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Original Article

Prevalence of intestinal parasitic infestation in HIV seropositive and seronegative patients in Ilorin, Nigeria

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Abstract

Objective: To determine the prevalence, severity and pattern of intestinal parasitic infestation in HIV-seropositive patients.
Methods: A Cross-sectional study from January 2007 to December 2008.Patients were recruited from the HIV clinics of the hospital. Paired blood and single stool specimens were collected from each patient. The stool sample was investigated for intestinal parasites while the blood sample was tested for antibodies to HIV-1 and 2. HIV-seropositive subjects also had $CD4^+$ cells count done.
Result: Ninety each of stool and blood samples were collected from HIV-seropositive and HIV-seronegative patients. Four species each of helminthes and protozoan parasites and three species of coccidian parasites were isolated from the stool of both HIV-seropositive and seronegative subjects. The prevalence of these parasites was two and a halve times higher among the HIV seropositive patients than the seronegative ones. The range of CD4 cells count was 20-680 cells/µl with a median of 259 cells/µl. Patients with $CD4^+$ count <200/µl had more coccidian parasites in their stool and also had higher prevalence of intestinal polyparasitism ranging from 2 to 4 different species per stool sample.
Conclusion: The frequency of both AIDS defining and non- AIDS defining intestinal parasitic infestation was higher among the HIV infected patients. Patients' $CD4^+$ cells count was an important determinant of the rate and number of parasitic infestation.

Keywords: $CD4^+$ cells, HIV/AIDS, ilorin, intestinal parasites, prevalence

Résumé

Objectif: Pour déterminer la prévalence, la gravité et la répétition d'une infestation parasitaire intestinale patients séropositifs au VIH.
Méthodes: Étude transversale de janvier 2007 à décembre 2008. Patients ont été recrutés dans les cliniques de VIH de l'hôpital. Appairée de sang et de selles unique spécimens ont été collectés sur chaque patient. L'échantillon de selles a été étudiée pour les parasites intestinaux, tandis que l'échantillon de sang a été testé pour des anticorps contre le virus HIV-1 et 2. Sujets séropositifs au VIH avaient également $CD4^+$ cellules nombre fait.
Résultat: Échantillons de quatre-vingt-dix de selles et de sang ont été prélevés sur des patients séropositifs au VIH et le VIH-séronégatifs. Quatre espèces chaque helminthes des parasites qui et trois espèces de parasites coccidian ont été isolés des selles de-séropositifs au VIH et sujets séronégatifs. La prévalence de ces parasites était deux et une moitié de fois plus élevée chez les patients séropositifs au VIH et ceux séronégatifs.
Nombre de la plage de cellules CD4 était 20-680 cellules/µl avec une médiane de cellules 259/µl. Les patients avec CD4 + nombre < 200/µl avait plus coccidian parasites dans leurs selles et également une prévalence supérieure de polyparasitism intestinale allant de 2 à 4 espèces différentes par exemple des selles.
Conclusion: Infestation parasitaire de la fréquence de définition du SIDA et définition non-SIDA intestinale était plus élevée chez les patients VIH infecté. CD4 des patients + nombre de cellules a été un important déterminant le

taux et le nombre d'infestation parasitaire.

Mots-clés: CD4+ cellules, le VIH/SIDA, ilorin, parasites intestinaux, prévalence

Introduction

The human immunodeficiency virus (HIV) causes progressive impairment of host cellular immune system leading to increased susceptibility to infectious agents and tumors. HIV infection is a major medical problem in Nigeria. The current estimated infection rate is 2.9 millions.[1] Persons with HIV are predisposed to frequent and prolonged infestation with protozoan parasites which often manifest as diarrhea.[2] The broad clinical spectrum of diseases caused by intestinal parasites in HIV patients ranged from asymptomatic infestation to severe, life threatening diarrhea, dehydration and malabsorption.[3,4]

T-lymphocyte cell (T-cell) mediated immunity is very important in maintenance of healthy colon.[5] Depletion of circulating T-cell as a result of HIV infection invariably leads to increased susceptibility to opportunistic intestinal protozoan parasites.[5-7] In addition, intestinal parasitic infestations are basic health problems prevalent in tropical developing countries. Reports[4,5] indicated that diarrhea occurred in 30-60% of HIV/AIDS patients in developed countries and in about 90% of HIV/AIDS patients in Africa.

There are limited number of reports[8-10] from Nigeria that have described the relationship between HIV and intestinal parasites. These studies, however, have limited information on intestinal coccidian opportunistic parasites such as *Cryptosporidium, Cyclospora* and *Isospora* species. It is also important to note that this report will be the first documentation on HIV/AIDS and intestinal parasites from this center. And it aims to determine the frequency and pattern of intestinal parasitic infestation, including protozoan species in HIV-seropositive patients in our center.

