Alexandria Journal of Medicine (2017) 53, 111–116

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Prevalence of intestinal parasites in newly diagnosed HIV/AIDS patients in Ilorin, Nigeria

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Received 3 January 2016; revised 11 April 2016; accepted 12 April 2016
Available online 6 May 2016

KEYWORDS

Intestinal parasites;
HIV/AIDS;
Newly diagnosed;
Treatment naive;
Adults

Abstract *Background:* Human immune-deficiency virus/acquired immune-deficiency syndrome predisposes to opportunistic parasitic infestations of the gastrointestinal tract. This study aimed to determine the prevalence of intestinal parasites in newly diagnosed treatment naïve HIV/AIDS patients.

Methods: This hospital-based cross-sectional study was carried out from December 2010 to June 2011. Questionnaires were administered to 238 HIV/AIDS subjects, and 238 age and sex-matched controls. $CD4^+$ T cell count was carried out on HIV-positive subjects. Stool samples were examined using direct microscopic and modified Ziehl-Neelsen methods. Positivity of intestinal parasites was taken as the presence of worms, oocyst, cyst, ova or larvae in the stool samples.

Results: Ninety males and 148 females were studied for the HIV-positive and HIV-negative controls respectively. Intestinal parasitic infestation in HIV-positive subjects was 68.5%, and was significantly higher than in the HIV-negative controls 49.2% ($P < 0.05$). In HIV-positive subjects, *Cryptosporidium* spp. was the commonest (55.0%) parasite isolated. Others were *Cyclospora cayetanensis* (41.2%), *Isospora belli* (3.0%), *Entamoeba histolytica* (8.4%), *Giardia lamblia* (3.7%), *Ascaris lumbricoides* (2.5%), *Strongyloides stercoralis* (1.7%), *Trichuris trichiura* (0.8%) and *Schistosoma mansoni* (0.4%). HIV-positive patients with $CD4^+$ T cell count of less than 200 cells/ul were more at risk of opportunistic parasites compared to the HIV-negative controls.

Conclusion: The prevalence of intestinal parasites in newly diagnosed HIV/AIDS individuals was high, and its association with $CD4^+$ T cell count was demonstrated. Routine screening for parasitic infestations at diagnosis is indicated to reduce the burden of the disease.

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Peer review under responsibility of Alexandria University Faculty of Medicine.

http://dx.doi.org/10.1016/j.ajme.2016.04.001
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1. Introduction

Human immune-deficiency virus/acquired immune-deficiency syndrome (HIV/AIDS) is a global public health problem.[1] The disease predisposes individuals to various opportunistic infections of which parasitic infestations of the gastrointestinal (GI) tract are prominent.[2] Several studies have shown that some intestinal parasites such as *Cryptosporidium* spp., *Cyclospora cayetanensis*, *Isospora belli*, *Entamoeba histolytica/dispar*, *Strongyloides stercoralis*, and *Giardia lamblia* are responsible for 60–80% of infestations.[3] Thus infestation of the GI tract by these organisms plays a crucial role in HIV/AIDS pathogenesis, and diarrhoea diseases assume a prominent role reaching up to 50% in developing countries.[2] The decrease in immunity by attack on the immune system especially the cluster of differentiation ($CD4^+$ T cells) component, macrophages, and defect in the production of immunoglobulin A (IgA) increases susceptibility to these parasites.[4] Gut-associated lymphatic tissue (GALT) in the GI tract is an important site for early HIV replication and $CD4^+$ T cell replication.[5]

