Original Article

Enteric parasitic infections in HIV-infected patients with low CD4 counts in Toto, Nigeria

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ABSTRACT

Objectives: Enteric parasites are a major cause of diarrhoea in HIV/AIDS patients with low CD4 counts. Parasitic infections in HIV-infected individuals can reduce their quality of life and life span, especially those who are severely immunosuppressed with a CD4 T-lymphocyte count < 200cells/µl. This study presents CD4 counts and enteric parasitic infections in HIV-infected individuals.

Methodology: A total of 480 subjects were recruited at the General Hospital, Nasssarawa Toto, Nigeria. Stool samples collected were analyzed macroscopically and microscopically for consistency and the presence of enteric parasites; while the accompanying CD4 values were enumerated using the coulter manual CD4 count method.

Results: The overall prevalence rate of enteroparasites was 24% (115/480). The parasites detected were *Hookworm* (6.5%), *Entamoeba histolytica* (4.4%), *Schistosoma mansoni* (2.9%), *Giardia lamblia* (2.5%), *Entamoeba coli* (2.3%), *Isospora belli (1.7%), Strongyloides stercoralis* (1.0%), *Faciola spp.* (1.0%), *Dipylidium caninum* (0.6%), *Cyclospora spp.* (0.4%), *Ascaris lumbricoides* (0.4%), and *Enterobius vermicularis* (0.2%). Most (80%) of the patients infected with these parasites had a CD4 count of less than 200 cells/µl. Similarly, majority (65%) of patients with diarrhoea had a CD4 count of less than 200 cells/µl. Patients with CD4 counts of 201 cells/µl and above were less affected. However, these differences were not statistically significant ($p>0.05$).

Conclusions: Low CD4 counts in HIV-infected patients can lead to enteric infections. This information strengthens the importance of monitoring CD4 counts and intestinal parasites. Routine CD4 testing will greatly improve the prognosis of HIV positive patients.

KEY WORDS: CD4 counts, diarrhoea, enteric parasitic infections, Acquired Immunodeficiency Syndrome.

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INTRODUCTION

Enteric parasitic infections can affect both the quality of life and life-span of HIV-infected individuals. These infections are widespread and about 25% of the world's population is chronically infected.[1] They could be higher in developing countries such as in Africa because of the higher prevalence of enteric infections in the general population.[1] Diarrhoea is a common clinical presentation of gastrointestinal infections in HIV/AIDS patients.[2] It occurs in about 30-60% of AIDS

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cases in developed countries and in about 90% of AIDS patients in developing countries.[2]

Generally, diarrhoea in humans is caused by enteric pathogens such as bacteria, parasites, fungi and viruses.[3] Parasites such as Cryptosporidium, Isospora, Microsporidia, Giardia, Entamoeba, Cyclospora, Blastocystis, Strongyloides, and Ascaris have been documented in AIDS patients.[4] These infections may be very severe in immunosuppressed patients with CD4 T-lymphocyte counts below 200 cells/µl. They cause chronic diarrhoea, accompanied by weight loss.[2]

In Nigeria, about 2.6-3.2 million adults (+15 years) were living with HIV/AIDS by the end of 2009.[5] In the same year, UNAIDS estimated that approximately 270,000 adults were newly infected with HIV. Furthermore, an estimate of between 130,000–260,000 AIDS-related deaths were recorded in adults and children in 2009.[5]

Some studies[4,6] have reported the prevalence of enteric parasites among HIV-infected individuals in Nigeria (28.4%) and other African countries such as Ethiopia (62.5%). However, most of these studies did not make a link between the reported prevalence of these enteric parasites and immunosuppression (low CD4 counts). The main aim of this study therefore was to study the prevalence of parasitic infections in HIV-positive individuals, with a low CD4 count. Such information would inform public health policy in designing intervention strategies in reducing intestinal parasites among HIV-positive persons and the general population.

METHODOLOGY

A total of 480 participants were recruited from among outpatients in the Toto General Hospital, Nigeria. Toto town is the headquarters of the Toto Local Government Area, in Nassarawa state, Nigeria. It has an area of about 2,903 kilometres squared and a population of about 119,077 at the 2006 census.[7] Upon taking their vital signs by the nurses, patients were briefed on the nature of the study and referred to the Laboratory. At the laboratory, further explanations by the research team regarding the nature and aim of the study was provided. Participation was voluntary and patients were assured of anonymity for their laboratory testing.