Materials and Methods

This cross-sectional study was carried out at the University of Ilorin Teaching Hospital (UITH), a tertiary and referral hospital in Kwara State, Nigeria. Patients were recruited from two entry points: the Medical outpatient clinic and the blood bank unit of the hospital. The HIV status of the subjects was confirmed with a double sequential enzyme-linked immunosorbent assay (ELISA) method.

Consecutive patients, who reported at these clinics either as a new registration or as a follow-up care case but yet to be commenced on antiretroviral medication, were recruited into the study. An informed consent was sought and obtained from the patients before samples were collected from them. A simple structured questionnaire was administered to ensure that the subjects were not on any antihelmintic, antibiotics and antiprotozoan drugs in the last six months. History of chronic diarrhea was ruled out and this was defined as loose to watery stool with or without mucus and blood occurring for more than three times a day.[2] Controls were the subjects who came for VCT and tested seronegative to both HIV 1 and 2 using ImmunoComb®11 HIV 1 and 2 Bispot kit (Orgenics Ltd, Tarne P.O.B 360, 70650 Israel). Two clinical specimens, stool and blood, were collected from each patient. The stool specimen was investigated for intestinal parasites by simple wet preparation for trophozoites, wet iodine preparation and formol-ether concentration technique[11-14] for helminthic ova and larva. A drop of stool concentrate was also stained by modified Ziehl-Neelsen staining technique for oocysts of *Isospora belli, Cryptosporidium* and *Cyclospora* species.

Three milliliters of blood was collected into EDTA bottle from the HIV seropositive cases. The sample was used for estimation of peripheral CD4+ cells count and this was done using Dynal®T4 Quant Kits (Dynal® Bitech Asa, Oslo, Norway). All the subjects were categorized by their immune status (CD4+ T-cell) according to the 2006 WHO revised classification system.[15] The patient's immune status was later correlated with the number and rate of detection of intestinal parasites in their stool samples.

Results

There were 43 (47.8%) and 47(52.2%) HIV seropositive males and females compared to 45 (50%) each of males and females seronegative control. The median age of the HIV seropositive patients was 35 years with a range of 15-56 years compared to median age of 37 years with a range of 17-55 years for the seronegative patients.

Eleven different species of intestinal helminthes and protozoans were recovered from the stool specimens of 79 (87.8%) HIV seropositives and 67 (74%) seronegative patients. No parasites were isolated from the remaining 11 (12.2%) HIV-seropositive

and 23 (25.6%) HIV-seronegative controls, Table 1. The prevalence of both opportunistic and non-opportunistic intestinal parasites was two and a half times higher among the HIV seropositive patients than the seronegative ones. Odd ratio=2.5, 95%CI= 1.05-5.85. There was no difference in the rate of detection of ova of hookworm; 7.8% and that of *Ascaris lumbricoide* 6.7% in both seropositives and their seronegative counterparts. However, HIV seropositive status more than seronegative status was associated with a higher prevalence of *Trichuris trichiura* (10% vs. 6.7%), *Entamoeba histolytica* (21% vs.15. 6%) and *Iodamoeba, buteschli* (7.8% vs.5.6%) as well as *Cyclospora* species (17.7% vs. 11.1%) respectively, Table 2. Similarly *Isospora belli* (11.1% vs.2.2%), *Strongyloides stercoralis* (18.9% vs.5. 6%), *Cryptosporidium* species (32.2% vs.8.9%) and *Giardial lamblia* (17.7% vs.5.6%) were more prevalent in the HIV infected patients than non infected ones.

Specifically, the prevalence of extracellular intestinal helminthes such as *Strongyloides stercoralis* and *Giardial lamblia* was about four folds each higher among the HIV seropositive patients compared with HIV seronegatives: OR=3.96, 95% CI= 1.3-13.0 and OR=3.7, 95% CI= 1.2-12.1, Table 2. That of the intracellular protozoan parasites was even higher; *Cryptosporidium* spp was about 5 times more common in HIV sero-positives than the seronegatives: OR=4.9, 95% CI= 2.0-12.5 while *Isospora belli* was even more than 5 times commoner, OR=5.5, 95% CI= 1.0-37.5.