Helper T cells which are very important in adaptive immunity, activate B cells to secrete antibodies, activate macrophages to destroy ingested microbes, and also activate cytotoxic T cells to kill infected target cells. These functions are impaired in patients with HIV/AIDS.[6] Furthermore, HIV/AIDS decrease the circulating pool of effector, and memory CD8+ T cells which combat viral infections. The end result is immunosuppression.[7] There have been reports of the prevalence of intestinal parasites in HIV/AIDS patients in other parts of Nigeria and the world.[8–14] Ibrahim et al.[8], and Babatunde et al.[9], in North central, Nigeria, found an increase in the prevalence of intestinal parasites in HIV-positive patients compared to HIV-negative controls. Similarly, Akinbo et al.[10], Hailemariam et al.[11], Alemu et al.[12], Jegede et al.[13], and Oyedeji et al.[14] all found an increase in the prevalence and multiple intestinal parasitosis in HIV-positive Nigerian and Ethiopian patients compared with HIV-negative controls. These studies were mostly on patients already on highly active anti-retroviral therapy (HAART). There is a paucity of data on similar studies on treatment naïve HIV/AIDS patients. It is thus important to find out the common intestinal parasites in newly diagnosed treatment naïve adult HIV/AIDS patients in Ilorin, Nigeria, since these parasites play a prominent role in diarrhoeal diseases, and HIV/AIDS pathogenesis.

This study therefore aimed to determine the prevalence of intestinal parasites in newly-diagnosed treatment naive HIV/AIDS patients.

2. Subjects, materials and methods

The study was a hospital-based cross-sectional study carried out over a six-month period (December 2010–June 2011), at the Infectious disease clinic of University of Ilorin Teaching Hospital (UITH), Ilorin, Nigeria. A standard structured questionnaire was administered to consecutive 238 newly diagnosed treatment naive adult HIV/AIDS patients, and 238 age and sex-matched HIV-negative controls whose blood and stool samples were tested at first enrolment. Individuals who had taken antibiotics, anthelmintic drugs and steroids prior to enrolment were excluded from the study.

2.1. Specimen collection

Blood sample: Ten millilitres of venous blood samples were collected aseptically into labelled EDTA specimen bottles. Rapid Enzyme-linked immunosorbent assay (ELISA) was carried out to detect antibodies to HIV 1 and 2 using rapid ELISA test kit (AcuuBio Tech Co., Ltd., China). $CD4^+$ T cell count was carried out with Partec 4000 cyflow counter Germany (2006) at the HIV laboratory of UITH.[15] The range of $CD4^+$ T cell count was classified using the 1993 Center for Disease Control and Prevention USA, immunological criteria.[16]

Stool sample: Stool samples were collected into sterile containers, labelled, and analysed within 24 h of collection. Stool samples were examined using direct microscopy for wet preparation, and iodine preparation.[17,18] Modified Ziehl-Neelsen methods, and Faecal concentration technique (using formol ether concentration method) were also used to increase the yield of intestinal parasites. Positivity of intestinal parasites was taken as the presence of worms, ova, trophozoites, oocysts and cysts in the stool samples.[17–19]

Data analysis: Epi-info™ version 3.5.1(CDC, USA 2008) software package was used for analysis. Statistical significance was taken as *p*-value less than 0.05.

Ethical approval was obtained from the Ethics and Research committee of UITH.

3. Results

There were 90 males (37.8%) and 148 females (62.2%) in both groups, with a male to female ratio of 0.6:1. The age range for both groups (HIV-positive subjects and HIV-negative controls) was from 16 to 65 years. The mean age of respondents was 36.1 ± 9.6 years and 35.9 ± 9.4 years for the HIV-positive subjects and HIV-negative controls respectively. The commonest age group was 26–35 years for both HIV-positive subjects and HIV-negative controls. The mean age of the respondents was similar ($P > 0.05$). Majority of the respondents were married, and secondary school level of education was the highest level of education attained by them. Most of the respondents were of Yoruba ethnic origin, and trading was the major occupation of the HIV-positive subjects, while most of the HIV-negative controls were self-employed (Table 1).

The prevalence of intestinal parasites in HIV-positive subjects and HIV-negative controls was 68.5% and 49.2% respectively. HIV-positive subjects had increased risk of intestinal parasites compared to HIV-negative controls ($P < 0.05$); OR = 2.25 CI (1.52–3.32).

