Occurrence of intestinal parasites amongst persons on highly active antiretroviral drug therapy in Calabar, Cross River State, Nigeria

Paul C. Inyang-Etoh, Mfoniso I. Udonkang, Adepelumi O. Adeboboye

Department of Medical Laboratory Science, Faculty of Allied Medical Sciences, College of Medical Sciences, University of Calabar, Nigeria

Abstract

Opportunistic and intestinal parasite infections are common health problem among HIV/AIDS patients. Early detection and treatment of these parasites are important to improve the quality of life of this category of patients. The occurrence of intestinal parasites among 400 patients on highly active antiretroviral drug therapy (HAART) aged 11-60 years was investigated. Standard parasitological techniques like direct microscopy, formol ether concentration and modified Ziehl-Neelsen staining techniques were used to analyze the stool samples. Intestinal parasite infections were positive in 116 (29%) of the subjects on HAART while control subjects had 12 (12%) and the difference was statistically significant (P<0.05). Subjects in the age group 21-30 years had the highest infection rate 54 (35.1%). There was no statistically significant difference in infection according to age (P>0.05). Females 76 (32.5%) had a higher prevalence rate than males 40 (24.1%). But there was no statistically significant difference in infection according to gender (P>0.05). Patients with CD4 count of less than 200 cells/mm^3 were observed to be more infected than those with CD4 count of more than 200 cells/mm^3. There was a strong positive correlation (r=0.94) between CD4 count and the occurrence of intestinal parasite infection. Protozoan parasites 84 (21.0%) accounted for a higher prevalence rate than helminthic parasites 32 (8.0%). These findings has revealed a high prevalence of intestinal parasite infection among patients on HAART thus the routine screening of stool samples from these category of patients for intestinal parasites is advocated for effective management of the disease.

Introduction

Intestinal parasitic infections are common in Sub-Saharan Africa where the majority of Human Immune Deficiency Virus (HIV)Acquired Immunodeficiency Syndrome (AIDS) cases are concentrated.[1] One of the major health problems in patients with HIV is superimposed infections due to reduced immunity. The lack of knowledge coupled with these intestinal parasites not being included in routine diagnostic testing have unknowingly contributed to these parasites being perceived as *uncommon*.[2] In the last few years, advances in HAART have led to improvements in immunological state with a resulting reduction in the frequency of certain intestinal parasites.[3-5] Unsanitary conditions, poverty and malnutrition promote transmission of intestinal parasite infections.[6,7] Some of these parasites may disseminate to other organs such as the bronchioles, bile ducts and liver, producing symptoms specific to the organs affected.[8] Patients with CD4 counts >200 cells/mm^3 usually have self-limiting infections, whereas most patients with counts <140 cells/mm^3 develop severe and persistent infections.[9] Intestinal parasitic infections that are asymptomatic or cause self-limiting diarrhea in immunocompetent individuals can cause profuse diarrhea in immunocompromized individuals, generally accompanied by weight loss, anorexia, malabsorption, and in some cases fever and abdominal pain.[10] This study is however designed to establish the prevalence and pattern of parasitic infections, and also correlate CD4 levels with its prevalence among persons on Highly Active Antiretroviral Drug Therapy in Calabar Metropolis, Nigeria.

Materials and Methods

Study area

This study was conducted in Calabar, the state capital of Cross River State. Basically, Calabar Metropolis is made up of two local government areas (LGAs), Calabar Municipality and Calabar South LGA in Cross River State which is within the rainforest belt of Nigeria. According to 2006 National Population Census, the total population of Calabar was estimated at 371,022.[11] Geographically, Calabar is located on 4°58'34" N and 8°20'50" E. The city is bounded westward by the Calabar River, to the North is bound by Odukpani Local Government Area, the near East stretches the Great Qua River and Akpabuyo Local Government Area and to the far South are swamps, creeks and the Atlantic ocean. Calabar Metropolis is a cosmopolitan city virtually embracing all ethnic groups in Nigeria and therefore presents a good case study.[12]

