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ORIGINAL ARTICLE

OPPORTUNISTIC INTESTINAL PROTOZOAN PARASITES AMONG HIV POSITIVE PATIENTS ON ANTIRETROVIRAL THERAPY AT NEKEMTE HOSPITAL, WEST ETHIOPIA

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ABSTRACT

Background and aim: *Opportunistic protozoan infections are among the most serious infections in patients with AIDS. The aim of this study was to assess the impact of ART on opportunistic intestinal protozoan infections in HIV/AIDS patients.*
Patients and Methods: *HIV/AIDS patients that had not begun ART and those that were on-ART at Nekemte Hospital, West Ethiopia were included. Stool specimens were collected from 296 HIV/AIDS patients (94 pre-ART and 202 on-ART) and processed using modified Ziehl-Neelsen staining for Cryptosporidium parvum and Isospora belli and Uvitex-2B staining method for microsporidia. CD4+ T-cell count was determined by using FACS analysis or flow cytometry.*
Results: *Out of the 296 study participants examined, 41 (13.9 %) and 14 (4.7 %) were positive for intestinal cryptosporidiosis and isosporiosis, respectively. Enterocytozoon bieneusi was detected in 7(4.8%) of the 145 participants examined. C. parvum 17(21.3%), I. belli 8(10%) and microsporidia 5(11.6%) were more common in pre-ART diarrhoeal patients than those on-ART. The prevalence of cryptosporidiosis, isosporiosis and microsporidiosis among pre-ART study participants was significantly higher than those on-ART ($P<0.05$). A large majority of diarrhoeal AIDS patients infected with intestinal cryptosporidiosis and isosporiosis were in stage III of the WHO HIV/AIDS staging. An increase in CD4+ T-cell count was observed with increase in the number of months of ART.*
Conclusion: *The results of this study show that intervention with HAART decreases the prevalence of opportunistic intestinal protozoan parasites by improving the immune status of the patients and hence would enhance the health and well-being of HIV/AIDS patients under the Ethiopian nutritional and health service system.*
Keywords: *Antiretroviral Therapy, CD4+ T-cells, Human Immunodeficiency Virus, Opportunistic intestinal protozoan parasite.*

INTRODUCTION

Opportunistic protozoan infections are among the most serious types of infection in patients with AIDS. They cause severe morbidity and mortality (1). HIV infection has been shown to predispose the patient to intracellular opportunistic intestinal protozoan infections such as *Cryptosporidium parvum*, *Isospora belli*, *Cyclospora cayetanensis*, *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis* (2).

Opportunistic intestinal protozoan infections are increasingly becoming prevalent in AIDS patients (3). Almost 80% of AIDS patients die of AIDS-related infections including intestinal parasites rather than of the HIV infection itself (4). Infections usually occur late in the course of HIV infection when $CD4^+$ T-cell count has been severely depleted mostly below 200 cells /mm (3,5).

In AIDS patients with diarrhoea, the prevalence of *C. parvum* ranges from 10% to 30% in developed countries and 30% to 50% in the developing world (6). In Ethiopia, the prevalence of cryptosporidiosis in HIV/AIDS patients was reported as 40% (7) and 25.9% (8), indicating its public health significance.

In the absence of effective and specific therapy against infection with *Cryptosporidium*, preventive measures are of great importance.

I. belli infections are essentially cosmopolitan in distribution but are more common in tropical and subtropical regions. In Ethiopia, the prevalence of *C. parvum*, *I. belli* and *Cyclospora cayetanensis* reported in ADIS patients with chronic diarrhoea was 11%, 7.4% and 3.7%, respectively (9). A 10.26% prevalence of double infection with *C. parvum* and

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I. belli in ADIS patients with diarrhoea has also been reported (10).