Samples were collected between October 2005 and March 2006. 2ml of venous blood was collected by a trained phlebotomist from a total of 480 consenting participants. Blood samples were screened for the presence of HIV antibody using the Smart Check HIV ½.[8] Reactive samples were confirmed using the Western blot assay.[4] CD4 counts were determined manually for all study participants.[9] In this method, a CD4 monoclonal antibody (MAb) was used. The MAb reagent was used to manually enumerate the absolute CD4 count by visible light microscopy. Briefly, 15ul of MAb reagent was added to 50 ul of EDTA blood in a test tube and mixed gently for 5 minutes. Then, 15ul of a blood-latex particle mixture was pipetted into another tube containing 50ul of Turk's solution and mixed gently for for 10 to 15 seconds. The sample was loaded into a hemocytometer chamber and then observed under the light microscope. Cells having three or more latex particles attached to them (rosettes) were counted as CD4+ lymphocytes.

About 2g of stool samples were also collected from all the participants using sterile universal leak-proof bottles. They were examined macroscopically and microscopically. Macroscopically, they were examined for colour, consistency, blood stains (diarrhoea) and worms or their segments. Microscopically, they were examined for the presence of various amoebae, flagellates and ciliates. The formol-ether concentration technique was specifically done for the identification of amoebae such as Entamoeba histolytica, Entamoeba coli, and Giardia lamblia. Briefly, 1g of faeces was mixed with 10% formol saline, treated with ether and centrifuged. Smears were made from the pellets, air-dried, fixed with methanol and examined under the microscope.[10] The modified Ziehl Neelsen (Z-N) staining method was used for the identification of Euccocidians such as species of Cyclospora and Isospora.

Permission for this study was obtained from Toto General Hospital, Nigeria. Informed consent was obtained from all adult (18 years and above) study participants before their enrolment in the study. For participants below 18 years, consent was obtained from them after permission from their parents or guardians; while for children below seven years of age, permission was obtained from their parents or guardians. The Ethical Committee of the Research and Publication Unit of the University of Abuja approved the study design and research.

Demographic and other data were analyzed using Statistical Package for Social Sciences (SPSS version 17.0; Chicago, USA). Significant differences between categorical variables were compared using Chi-square. A p-value of < 0.05 was considered statistically significant.

RESULTS

The mean age of the study participants was 32.68 years (range 3–70 years). The proportion of women

Table–I: The relationship between gender, parasitic infection and diarrhoea.

Sex	*Total tested*	*Infected with parasites*	*Not infected*	*Had diarrhoea*	*No diarrhoea*
Female	313	72 (63.0%)	124 (34.0%)	64 (36.4%)	103 (33.9%)
Male	167	43 (37.0%)	241 (64.0%)	112 (63.6%)	201 (66.1%)
Total	480	480		480	

X^2 =2.0, df=1, p=0.157

and men was 65.2% and 34.8%, respectively. The mean CD4 count of the subjects was 189.66 (interquartile range 81.50-258.75) cells/µl.

The overall prevalence rate of intestinal parasites was 24% (115/480). Diarrhoea was observed in 36.6% (176/480) of the participants. The rate of parasite infection in males and females was 37.0% and 63.0%, respectively. Infection was accompanied with diarrhoea in 63.6% males and 36.4% females. However, there was no significant difference (χ^2=2.0, df=1, p=0.157) between males and females in the rate of parasitic infections and the occurrence of diarrhoea (Table-I).

Most (80%) of the patients infected with parasites had a CD4 count of less than 200 cells/µl. Similarly, majority (65%) of patients with diarrhoea also had a CD4 count of less than 200 cells/µl. Patients with CD4 counts of 201 cells/µl and above were less affected (Table-II). However, this difference was not statistically significant (p>0.05).

The parasites detected were Hookworm (6.5%), Entamoeba histolytica (4.4%), Schistosoma mansoni (2.9%), Giardia lamblia (2.5%), Entamoeba coli (2.3%), Isospora belli (1.7%), Strongyloides stercoralis (1.0%), Faciola spp. (1.0%), Dipylidium caninum (0.6%), Cyclospora spp. (0.4%), Ascaris lumbricoides (0.4%), and Enterobius vermicularis (0.2%) (Table-III).