Of the 90 patients studied, 67 (74.4%) had their $CD4^+$ cells estimated. It ranged from 20 cells to 680 cells per microliter of blood, with a median of 259 cells per microliter. Twenty six patients; about 40% of the studied population had severe immunosuppresion; $CD4^+$ $\leq$200/ μl while 37 patients; 55.2% had moderate immune depression; $CD4^+$ count of between 201-499cells/ μl. Only 4 patients; 6% had mild disease with $CD4^+$ count $\geq$ 500/ μl, Table 3. Generally, the CD4 cells count of less than 200 cells/Ul was highly correlated with the number of parasites a seropositive patients haboured, The Spearman's correlation coefficient was 67% and *P*- value was 0.039. The rate of detection of parasites that were AIDS defining was also higher among patients whose $CD4^+$ count was less than 200cells/ ul. Fifty percent of all the cases of isolated *Isosporal belli* and 62% each of *Cryptosporidium* and *Cyclosporal* spp were from these severely immunocompromised hosts. So were 53% and 56% of *Strongyloides stercoralis* and *Giardial lamblia,* respectively.

Polyparasitism was also a common feature in this class of patients. Fifteen; 57.7% of the 26 patients with $CD4^+$ count less than 200 had two parasites

Table 1: Prevalence of intestinal parasites and patients' HIV sero-status

Parasites	HIV-seropositive (%)	HIV-seronegative (%)
Helminthes		
Hookworm	7(7.8)	7 (7.8)
Ascaris lumbricoides	6 (6.7)	6 (6.7)
Trichuris trichiura	9 (10)	6 (6.7)
Strongyloides stercoralis	17 (18.9)	5 (5.6)
Protozoan		
Entamoeba histolytica	19 (21.1)	14 (15.6)
Entamoeba coli	12 (13)	11 (12)
Giardia lamblia	16 (17.7)	5 (5.6)
Iodamoeba beutschlii	7 (7.8)	5 (5.6)
Coccidian		
Cryptosporidium spp	29 (32.2)	8 (8.9)
Cyclospora spp	16 (17.7)	10 (11.1)
Isospora spp	10 (11.1)	2 (2.2)

Table 2: Risk of acquisition of some parasites in HIV seropositive patients

Parasite	OR	95%CI	χ^2	*P*-value
Hookworm	0.92	0.53-1.58	0.11	0.75
Ascaris lumbricoidis	0.84	0.51-1.65	0.09	0.78
Trichuris trichiura	1.12	0.72-1.75	0.23	0.62
Strongyloides stercoralis	3.96	1.3-13.0	6.3	0.01
Entamoeba histolytica	1.7	0.7-4.0	1.3	0.2
Giardia lamblia	3.7	1.2-12.1	5.4	0.02
Cryptosporidium spp	4.9	2.0-12.5	13.5	0.0002
Cyclospora spp	1.7	0.7-4.4	1.1	0.3
Isospora belli	5.5	1-37.5	4.4	0.02

OR= odd ratio, 95% CI=confidence interval, χ^2= chi square

Table 3: Percentage distribution of some parasites by the patients' CD4+ counts

CD4+ cells counts	*Strongyloides stercoralis*	*Cryptosporidium* spp	*Giardial lamblia*	*Cyclospora* spp	*Isospora belli*
$\leq$ 200	6(53)	18(62)	9(56.3	10(62.5)	2(50)
201-499	3(17.6)	6(20.7)	3(18.7	4(25)	3(30)
$\geq$ 500	3(17.6)	2(7)	1(6.3)	0(0)	0(0)
Not done	2(11.8)	3(10.3)	3(18.7	2(12.5)	2(20)
Total	17(100)	29(100)	16(100)	16(100)	10(100)

Not done = CD4+ cells count was not done

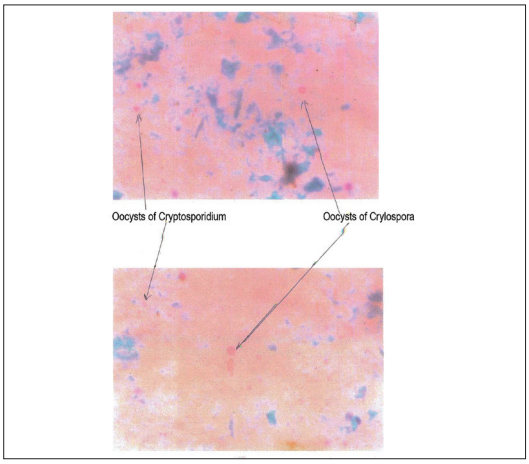

Figure 1: Ziel-Neelsen stained stool smear of oocysts of *Cryptosporidium* species and oocysts of *Cyclospora* species

isolated from their stool; Figure 1 while 5 patients; 19.3% had three and 3 patients; 11.5% had four different parasites in their stool, Table 4.