Human infections with microsporidia have been reported from all over the world, and the majority of cases have involved HIV infected patients (11). In Ethiopia, intestinal microsporidiosis due to *E. bieneusi* (17.2%) (12) and *E. intestinalis* (22.5%) (13), were reported to be highly associated with chronic diarrhoea in AIDS patients. In addition, a recent study showed 18.2% (10) prevalence of microsporidiosis in diarrhoeal HIV/AIDS patients. Successful treatment of microsporidiosis in immunodeficient patients is limited and no effective therapy is known(11)

HIV infects the T helper cells because it has the protein CD4 on its surface which HIV uses to attach itself to the cell before gaining entry (14). CD_4^+ T-cell depletion is the hallmark of the deteriorating immune system (15). In Ethiopia, the normal CD_4^+ T-cells count in HIV-negative individuals ranges from 366-1235/mm (3,16), while in HIV-positive stage-I individuals it ranges from 89-966/mm (17). The immune system, which has been trying to battle the HIV infection, eventually weakens and this will make the person more susceptible to opportunistic intestinal parasites.

The classification of the HIV disease can be undertaken for several purposes and should be distinguished from disease staging. Staging is disease classification that aims primarily to make groupings that have different prognoses which can be used in guiding treatment decisions (18).

Antiretroviral drugs are usually designed to inhibit viral proteins which have a role in the replication of HIV. They significantly delay the progression of HIV to AIDS and allow people living with HIV to live relatively normal and healthy lives.

The center for Disease Control recommended starting HAART if there is history of opportunistic infections or other severe HIV disease, or if the CD4+ T-cell count is less than 200/mm (19).

Since the introduction of HAART in 1996, the mortality and morbidity for a wide variety of opportunistic viral, bacterial, fungal and parasitic infections have decreased dramatically among HIV-infected individuals in economically developed countries (20,21), hence improving the quality of life in AIDS patients. In Ethiopia, ART use was started very recently despite a higher infection rate in the country. Despite formidable logistical handles, the number of individuals able to access this treatment in Ethiopia is expanding and the overall situation has been improving (22). The aim of this study was to assess the impact of ART on opportunistic intestinal protozoan infections in HIV/AIDS patients.

METHODS

Study design, area and subjects: A cross sectional study was conducted from June to December 2006 at Nekemte Hospital, East Wollega Zone of Oromia Regional State, West Ethiopia, involving HIV seropostive patients who came to the hospital. The patients were either on ART or eligible for it. All patients in ART clinic during the study period and willing to participate in the study were included. Informed consent was obtained from the patients or families for children and very sick patients which was approved by the hospital administration. Ethical approval was obtained from the Ethical Committee of Addis Ababa University, Department of Biology.

***Stool collection and pr*ocessing**: A single fresh stool was collected with a labeled stool cup from the participants following standard procedures. A portion of the stool was preserved with SAF (15g sodium acetate, 20ml glacial acetic acid, 40ml formalin and 925ml distilled water) in a proportion of 1g of stool in 3ml of SAF and was transported to the Parasitological Laboratory of the Ethiopian Health and Nutrition Research Institute (EHNRI).

Modified Ziehl-Neelsen method: For the detection of oocysts of *C. parvum* and *I. belli*, the modified Ziehl-Neelsen method was followed. In this method, a thin smear was prepared directly from fresh as well as from sediments of concentrated stool and allowed to air dry. Then the slides were fixed with methanol for 5 minutes and stained with carbolfuchsine for 30 minutes. The slides were then washed with tap water and decolorized with acid alcohol (1ml HCl and 99ml of 96% ethanol) for 1-3 minutes. The slides were washed in tap water and counter-stained in methylene blue for another 1 minute. Finally, they were washed in tap water and allowed to air dry. The slides were then examined under a light microscope with X1000 magnification. Each slide was examined for 10 minutes to decide whether it was negative or positive.

Uvitex-2B staining method: For the identification of microsporidial spores, a portion of each sample from a preserved stool was processed by a water-ether

sedimentation method and stained with Uvitex-2B at the Ethiopian Health and Nutrition Research Institute. In brief, one gm of fresh stool was mixed thoroughly with 8ml of distilled water in a 15ml conical test tube. After sieving it with cotton gauze, 3ml ether was added and the mixture shaken for one minute and centrifuged at 2000g for 2 minutes. From the sediment, thin smears were prepared on a microscope slide and were allowed to air-dry. The slides were then fixed with methanol for 2 minutes, allowed to air-dry and finally stained with Uvitex-2B (Ciba, Giegy, and Basel, Switzerland) for 10 minutes. The slides were then washed in PBS (8.0gm NaCl, 0.2KCL, 1.44gm Na_2HPO_4, 0.24gm KH_2PO_4, PH=7.2) for 5 seconds, counter-stained with Evans blue (Sigma) for 30 seconds, then washed in PBS for 5 seconds, and finally gently rinsed in running tap water. The slides were observed under fluorescent microscope with 50-W mercury high pressure lamp fitted with excitation filter 355-425nm and a suppression filter of 460nm (Leitz, Ploemopak Filter Block D, Germany) at a magnification of x1000. Each slide was examined for about 10 minutes to decide whether it was positive or negative.