Correspondence: Paul C. Inyang-Etoh, Medical Laboratory Science Department, Faculty of Allied Medical Sciences, College of Medical Sciences, University of Calabar, P. M. B. 1278, Calabar, Cross River State, Nigeria.
Tel.: +234.803.723.7567.
E-mail: inyangetoh@yahoo.com

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Contributions: PCI, conception and design of the research work, arrangement and presentation of manuscript, data analysis and interpretation, final approval of manuscript; MIU, collection and examination of stool sample (direct smear and concentration method), modified technique for stool examination; AOA, collection and examination of stool sample (direct smear and concentration method), collection of blood for HIV screening and CD4 count.

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Highly active antiretroviral drug therapy

Highly active antiretroviral drug therapy (HAART) is the name given to aggressive treatment regimens used to suppress HIV viral replication and the progression of HIV disease. The usual HAART regimen combines three or more different drugs such as two nucleoside reverse transcriptase inhibitors (NRTIs) and a protease inhibitor (PI), two NRTIs and a non-nucleoside reverse transcriptase inhibitor (NNRTI) or other such combinations. These HAART regimens have proven to reduce the amount of active virus and in some cases can

lower the number of active virus until it is undetectable by current blood testing techniques.

Study population

The study population were unselected 400 subjects (116 males and 134 females) aged 11-60 years attending the HIV Clinic at the University of Calabar Teaching Hospital (UCTH) and the State General Hospital which are both located in Calabar Metropolis. Moreover 100 HIV negative (healthy) subjects (40 male and 60 female) were used as controls.

Ethical approval and informed consent

Subjects for the study were enrolled after due approval from the Ethical Research Committee of the Cross River State Ministry of Health. Patients or Guardians were made to sign a consent form before enrollment.

Collection of samples

A total of 400 samples were collected into clean, leak-proof, wide-mouthed universal container issued to each of the patients. The samples were then transported to the parasitology laboratory in University of Calabar Teaching Hospital (UCTH) as soon as possible for parasitological analysis. Samples that were not analyzed immediately were preserved in 10% formol-saline. Blood samples were also collected from same number of patients for confirmation of HIV status and CD4 count.

Laboratory diagnosis

Macroscopic examination

Samples were examined macroscopically in the laboratory noting their color, consistency and the presence of blood, mucus, adult worm or segmented worms.

Direct microscopic examination

A peanut size of the stool sample was collected from the sample container and homogenized with a few drops of normal saline. An aliquot of the homogenate was used in making the direct smear. On a separate slide, a drop of the homogenized sample was placed at the center of the two slides. A drop of saline was added to the first slide, and also a drop of iodine to the other slide. A coverslip was then placed over each sample on the side. The preparation was then examined microscopically using 10× objective, and a confirmation of any identified parasite was done with the 40× objective.

Formol-ether concentration technique

This was performed according to the methods.[13] Half a teaspoonful of stool was thoroughly mixed in 10 mL of water and strained via two layers of gauze in a funnel; the filtrate was centrifuged at 2000 rpm for 2 minutes. The supernatant was discarded and the sediment resuspended in 10 mL of normal saline. It was again centrifuged and the supernatant discarded. The sediment was then resuspended in 7 mL formol saline and allowed to stand for 10 minutes for fixation. To this, 3 mL of ether was added. The tube was stoppered and shaken vigorously to mix. The stopper was then removed and the tube centrifuged at 2000 rpm for 2 minutes. The tube was allowed to stand for two minutes. Four layers became visible, the top layer comprised ether, second was a plug of debris, and the third was a clear layer of formal saline, while the fourth had the sediment. The plug of debris was detached from the side with the aid of a glass rod and the liquid poured off leaving a small amount of formal saline for resuspension of the sediment. The sediment was then poured on a clean glass slide, covered with a coverslip and examined microscopically.