The most frequently detected parasites in the HIV-positive subjects and HIV-negative controls were *Cryptosporidium* spp. and *C. cayetanensis*. These were significantly higher in the HIV-positive subjects than in HIV-negative controls (55% vs 16.8%) ($X^2 = 73.97$: $P < 0.05$) and (41.2% vs 8.4%) ($X^2 = 66.81$; $P < 0.05$) respectively. There were no statistically significant differences in the prevalence of *I. belli* (3.0% vs 0.8%; $X^2 = 1.81$: $P > 0.05$), *E. histolytica* (8.4% vs 8.8%) ($X^2 = 1.0$: $P > 0.05$), *G. lamblia* (3.7% vs 0%), *Hookworm* spp. (0% vs 0.8%), *S. stercoralis* (1.7% vs 0.8%; $P > 0.05$), *Ascaris lumbricoides* (2.5% vs 0%), *Trichuris trichiura* (0.8% vs 0%), *Schistosoma mansoni* (0.4% vs 0%) in both groups (Table 2).

Table 1 Socio-demographic characteristics of respondents.

	HIV-positive subjects frequency *n* (%)	HIV-negative controls frequency *n* (%)
Age (years)		
16–25	42(17.6)	42(17.6)
26-35	89(37.4)	89(37.4)
36–45	68(28.6)	68(28.6)
46–55	33(13.9)	33(13.9)
56–65	6(2.5)	6(2.5)
Gender		
Female	148(62.2)	148(62.2)
Male	90(37.8)	90(37.8)
Marital status		
Single	59(24.8)	89(37.4)
Married	169(71.0)	148(62.2)
Divorced	7(2.9)	1(0.4)
Widowed	3(1.3)	0(0)
Ethnicity		
Hausa	10(4.2)	12(5.0)
Yoruba	171(71.9)	191(80.3)
Ibo	12(5.0)	9(3.8)
Others	45(18.9)	26(10.9)
Level of education		
No formal education	65(27.3)	27(11.3)
Primary	70(29.4)	52(21.8)
Secondary	75(31.5)	83(34.9)
Tertiary	19(8.0)	68(28.6)
Quranic	9(3.8)	8(3.4)
Occupation		
Unemployed	37(15.6)	30(12.6)
Self employed	67(28.1)	61(25.6)
Students	7(2.5)	45(18.9)
Traders	77(32.4)	37(15.6)
Civil servants	42(17.6)	44(18.5)
Professionals	9(3.8)	21(8.8)

Some respondents were infected with up to four different parasites. HIV-positive subjects had more multiple parasitic infestation rates (more than one) than the HIV-negative controls 33.2% (79/238) and 25.6% (61/238) respectively ($X^2 = 34.13$; $P < 0.05$) (Fig. 1).

The range of $CD4^+$ T cell count in HIV-positive patients was 13–883 cells/ul. The mean $CD4^+$ T cell count was 257 ± 201.7. HIV-positive patients with $CD4^+$ T cell count < 200 cells/ul had increased risk of intestinal parasitic infestation ($p = 0.017$; OR 1.95: CI = I.09–3.55) compared to those with $CD4^+$ T cell count of 200–499 ($p = 0.760$; OR = 1.09, CI = 0.59–2.04) and those with $CD4^+$ T cell count > 500 ($p = 1.00$; OR = 0.24, CI = 0.10–0.54) (Table 3).

4. Discussion

Intestinal parasites, especially the opportunistic ones are a common cause of morbidity and mortality in HIV/AIDS patients.[8] The prevalence of intestinal parasites in HIV-positive subjects in this study was found to be high (68.5%). Studies carried out in other parts of Nigeria showed diversity in findings. The figure of 68.5% is lower than the 87.8% reported in an earlier study carried out in our centre by Babatunde et al.[9] Ibrahim et al. in North-central Nigeria, documented a figure of (50.0%).[8] Akinbo et al. in South-south Nigeria reported 15.3%.[10] This variation in prevalence is not different from that documented in Africa and other parts of the world. Hailemariam et al.[11] and Alemu et al.[12] in Ethiopia reported a prevalence of 52.6% and 80.3% respectively. Kipyegen et al. in Kenya documented a parasitic prevalence in HIV-positive patients which is to be 50.0%).[20]

Da silva et al. in Brazil found a prevalence rate of 27.0%.[21] Mohandas et al. in India found out that 30.0% of HIV-positive patients harbour intestinal parasites.[22]