Discussion

There was a high prevalence of both the helminthic and the protozoan intestinal parasitic infestation in both the HIV-seropositive cases and their seronegative controls with a higher propensity among the later group. The prevalence rate was 87.8% among the HIV sero-positives and 74% among the sero-negative controls. This observation underscores the earlier documented[5,16,17] endemicity of intestinal parasites in the developing parts of the world.[18-20] This has been attributed to a poor environmental sanitation practice and personal hygiene[21] of the people. However, occupational hazard could also be a factor especially among the farmers who have high rate contact with contaminated soil and even the traders who are fond of moistening their fingers with saliva when counting money.[22]

The prevalence of intestinal parasites in HIV seropositive patients was twice that of the seronegative controls. This, however, differs for different parasites. The rate was four times higher for helminthes such as *Strongyloides stercoralis* and *Giardia lamblia* while it was five to five and a halve times higher for coccidian parasites that are AIDS defining such as *Cryptoridium* spp, *Cyclospora* and *Isospora belli.* Some studies in Honduras,[23] Zambia[24] and Tanzania[25] found a higher prevalence of intracellular parasites, specifically cryptosporidium and strongyloides stercoralis in HIV positive cases similar to our observation in this report. They also recorded a higher prevalence of extracellular parasites such as Ascaris lumbricoides, necator

Table 4: Frequency of intestinal parasites detection by the patients' CD4+ counts

Number of parasites in stool	CD4+ cells count/ul		CD4+ not done	Total
	≤200	200-499 ≥500		
One	2(7.7)	17(46) 2(50)	4(17.5)	25(27.8)
Two	15(57.7)	10(27) 1(25)	10(43.5)	36(40)
Three	5(19.3)	4(10.8) 0(0)	3(13)	12(13.3)
Four	3(11.5)	2(5.4) 0(0)	3(13)	8(8.9)
None	1(3.8)	4(10.8) 1(25)	3(13)	9(10)
Total	26(100)	37(100) 4(100)	23(100)	90(100)

None = no parasite found in stool

americanus and entamoeba hystolytical among the HIV negative cases. The postulated reason for this latter observation i.e lower prevalence of luminal parasites in HIV seropositives patients compared to the HIV seronegative ones was that the colonization of the intestinal tract by parasites might have been influenced by HIV enteropathy,[26,27] thereby causing both structural and functional impairment of the gut and thus making the luminal environment unfavorable for these parasites to thrive. Increased cytokines production by Helper T cells (Th2) during HIV replication was also considered contributory to reducing parasites survival in the HIV positive patient's gut.[27] The above is the concept of missing parasites infestation in HIV/AIDS.[28]

However, studies[29-32] in some other parts of the world have found higher prevalence rate of non AIDS-defining parasites such as *Giadial lamblia*, strongyloides stercoralis and entamoeba hystolytical in HIV seropositives patients similar to our experience in this report. The possible explanation for this was that though these parasites may be non opportunistic but heavier parasites load could accumulate in HIV seropositive individuals who are severely immunocompromised and suffers from delayed clearance of these parasites.[29]

The more immune compromised these patients were, the higher would be the prevalence of these parasites in their stool. This was evident in the fact that 50-60% of the total parasites isolated were from the stool samples of the patients with $CD4^+$ cells count of < 200/ul. It was this same group of severely immunosuppressed HIV sero-positive patients that had two to four parasites of different species in their stool specimens. This observation perhaps highlights the importance of T-cell mediated immunity in the host defense against intestinal parasites.[3,4,7,32-34] Depressed hosts' humoral immunity also have a significant contributory role in this aspect with special regards to increased susceptibility to *Giardial lamblia*. This has been known to occur at a far advanced state of immunosuppression[32]

which was in tune with our own experience too. Another important observation in this report that is consistent with other authors' experience[9,20,35] was the higher frequency of diarrhea stool in those with lower $CD4^+$ counts. Apart from the effects of HIV enteropathy, the presence of intestinal parasites has been known to contribute to this phenomenon[3,4,7] which on the longer term could result to the wasting syndrome called slim disease.

In the course of this study, there were some limitations that we need to highlight and these included the use of the light microscope in the laboratory diagnosis of the intestinal parasite which is not as sensitive as the modern method such as polymerase chain reaction. This facility is currently not available in our center. Secondly, only a single stool sample was collected from each of the participating patients and this could have affected the yield of the parasites since a multiple stool specimen examination has been recommended for a better parasites yield since oocysts excretion could be low and even variable and trophozoites could easily be killed. Thirdly, we were unable to differentiate between species of different parasites such *Entamoeba hystolytical* fromn *Entamoeba dispar* and between different species of *Cryptosporidium* and even *Cyclospora* because of the dearth of sensitive parasites detection techniques such as the Polymerase chain reaction technique, Isoenzyme analysis and Antigen detection techniques.

Conclusion

It is evident from this report that intestinal parasites were highly prevalent among the HIV infected patients. This was particularly so in patients with very low $CD4^+$ cells count.

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