Identification of parasite species

The microscopic examination of direct smear and stool deposits after an iodine stained, formol-ether concentration method revealed the characteristic ova of nematodes. *Ancylostoma duodenale* was differentiated from *Necator americanus* by its buccal cavity which bears two hook like teeth on the top and two triangular cutting plates on the bottom while the mouth of *N. americanus* has four cutting plates, two on the ventral and two on the dorsal surfaces.

Special technique

This consists of the modified Ziehl-Neelsen staining technique. It is modified, because this technique does not require heating as compared to the conventional Ziehl-Neelsen staining procedure. It is used specifically for the identification of coccidian parasites such as *Cryptosporidium parvum, Isospora belli* and *Cyclospora species* in stool samples.

Procedure

We proceeded as follows: i) a smear was prepared from the sediment obtained by the formal ether concentration technique. It was air-dried and fixed with methanol for 2 minutes. ii) It was then stained with unheated carbol fuchsin for 15 minutes, and washed off the stain with water. iii) The smear was decolorized with 1% acid alcohol for 10seconds, and washed off with water. iv) It was then counterstained with 0.5% malachite green for 30 seconds, washed off with water and the slide was allowed to stand in a draining rack for the smear to dry. v) It was examined microscopically using a lower power magnification to detect oocysts and the oil immersion objective to identify them.

HIV screening test

HIV screening was done using the serial algorithm of screening with determine and confirmed result with Unigold.

Alere determine HIV1/2 is an immune-chronmatographically test for the qualitative detection of antibodies to HIV-1 and HIV-2.

HIV confirmatory test using unigold

All the test samples that were positive with determine were confirmed with a second test using Uni-Gold. For testing, two drops of whole blood from the pricked finger were allowed to fall into the sample port, followed by two drops of wash buffer and allowed to react. Antibodies of HIV-1 or HIV-2 proteins were bound to the colloidal gold linked antigens. The antibody protein colloidal gold complex moves chromatographically along the membrane to the test and control regions of the test device. A positive reaction is visualized by a pink band in the test region of the device and in the control line. A negative reaction occurs in the absence of human immunoglobin antibodies to HIV in the analyzed specimen. Consequently no visually detectable band develops in the test region of the device.

Analysis of whole blood for CD4 cell count

CD_4 count was analyzed using Partec CyFlow counter made in Germany. Briefly, 20 mL fresh whole EDTA blood was added to 20 mL CD_4/MAb in a test tube and incubated in the dark at room temperature for 15 minutes, and 800 mL of buffer was added, connected to the CyFlow and allowed to run. Automatically generated report of the blood sample becomes displayed on the screen of the machine.

Data analysis

Difference in proportion was evaluated using the Chi-square test. Pearson's correlation co-efficient was used to determine the correlation of the variables. Statistical significance difference was achieved if $P<0.05$.

Results

The results of the study on the intestinal parasite load among 400 patients on HAART aged 11-60 years is presented below. Table 1 shows the prevalence of intestinal parasites among patients on antiretroviral drug therapy according to age. Out of a total of 400 HIV seropositive individuals on antiretroviral drug therapy who participated in the study, 116 (29%) were positive for intestinal parasite.

Subjects aged 21-30 had the highest prevalence rate (35.1%) while those aged 11-20 years had the lowest prevalence rate of (18.2%). There was no statistically significant difference in infection according to age (χ^2 =7.51 df(4) P>0.05). The distribution of intestinal parasites among patients on antiretroviral drug therapy according to their CD4 cell counts is shown on Table 2. Protozoan parasites 84 (21.0%) accounted for a higher prevalence rate than helminthic parasite infections 32 (8.0%).

Figure 1 shows the gender effect on the prevalence of intestinal parasites in patients on antiretroviral drug therapy. Females 76 (32.5%) had a higher prevalence rate than males 40 (24.1%). But there was no statistically significant difference in infection according to gender (χ^2 =5.2 df(1) P>0.05).