HIV-positive subjects in this study were all newly diagnosed and treatment naïve in contrast to the other studies mentioned above. Furthermore, the study subjects in aforementioned studies, were a combination of those on treatment and treatment naïve. They may have used anthelmintic drugs such as Albendazole, Piperazine, Levamisole, and HAART. This may have boosted their immunity and subsequently altered the intestinal flora in the gut.[14] Sampling techniques and observer differences in the examination of stool samples may be another factor responsible for these differences in prevalence even though most of the studies involved the use of direct wet mount, formalin-ether sedimentation concentration, and modified Ziehl Neelsen methods. However, Hailemariam et al.[11] did not use Ziehl Neelsen method.

Table 2 Distribution of intestinal parasitic species in respondents.

Parasites	HIV-positive	HIV-negative	Chi-squared	*p*-value	Comment
(1) *Cryptosporidium* spp.	131(55.0)	40(16.8)	73.97	< 0.05	S
(2) *Cyclospora* spp.	98(41.2)	20(8.4)	66.81	< 0.05	S
(3) *Isospora belli*	7(3.0)	2(0.8)	1.81	> 0.05*	NS
(4) *E. histolytica*	20(8.4)	21(8.8)	1.0	> 0.05	NS
(5) *Giardia lamblia*	9 (3.7)	0(0)	–	–	–
(6) *Hookworm* spp.	0(0)	2(0.8)	–	–	–
(7) *S. Stercoralis*	4(1.7)	2(0.8)	–	> 0.05*	–
(8) *A. lumbricoides*	6(2.5)	0(0)	–	–	–
(9) *Trichuris trichiura*	2(0.8)	0(0)	–	–	–
(10) *S. mansoni*	1(0.4)	0(0)	–	–	–

S-significant.
NS-not significant.
* *p* value from Fishers exact test.

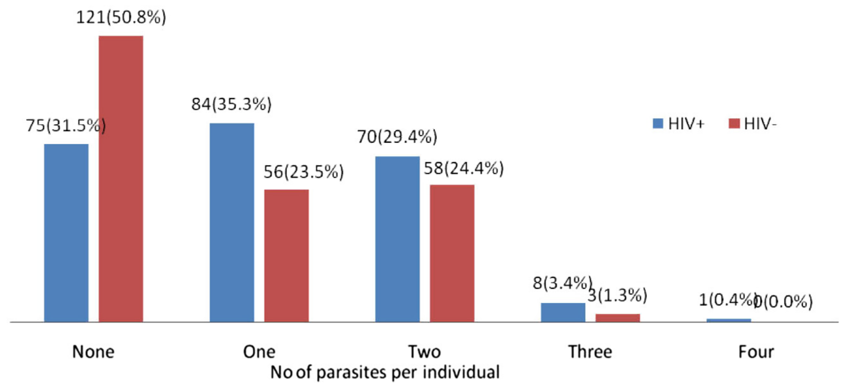

Figure 1 Multiple parasitic infestation rate in respondents.

Table 3 Distribution of intestinal parasites in HIV-positive subjects at different values of $CD4^+$ T cell count (cells/ul).

$CD4^+$ T Cell count	*n* (%) *n* = 238	Intestinal parasites *n* (%) *n* = 163	Odds ratio (95% CI)	*p*-value
< 200	125(52.5)	94(57.7)	1.95(1.09–3.55)	0.017
200–499	79(33.2)	55(33.7)	1.09(0.59–2.04)	0.760
> 500	34(14.3)	14(8.6)	0.24(0.10–0.54)	1.000