Blood collection and CD4+ cell count: Venous blood was collected and the CD4+ cells were analyzed by using FACS analysis or flow cytometry (Becton Dickinson Immunocytometry system, and Jose, CA., USA). Briefly, 100µl of whole blood was mixed with 10µl of each monoclonal antibody combination in separate tubes and incubated at room temperature for 20 minutes.

Red blood cells were then lysed by adding 2ml of fluorescence activated cell sorter lysing solution (Becton Dickinson). After vortexing, tubes were incubated in the dark at room temperature for 10 minutes and centrifuged at 300xg for 5 minutes. The cell pellet was washed once with 2ml of isoton, resuspended in 500µl of isoton, and analyzed with simulset software (Becton Dickinson).

Data analysis: Data were entered and analyzed using the SPSS software version 13. Chi square test was employed to measure the strength of association. Values were considered to be statistically significant when the p-value was less than 0.05.

RESULTS

A total of 296 HIV-positive subjects were included in the study. Pre-ART subjects comprised 94 (31.8%) and the remaining 202 (68.2%) were on-ART. One hundred twenty-one (40.9%) were males while 175 (59.1%) were females. Their age range was 5-44 years. A significant majority of the study participants were in the age range of 15-24 years (Table 1).

Table 1: Age and sex distribution of study participants by ART status in Nekemte Hospital, West Ethiopia, June – December, 2006.

ART status		Sex	Age in year				Total
			<=14	15-24	25-34	35-44	
Pre-ART (n=94)		male	8 (8.5)	17 (18.1)	8 (8.5)	5 (5.3)	38 (40.4)
		female	20 (21.3)	29 (30.9)	4 (4.3)	3 (3.2)	56 (59.6)
		total	28 (29.8)	46 (48.9)	12 (12.8)	8 (8.5)	94 (100.0)
On-AR (n=202)	1-3 months (n=67)	male	7 (10.4)	8 (11.9)	15 (22.4)	1 (1.5)	31 (46.3)
		female	8(11.9)	15 (22.4)	9 (13.4)	4 (6.0)	36 (53.7)
	4-5 months (n=57)	male	0 (0.0)	6 (10.5)	15 (26.3)	2 (3.5)	23 (40.3)
		female	5 (8.8)	18 (31.6)	6 (10.5)	5 (8.8)	34 (59.7)
	6 months (n=78)	male	1 (1.3)	14 (17.9)	11 (14.1)	3 (3.8)	29 (37.2)
		female	13 (16.7)	27 (34.6)	9 (11.5)	0 (0.0)	49 (62.8)
	Total		34(16.8)	88 (43.6)	65 (32.2)	15 (7.4)	202 (100.0)

Among the 296 stool specimens examined by modified Zhiel-Neelsen method, 41 (13.9%) and 14 (4.7%) were positive for *C. parvum* and *I. belli*, respectively. Samples from 145 subjects were examined by Uvitex-2B staining method, and 7 (4.8 %) participants were found to be positive for intestinal microsporidia (Table 2). The prevalence of *C. parvum, I. belli* and microsporidia in pre-ART study participants was significantly higher than those on ART ($p<0.05$) (Table 2). The prevalence of *C. parvum* and *I. belli* decreased with an increase in duration of ART (Table 2).

Table 2: Prevalence of opportunistic intestinal protozoan parasites in HIV positive individuals by ART status in Nekemte Hospital, West Ethiopia, June - December 2006.