Figure 2 shows the distribution of intestinal parasites among patients on antiretroviral drug therapy according to species. Patients with CD4 count of less than 200 cells/mm^3 were observed to be more infected than those with CD4 count of more than 200 cells/mm^3. There was a strong positive correlation (r=0.94) between CD4 count and the occurrence of intestinal parasite infection.

Discussion and Conclusions

Intestinal parasite infection has been one of the major contributing factors to the morbidity and mortality of HIV/AIDS patients in most developing countries where HIV/AIDS cases are common. With the advent of HAART, HIV-associated morbidity and mortality has dramatically decreased in many high-income countries. This study was an attempt to establish the enormity of the prevalence of intestinal parasites among patients on HAART Calabar, Cross River state.

The 29% prevalence rate in intestinal parasite infection observed in this study is higher than 8.7% earlier reported by Etok *et al.*,[14] at the General Hospital, Calabar and 17.6% reported by Missaye *et al.*,[15] in South Wollo zone of Amhara region state, North-East Ethiopia. The reason for the high prevalence rate (29%) of intestinal parasites infection in the study area may be due to the increase in influx of tourist and also the corresponding increase in the number of this category of patients from the rural areas and the associated poor nutrition and sanitary conditions of the people from rural settings. However, this value is

Table 1. the prevalence of intestinal parasites among patients on antiretroviral drug therapy according to age group.

Age range (years)	Test subjects		Control subjects	
	Examined	Positive for parasites (%)	Examined	Positive for parasites (%)
11-20	22	4 (18.2)	5	1 (20)
21-30	154	54 (35.1)	38	5 (13.2)
31-40	146	36 (24.7)	36	4 (11.1)
41-50	64	18 (28.1)	18	2 (11.1)
51-60	14	4 (28.6)	3	0 (0.0)
Total	400	116 (29)	100	12 (12.0)

Table 2. Distribution of intestinal parasites among patients on antiretroviral drug therapy according to their CD4 cell counts (N=400)

Parasites	CD4 count (cells/mm^3)		
	<200 (n=122)	201-400 (n=196)	>400 (n=82)
Protozoa			
Giardia intestinalis	4 (3.3%)	0 (0%)	0 (0%)
Entamoeba histolytica/dispar	10 (8.2%)	24 (12.2%)	6 (7.3%)
Cryptosporodium sp	32 (26.2%)	6 (3.1%)	0 (0%)
Isospora belli	2 (1.6%)	0 (0%)	0 (0%)
Total	48 (39.3%)	30 (15.3%)	6 (7.3)
Helminthes			
Trichuris trichiura	6 (4.9%)	0 (0%)	0 (0%)
Ascaris lumbricoides	2 (1.6%)	14 (7.1%)	0 (0%)
Ancylostoma duodenale	2 (1.6%)	6 (3.1%)	0 (0%)
Strongyloides sp	2 (1.6%)	0 (0%)	0 (0%)
Total	12 (19.5%)	20 (10.2%)	0 (0%)
Grand total	60 (49.0%)	50 (25.5%)	6 (7.3%)

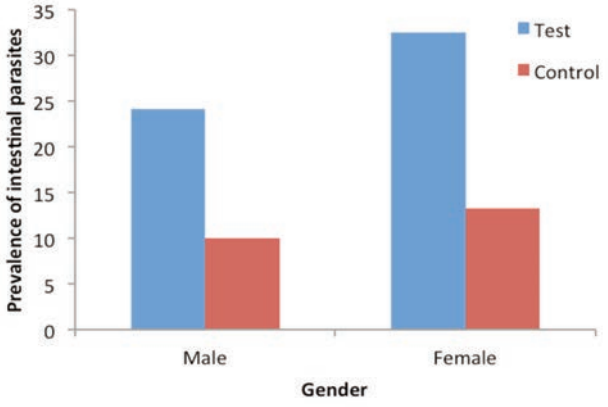

Figure 1. The gender effect of prevalence of intestinal parasites in patients on antiretroviral drug.