Cryptosporidium spp. was found to be the most predominant intestinal parasite in HIV-positive subjects (55.0%). Studies carried out in Ile-Ife, South-west Nigeria, by Oyedeji et al.[14] and Adesiji et al.[23] found the most predominant intestinal parasite in HIV-positive subjects to be *Cryptosporidium* spp. (19.2% and 52.7%), and Thomas et al. in Lagos, South-west Nigeria, documented similar findings.[24] However, this is in contrast to the findings by Nwokediuko et al. in Enugu, South-east Nigeria[25] and Oyerinde et al. in Lagos[26], who reported a rarity of C*ryptosporidium* spp. This is unusual, as C*ryptosporidium* spp. infection has been documented even in apparently normal individuals by Ikeh et al.[27], Nwabuisi[28], and Banwat et al.[29] all in North-central Nigeria. The prevailing environmental conditions may not be favourable for the transmission of these parasites in that part of the country, which may have accounted for the reported low incidence of C*ryptosporidium* spp. in those regions. These studies were noted to have been carried out on patients with diarrhoea; hence, they may have taken anthelmintic drugs such as Albendazole, Piperazine, and Levamisole prior to enrolment into the studies. The findings of Oyedeji et al.[14] were influenced by the prior use of ant-helminthics and Co-trimoxazole. Jegede et al.[13] in North central, Nigeria, did not find any statistical difference in the spectrum of intestinal parasites between HIV-positive and HIV-negative controls.

However, studies in Nigeria by Wokem et al.[30] and Ethiopia by Hailemariam et al.[11] found *A. lumbricoides* to be the most predominant parasites in HIV-positive subjects. *S. stercoralis* was found to be most predominant in HIV-positive subjects in Thailand.[31] Further studies need to be carried out in those geographical areas to validate these findings as opportunistic parasites are expected to thrive more in HIV-positive patients.

From this study, *C. cayetanensis* was detected in a significant number of HIV-positive subjects compared to HIV-negative controls (41.2% vs 8.4%). This differs from reports in some parts of Nigeria and the world at large, in which the prevalence was found to be low or undetected.[23,24,32,33] The geographical variation and the remarkable similarity in morphologic features with *Cryptosporidium* spp. could account for these differences. The presence of *Cryptosporidium* spp. and *C. cayetanensis* in the HIV-negative controls may be due to the fact that asymptomatic carrier state can occur in immune-competent individuals and they only become symptomatic with diarrhoeal disease, when they become immune-deficient.[28,30,34,35] Reports by Nwabuisi[28] in Ilorin, and Banwat et al.[29] in Jos, North-central Nigeria, have shown that infection with *Cryptosporidium* spp. and *C. cayetanensis* can occur in immuno-competent individuals. Poor personal, and environmental hygiene which could facilitate transmission of the cyst and ova of parasites may be responsible in this part of the world.

The rate of multiple parasitic infestations was 33.2% and 25.6% in HIV-positive and HIV-negative controls respectively. A report from Jos by Ibrahim et al.[8], found multiple parasitic infection rates in HIV-positive subjects to be lower (13%), while Hailemariam et al.[11], and Alemu et al. documented a high multiple parasitic infestation rate of 91.3%.[12] This may be attributed to depressed immunity in the HIV-positive subjects, which predisposed them to mixed infections including the opportunistic and non-opportunistic parasites.

HIV-positive patients with $CD4^+$ T cell count < 200 cells/ul had increased risk of intestinal parasitic infestation ($P < 0.05$) compared to those with $CD4^+$ T cell count of > 200. Majority of HIV-positive subjects with $CD4^+$ T cell count of < 200 cells/ul had more than double the risk of developing diarrhoea ($P < 0.05$) compared to those with $CD4^+$ T cell count of > 200. This is in keeping of reports from earlier studies carried out by Babatunde et al.[9], Wiwanitkit[31] and Assefa et al.[32] All these findings have shown that immunity plays a major role in the survival and proliferation of intestinal parasites in HIV-positive patients.

A major limitation in this study, is the fact that other staining techniques such as modified trichome staining technique were not used which could have detected Microsporidia spores.

In conclusion, the prevalence of intestinal parasites in treatment naive adult HIV/AIDS individuals was found to be high. HIV-positive individuals with $CD4^{+}$ T cell count less than 200 cells/ul were more at risk of infestations with intestinal parasites, and multiple intestinal parasites than those with more than 200 cell/ul. Apparently normal individuals also harbour opportunistic parasites.

Conflict of interest

None.

Source of funding

None

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