Parasite identified	Pre-ART (0 month) (n=94)	On-ART				Total (n=296)	P-value
		1-3 months (n=67)	4-5 months (n=57)	6months (n=78)	All on- ART (n=202)		
C. parvum	24 (25.5%)	12 (17.9 %)	3 (5.3 %)	2 (2.6 %)	17 (8.4 %)	41 (13.9%)	0.000
I. belli	11 (11.7%)	3 (4.47 %)	0 (.0%)	0 (.0%)	3 (1.5 %)	14 (4.7%)	0.000
Microsporidia	6 (7.5 %)[a]	1 (1.5%)[b]	-	-	1 (1.5 %)[c]	7 (4.8%)[d]	0.042

[a]n=80, [b]n=65, [c]n=65, [d]n=145

Eighty of the participants demonstrated diarrhea. Among them, 23 (28.8 %) and 9 (11.3%) were found positive for *C. parvum* and *I. belli*, respectively. Microsporidia were detected in 6 (13.9 %) of the 43 diarrheal samples examined (Table 3).

The prevalence of opportunistic intestinal parasites was significantly higher in pre-ART diarrheal subjects than that on-ART ($P<0.05$). In addition, a large number of opportunistic intestinal parasites was detected in diarrhoeal subjects when compared to non-diarrhoeal ones (Table3).

A large number of diarrhoeal HIV/AIDS patients infected with the opportunistic intestinal protozoal parasites was in stage III of the WHO HIV/AIDS clinical staging (Table 4).

A significant majority of the pre-ART subjects had CD4 counts of less than 200 cells/mm^3 while those on-ART had greater than 200 CD4 cells (Table 5).

Table 3: Prevalence of opportunistic intestinal protozoan parasites in HIV positive individuals by status of diearrhea and ART in Nekemte Hospital, West Ethiopia, June – December, 2006.

Diarrhoea status	Opportunistic intestinal parasites	ART status		Total	P-value
		Pre-ART	On-ART		
Diarrhoeal (n=80)	C. parvum	17 (21.3%)	6 (7.5%)	23 (28.8%)	0.010
	I. belli	8 (10%)	1 (1.3%)	9 (11.3%)	0.016
	Microsporidia[a]	5 (11.6%)	1 (2.3%)	6 (13.9%)	0.003
Non-diarrhoeal (n=216)	*C. parvum*	7 (3.2%)	11 (5.1%)	18 (8.3%)	0.139
	I. belli	3 (1.4%)	2 (.9%)	5 (2.3%)	0.062
	Microsporidia[b]	1 (1.0%)	0 (.0%)	1 (1.0%)	0.194

[a]n=43, [b]n=102

Table 4: Prevalence of opportunistic intestinal protozoan parasites in HIV positive individuals by diarrhoeal status and WHO HIV/AIDS staging in Nekemte Hospital, West Ethiopia, June - December 2006 (n=240).

Opportunistic intestinal parasites	WHO staging	Diarrhoeal status		P-value
		Diarrhoeal	Non- diarrhoeal	
C. parvum	I[a]	0 (0.0%)	1 (12.5%)	0.686
	II[b]	1 (2.3%)	4 (9.1%)	0.660
	III[c]	16 (9.2%)	7 (4.0%)	0.000
	IV[d]	3 (21.4%)	-	0.024
I. belli	III[c]	7 (4.0%)	3 (1.7%)	0.002
Microsporidia	III[e]	6 (8.0%)	-	0.002

[a]n=8, [b]n=44, [c]n=174, [d]n=14, [e]n=75

Table 5. Comparison of CD4+ T-cell counts in HIV positive individuals by ART status in Nekemte Hospital, West Ethiopia, June - December 2006.

CD4+cell count/mm^3	pre-ART (n = 94) **(0 months)** n (%)	on-ART(n = 202) **(6 months)** n (%)	P-value
<200	87 (92.6%)	53 (26.2%)	0.000
200-499	5(5.3%)	127 (62.9%)	0.000
>=500	2 (2.1%)	22(10.9%)	0.002

DISCUSSIONS

The results of this study showed a 13.9%, 4.7%, and 4.8% prevalence of intestinal cryptosporidiosis, isosporiosis and microsporidial infection, respectively, among HIV positive subjects at Nekemte Hospital, West Ethiopia. In addition, their prevalence in pre-ART and on-ART study participants was different, whereby a significant reduction in their prevalence was observed in those who were under HAART. A decrease in the prevalence of cryptosporidiosis due to interventions by HAART has been reported from studies elsewhere (20,21).

