intestinal parasites, have reported a prevalence of non-opportunistic parasites ranging from 5-30% [11] and 30-60% [12, 13, 14]. The occurrence of these parasites was significantly associated with low CD4 counts (<200 cells/ul). Similarly in a study done in Benin City, Nigeria the occurrence of intestinal parasites was significantly associated with CD4 counts <200 cells/ul [15]. In other studies however, only opportunistic parasites have been associated with low CD4 counts [7]. The association between the occurrence of intestinal parasites and the level of immunity in HIV-infected individuals has been well documented in other reports [16, 11, 17, 18].

Our study showed that there was no significant difference between the prevalence of intestinal parasites in those on ART compared to those not on ART. This is in contrast to the findings of a study done in Ethiopia which found significantly lower rates of intestinal parasitic infection in those on ART [19]. Another study comparing the prevalence of intestinal parasites before and after HAART found significant reductions in the prevalence of intestinal parasites with the use of ART [20]. These observed discrepancies might be explained by the difference in study sample sizes, study designs and geographic locations.

In our study, three of the participants with CD4 counts >500 were found to have intestinal parasites. One of them was found to have *Giardia lamblia*, while two of them had Ascaris lumbricoides. Similar findings were obtained in a study done in Thailand were three of the patients with CD4 cell counts >500 were found to have non-opportunistic intestinal parasites [21]. Ascaris lumbricoides occurrence was also found to be high in seronegative individuals in one study comparing intestinal parasites in seronegative and seropositive individuals [22]. One study found no intestinal parasites in HIV-infected individuals with CD4 count >500 cells/ul [7, 18] contrary to our finding.

Our study involved HIV-infected patients treated or untreated with ART presenting with diarrhoea. Other studies found higher prevalence of opportunistic parasites in patients with diarrhoea [21], with similar rates of non-opportunistic parasites as found in our study [7]. Another study found high prevalence of non-opportunistic parasites that was neither associated with the level of immunity nor with diarrhoea [21].

A limitation of our study is that some important laboratory tests were not performed. We did not perform modified ZN-staining, water-ether sedimentation method or adhesive tape/anal swab for Microsporidia and Enterobius vermicularis respectively, as well as the Baerman technique or culture for *Strongyloides stercoralis*. Some important intestinal parasites, both opportunistic and non-opportunistic, could have therefore been missed. Other risk factors for acquisition of intestinal parasites such as hygiene, socio-economic and environmental

factors were not included in this study. The sample size for this study was too small to make significant statistical inferences.

Non-opportunistic intestinal parasites were common in this population and significantly associated with low CD4 cell count. These infections therefore should not be overlooked in HIV-infected patients especially those with low CD4 counts and presenting with diarrhoea.

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Author contributions

TR, AE and BM conceived and designed the experiments. TR performed the experiments. TR, BM, BK analyzed the data. TR, BM, BK and AE did the literature search and wrote the manuscript. All authors have read and approved the publication of the final manuscript.

Conflict of interest: Authors declare that they have no conflict of interest

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