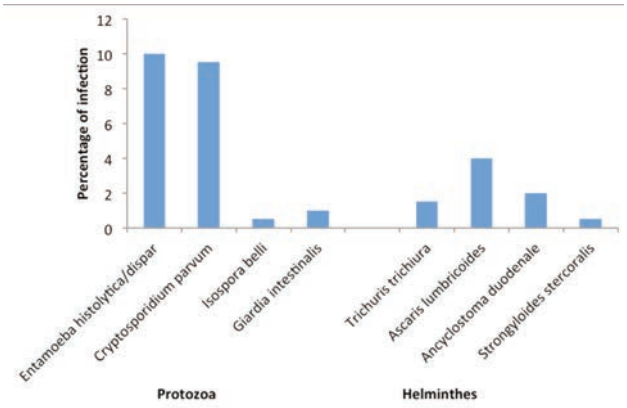

Figure 2. Distribution of intestinal parasites among patients on antiretroviral drug.

lower than the 59.5% reported by Nkenfou *et al*.,[16] in Dischang, Cameroon and 48% reported by Adamu and Petros, in Adama, Afar and Dire-Dana in Ethiopia.[17] In other parts of Nigeria, results on similar studies have been reported. Abaver *et al.,*[18] reported 24.7% in Abuja, Abaver *et al*.,[19] reported 24% in Nasarrawa, Sanyaolu *et al*.,[20] reported 33.8% in Lagos, Ibrahim *et al*.,[21] reported 61.4% in Jos and Inabo *et al*.,[22] reported 70.6% in Zaria.

Young adults aged 21-30 years had the highest number of participants (154) and in turn the highest prevalence of parasitic infection (35.1%). This depicts the high sexual activity and vulnerability, economic dependence and occupational exposure among this age group in the study area. This is in tandem with the report from NACA, that young Nigerian adults aged 15-24 accounted for most of the HIV prevalence in 2010.[23] Female participants (32.5%) were observed to be more infected than the males (24.1%) but there was no significant difference in the presence of infection according to gender (P>0.05). This result is in tandem with that reported by Inabo *et al*., and Etok *et al.*, who had 78.6% 10.3% infection rate in female participants respectively.[14,22] Women have been reported to be the most vulnerable group and in Nigeria they were reported to have accounted for 170,431 new HIV infections in 2011.[23] Cultural factors and education were said to be a contributing factor.

Protozoan infections have remained a threat to the health of HIV/AIDS patients. In this study, protozoan infection (21.0%) was more prevalent than helminthes (8.0%). This finding is similar to what was reported in Zaria by Inabo *et al*.,[22] This study also correlate the prevalence of intestinal parasites of the participants with their CD4 counts. The prevalence of intestinal parasites of 15.0% was observed to be corresponding with low CD4 counts (<200), 12.5% was observed in the moderate CD4 counts (201-400) and 1.5% in high CD4 counts (>400).

Protozoa was the most common parasite infection among subjects in the low CD4 counts group. Protozoan infection was observed to be 12.0% while 3.0% was for helminthes infection in the low immunity group. Opportunistic parasite infections were observed to be more common in the low immunity group (8.5%) as compared with 1.5% in the moderate immunity group. This result shows that though the patients are placed on HAART, reduction in the CD4 count of <200 results in susceptibility to opportunistic infections, which took advantage of deficient cell-mediated and humoral defense mechanisms as well as the limitation of HAART to curb secondary infections. Also, the absence of opportunistic intestinal infections in the high immunity group indicates that there was no breakdown in immunity to allow the establishment of these parasites.

Crytosporidium spp had a higher prevalence (27.6%) in the low immunity group. This is lower than that reported by Adamu and Petros, and Malaji *et al.,* who reported 62.5% and 54% in Ethiopia and India respectively.[17,24]

This study has shown that patients on HAART are vastly infected with intestinal parasite infection and the need to put in place, a process of routine screening and treatment for intestinal parasites among this category of patients for improved management of their ailment.

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