Diarrhea was the most frequent disease manifestation among AIDS patients. The diarrhoeal cases recorded in the present study were associated with the presence of opportunistic intestinal parasites. This finding was in concurrence with what was reported for cryptosporidiosis-induced diarrhoea in both developing and developed countries (23).

It was noted that most *C. parvum* associated diarrhoeal cases were detected in pre-ART individuals, rather than in those who were on-ART. This indicates that the finding might be due to the effect of HAART, which by improving the immune status of the HIV-positive individuals may have led to curbing the pathogenesis of *C. parvum* infections. This is consistent with earlier reports (24).

The prevalence of *C. parvum* in this study was 25.5%. This was in agreement with reports by Mengesha (7) and Fisseha *et al* (8) which showed a 40% and 25.9% prevalence of *C. parvum* in AIDS patients, respectively.

The role of *I. belli* as an opportunistic intestinal parasite was clear in this study as it was detected mainly among pre-ART HIV positive diarrhoeal patients. This was in agreement with the study reported by Awole *et al* (9) from Addis Ababa where they detected a prevalence rate of 11% for *C.parvum* and 7.4% for *I. belli* among diarrheic patients. A previous study by Endeshaw *et al.* (13) showed a 10.26% of double infection by *C. parvum* and *I. belli* in AIDS patients with diarrhea. However, this case was not true in the present study.

In the present study the prevalence of *E. bieneusi* was 11.6%. This was lower than the 22.5% prevalence reported in AIDS patients with diarrhea from Addis Ababa previously (13). The detection of only one microsporidial agent (*E. bieneusi*) may have resulted from differences in the immune status of the study participants and the differences in the study localities.

In this study, the majority of diarrhoeal HIV-positive study participants with cryptosporidiosis and isosporiosis infections were at stage III and IV of WHO HIV/AIDS staging, indicating that infections with opportunistic intestinal parasites mostly occured at the latest stages of HIV infection (25).

As the duration of HIV/AIDS patients on-ART increases, the prevalence of infections with opportunistic intestinal parasites has been shown to decrease, as reported by other works (18,19). Similarly, the prevalence of infection with *C. parvum* and *I. belli* was shown to significantly decrease over six months of ART follow-up period used in the present study. It is worth noting that the use of HAART has been associated with a gradual reconstitution of the immune system and reduction of HIV associated morbidity and mortality (26). A Similar outcome was registered in the present study by the use of HAART, as evidenced by an increase in CD4+T- cell count during the 6 months following ART.

In conclusion, the major opportunistic intestinal parasites detected in pre-ART diarrhoeal HIV positive patients were *C. parvum, I. belli* and *E. bieneusi*, and their prevalence decreased as the duration on-ART increased from 0 to 6 months which coincided with an increase in CD4+ T-cell count indicating the effective role of HAART in reducing opportunistic infections. For opportunistic intestinal protozoan parasites no proven therapy exists, therefore, easy access to ART is the only option and its early initiation in HIV-positive patients is urgently required.