DISCUSSION

In this era of HIV/AIDS, parasitic diarrhoea has gained tremendous importance because it is one of the leading causes of morbidity and mortality.[11] Diarrhoea can be fatal in this vulnerable group of individuals. Parasitic infections particularly helminthes cause chronic immune activation, promoting HIV infection and disease progression.[12,13]

The presence of diarrhoea and infections in HIV-infected patients with low CD4 counts can be attributed to primary HIV enteropathy and the associated reduced mucosal immunity. Similar findings have been reported in some emerging developing countries such as India.[10,13] Enteric parasitic infections are generally less common in patients with high CD4 counts as a result of several factors. Effective treatment with Highly Active Antiretroviral Therapy (HAART) is accompanied with an increase in the CD4 levels. The accompanying influx of CD4 positive cells into the lamina propria can help eradicate opportunistic infections.[14]

Parasitic infections detected in this study have been reported among HIV/AIDS patients in Sub-Saharan African countries and elsewhere. For instance, a study by Mariam et al found 11.5% of Strongyloides stercoralis in HIV/AIDS patients in Ethiopia.[4] A research conducted on intestinal parasitic infections in HIV/AIDS patients at a teaching hospital in central Brazil by Silva et al[15], shows that HIV-positive patients were more infected with

Table-II: The relationship between CD4 count, infection with parasites and diarrhoea.

Total No. of patients	*CD4 Count (cells/µl)*	
	0 – 200 cells/ul	201 cells/ul and above
Infected with parasites (n=115)	92 (80%)	23 (20%)
Patients with diarrhoea (n=176)	115 (65%)	61 (35%)

X^2 = 2.0, df=1, p>0.05

Table-III: Association between parasites detected and diarrhoea.

Parasites detected	*Number of patients*		
	Non-diarrheic (n=304)	*Diarrheic (n=176)*	*Total detected*
Ascaris lumbricoides	0	2 (0.4%)	2 (0.4%)
Entamoeba histolytica	3 (1.0%)	18 (10.2%)	21 (4.4%)
Entamoeba coli	3 (1.0%)	8 (4.5%)	11 (2.3%)
Giardia lamblia	2 (0.7%)	10 (5.7%)	12 (2.5%)
Strongyloides stercoralis	2 (0.7%)	3 (1.7%)	5 (51.0%)
Schistosoma mansoni	4 (1.3%)	10 (5.7%)	14 (2.9%)
Isospora belli	3 (51.0%)	5 (2.8%)	8 (1.7%)
Dipylidium caninum	2 (0.7%)	1 (0.6%)	3 (0.6%)
Enterobius vermicularis	0	1 (0.6%)	1 (0.2%)
Hook worm	16 (5.3%)	15 (8.5%)	31 (6.5%)
Cyclospora spp.	0	2 (1.1%)	2 (0.4%)
Faciola spp.	2 (0.7%)	3 (1.7%)	5 (1.0%)
Uninfected	267 (87.8%)	98 (55.7%)	365 (76.0%)

X^2 = 76.7, p= 0.000, df=12

intestinal parasites than the control group. The most frequent parasites that they discovered in the HIV-positive patients were Strongyloides stercoralis, Isospora belli, Cryptosporidium spp. and Hookworm. Their findings also showed that common intestinal parasites among the control group were Strongyloides stercoralis, Gairdia lamblia and hookworm.

Some studies have also reported the presence of intestinal parasites in HIV/AIDS patients in other parts of Nigeria.[6,16-18] Akinbo et al[18] have reported the presence of Entamoeba histolytica, Giardia intestinalis, Isospora belli, Ascaris lumbricoides, Hook worm, Strongyloides stercoralis, and Trichuris trichiura among HIV patients in Benin City, Nigeria. Some of these intestinal parasites were also detected in the present study. Gender affected the presence of intestinal parasites, and a higher prevalence was observed in females than in males. The reason for the association between gender and intestinal parasites may be attributed to more males being exposed than females based on occupational grounds. This finding is also consistent with Akinbo et al.[18] Parasitic infections are of great significance in the health of patients living with HIV/AIDS. Sub-Sahara Africa is home to the greatest number of HIV infections and chronic diarrhoea due to intestinal parasites which are also common in this region.[19-21] Protozoa and many other enteric parasites have been associated with diarrhoea, and even weight loss in HIV-infected individuals.

The high rate of enteric parasitic infections in Toto town could be due to poor hygienic and sanitary conditions. Another likely reason could be the lack of potable drinking water in most communities. Providing potable drinking water, adequate health education on hygienic practices and sanitary facilities for the Nigerian population could help in curbing this problem.

We have highlighted the presence of diarrhoea, low CD4 counts and enteric parasites among HIV-infected patients in a health institution in Toto, Nigeria; of which Hookworm was the most prevalent. Routine CD4 testing alongside with other health care packages could greatly improve the management of HIV patients in resource poor settings such as in Africa.

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