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REFERENCES

1. Curry A, Turner A.J. Lucas S. Opportunistic Protozoan infections in human immunodeficiency virus disease: Review highlighting diagnostic and therapeutic aspects, 1990, Middlesex School printing press, London.
2. GoodgameR.W.Understanding intestinal spore-forming protozoa: *cryptosporidia,* microsporidia, *isospora, and cyclospora*. *Ann. Intern. Med*. 1996; 124 (4): 429-441.
3. Shah UV. Purohit BC, Chandralekha D, Mapara MH. Co- infection with *Cryptosporidium, Isospora* and *S. stercoralis* in a patient with AIDS- a case report. *Indian J Med Microbial* .2005; 21: 137-138.
4. Kelly P. Diarrhoea and AIDS: recent developments in the African settings. *African Health*. 1998; 1: 16-18.
5. Juranek D.D. "Cryptosporidiosis: sources of infection and guidelines for prevention." *Clin Infect Dis.* 21 *Suppl.* 1995; 1: S57-61.
6. Petersen C. Cryptosporidiosis, Cyclosporiasis, and Isosporiasis in the setting of HIV infection. Center for HIV Information. 1998, HIV InSite Knowledge Base Chapter.
7. Mengesha B. Cryprosporidiosis among medical patients with AIDS in Tikur Anbessa Teaching Hospital. Ethiopia *E. Afr. med.J*. 1994; 71:376-378.
8. FissehaB, PetrosB,Woldemichael T, Mohammed H. Diarrhea-associated parasitic infectious agents in AIDS patients within selected Addis Ababa Hospitals.*Ethiop.J.Health Dev*. 1999; 13:1-5.
9. Awole M, Gebre-Selassie S, Kassa T, Kibru G. Prevalence of Intestinal parasites in HIV infected adult patients in South Western Ethiopia. *J Health Dev*. 2003; 17(1):71 - 78.
10. Endeshaw T, KebedeA,Verweij J.J. *et al*. Intestinal Microsporidiosis in Diarrheal Patients Infected with Human Immunodeficiency Virus-1 in Addis Ababa, Ethiopia .*Jpn.J.infect.Dis*. 2006; 59:306-310.
11. Franzen C, Muller A. Molecular techniques for detection, species differentiation, and phylogenetic analysis of Microsporidia. *Clin. Microbial. Rev*. 1999; 12: 243-285.
12. 12.Endeshaw T. Opportunistic and other intestinal parasites among HIV/AIDS Patients in Ethiopia ,2005,Ph.D Dissertation, Dept. Biology, Addis Ababa University.PP:1-122.
13. EndeshawT,KebedeA,Verweij J.J. *et al.* Detection of intestinal Microsporidiosis in Diarrhoeal patients infected with the Human Immunodeficiency virus (HIV-1) using PCR and Uvitex- 2B stain. *Ethiop Med J.*2005; 43: 97-101.
14. 14.DeWolf F, Roos M, Lange J. M .A. *et al.* Decline in CD4+ cell numbers reflects increase in HIV-1 replication. *AIDS Res Hum Retroviruses.* 1988;4:433–0.
15. Phillips AN, Lee CA., Elford J. Serial CD4 lymphocyte counts and development of AIDS. *Lancet* . 1991 ;337: 389-2.
16. Tsegaye A, Messele T, Tilahun T. *et al*. Immunohematological reference ranges for adult Ethiopians.*Clin.Diag.LabImmunol*. 1999 ;6:410-4.
17. Elias K, Tobias F, Rinke deWit. et al. Evaluation of the World Health Organization staging system for HIV infection and disease in Ethiopia:association between clinical stages and laboratory markers.*AIDS.* 1999; 13:381-389.
18. Osmond D.H. Classification and Staging of HIV Infection. *HIV InSite* . 1998;1:1-13.
19. Hansasuta P,Row S.L. HIV-1 transmission and acute HIV-1 infection. *B.Med. Bulletin.* 2001;*58* 109-127.
20. Conteas N.C ,Berlin O.G , Speck, C.E.*et al.* Modification of the clinical course of intestinal microsporidiosis in AIDS patients by immune status and anti- HIV-1 Theraphy. *Am.J.Trop. Med. Hyg*. 1998; 58: 555-558.
21. Pozio E, Morales M.A.G. The impact of HIV protease inhibitors on opportunistic parasites. *Parasitol*. 2005;21: 58-63.
22. Lawan S.D,Meyer L,Bekker L.G,Wood R. CD4 cell count recovery among HIV-infected patients

with very advanced immunodeficiency commencing antiretroviral treatment in sub-Saharan Africa. *Infec.Dis.* 2006;6:1-14.

23. Chen X.M, Keithly JS, Paya. C.V, LaRusso N.F. *Crptosporidiosis. N. Engl. J. Med.*2002;346:1723-31.
24. Hunter R.P, Nicholis G. Epidemiology and clinical features of *Cryptosporidium spp*.infection in immunocompromised patients. *Clin. Microbiol. Rev*. 2002; 15:145-154.
25. World Health Organization ,2005. Revised World Health Organization Clinical Staging of HIV/AIDS for Adults and Adolescents.
26. Hogg R,S.O,Shaughnessy M,V,Gatanic N.,B.*et al*. Decline in deaths from AIDS due to new antiretrovirals.Lancet 1997: 12: 349:12