Original article

Intestinal parasites among HIV/AIDS patients attending University of Gondar Hospital, northwest Ethiopia

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Abstract

Background: Opportunistic intestinal parasitic infections are the major public health problem among human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS) patients. In the absence of anti-retroviral therapy (ART), HIV/AIDS patients in developing countries such as Ethiopia continue to suffer from the consequences of intestinal parasites. This study aimed to determine the prevalence of opportunistic and other intestinal parasites among on-ART HIV/AIDS patients.

Methods: A comparative cross-sectional study was conducted from December 2015 to January2016 among pre-ART and on-ART adult HIV/AIDS patients at University of Gondar Hospital, northwest Ethiopia. A pre-tested structured questionnaire was used to collect data on socio-demographic and associated risk factors. Systematic random sampling was used to select the study participants. Stool samples were collected and processed using a direct, wet-mount, formol-ether concentration technique and modified Ziehl-Neelsen staining technique. The CD4 counts were estimated by using the BD FACS Count system. Data were entered and analyzed using SPSS 20 software.

Results: A total of 150 study participants (48ARTnaïve and102 on ART) were included in the study. The overall prevalence of intestinal parasitic infections was 45.3% – 25.3% in pre-ART patients and 20% in on-ART patients. Two thirds (8/12) of opportunistic parasitic infections were found in the ART-naïve patients and significantly associated with CD4 counts <200 cells/mm^3. Those who have no toilet [AOR=5.21, 95% CI: 1.82, 16.03], source of water from stream [AOR = 2.8; 95% CI: 1.05, 7.72], who have diarrhea [AOR = 11.38; 95% CI: 4.69, 15.61], WHO stage III [AOR =5.3; 95% CI: 2.47, 11.56] and ART status [AOR = 4.2; 95% CI: 2.02, 8.77] were significantly associated with the prevalence of intestinal parasites.

Conclusions: The prevalence of intestinal parasites was found to be higher in ART-naïve than on-ART patients. High proportions of intestinal parasites were associated with lower CD4 counts, ART naïve, diarrhea, WHO stage III, absence of toilet and source of water from stream. Therefore, public health measures and adherence to ART for ART naïve should be strengthened to improve the quality of life of these patients. [*Ethiop.J. Health Dev.* 2019; 33(2):65-72]

Key words: Opportunistic intestinal parasites, CD4 T-cells, Anti-retroviral therapy, diarrhea, Gondar

Background

Intestinal parasitic infection is a major source of morbidity in tropical countries, especially among human immunodeficiency virus (HIV) patients. Opportunistic infection poses major health problems among HIV patients, particularly in the late stage of the disease, when the immune system is severely depleted (1, 2).

HIV and AIDS is the leading cause of morbidity and mortality worldwide(3). The prevalence of intestinal parasites is high in sub-Saharan Africa, where the majority of HIV/AIDS cases are concentrated, and factors including poverty and malnutrition could promote transmission of both infections in the region (4).

Currently, the rapid expansion of the HIV/AIDS pandemic has brought about a significant change in the fauna of intestinal parasites all over the world, especially in developing countries. Several factors also contribute to the expansion and newly emerging intestinal parasites (5,6). With the HIV/AIDS pandemic, many intestinal parasites, previously considered sporadic or zoonotic infections, have become opportunistic parasites, causing numerous cases of life-threatening diarrhea(5).

Intestinal parasitic infections play an important role in the progression of HIV infection by disturbing the immune system while it is already involved in the fight against HIV(7). In HIV-infected individuals, the progressive decline in their immunological responses makes them extremely susceptible to a variety of opportunistic and other intestinal infections. In recent years, numerous studies have outlined the emergence of important gastrointestinal protozoa, such as *Cryptosporidium parvum*, *Cystoisospora*, *Cyclospora cayetanensis*, *Microsporidia*, *Entamoeba histolytica/dispar* and *Giardia lamblia*, which account for a significant number of cases of diarrhea in this population(8,9). Non-opportunistic parasites, such as *Entamoeba histolytica* and *Giardia lamblia*, are also parasites causing diarrheal diseases that occur in individuals living with HIV/AIDS (10).

Unfortunately, in the absence of anti-retroviral therapy (ART), HIV-positive individuals in developing

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countries continue to suffer the consequences of opportunistic parasites(11). Patients with very low CD4 cell counts who enroll onto ART programs have a heightened risk of morbidity and mortality before ART(12).ART increases the length and quality of life and productivity of HIV patients by improving survival and decreasing the incidence of opportunistic infections through a reduction of the viral load and increase in the level of CD4cells (13). There is evidence that the control of these opportunistic parasitic infections in HIV-positive people under highly active anti-retroviral therapy(HAART) is also induced by the inhibition of the aspartyl proteases of the parasites and by the reconstitution of the immune system of the patient(14,15). However, patients in resource-limited settings typically start ART programs with advanced symptomatic disease and very low blood CD4 cell counts, which predisposes them to high rates of both clinical and subclinical opportunistic infections(12).

A considerable decline in the mortality of adult AIDS patients was shown to be related to the start of ART in Addis Ababa, Ethiopia and other parts of the country(16). Although, there has been an improvement in the survival of HIV patients, parasitic infections pose a serious challenge with regard to reducing the morbidity and mortality of these individuals.

Most of the health institutions in Ethiopia lack appropriate diagnostic methods to detect low levels of parasite burden. In addition, some of the diagnostic methods for specific intestinal parasites, especially for the newly emerging opportunistic intestinal parasites, are not available to peripheral health institutions. There is a paucity of information regarding the prevalence and associated risk factors of opportunistic and other intestinal parasites among HIV/AIDS patients in Ethiopia. Therefore, this study aimed to determine the prevalence of opportunistic and other intestinal parasites among ART-naïve and on-ART HIV/AIDS patients attending University of Gondar Hospital ART clinic, northwest Ethiopia.

Methods and materials

Study design, area and period: An institution-based, comparative, cross-sectional study was conducted from December 2015 to January2016 on the prevalence of opportunistic and other intestinal parasites among HIV/AIDS patients who were ART naïve and on ART, attending University of Gondar Hospital ART clinic. Gondar city is in Amhara regional state, located 750kmnorthwest of Addis Ababa, the capital city of Ethiopia. The hospital is a tertiary-level teaching and referral hospital providing more than 450 beds for inpatients and rendering referral health services for over 5 million inhabitants in northwest Ethiopia. Large numbers of people from the surrounding zones and nearby regions visit the hospital for different medical services. The hospital consists of an ART clinic, an operating room, intensive care unit (ICU), fistula center, 13 different wards and outpatient departments.

Population: The source population for this study was HIV-positive individuals linked to the ART clinic during the study period.

Inclusion/exclusion criteria and study variables: All HIV-positive individuals linked to the ART clinic (ART naïve and on ART) were included in the study. Those HIV patients who were on anti-parasitic treatment for the previous two weeks were excluded.

The prevalence of opportunistic and other intestinal parasites among HIV patients was used as the dependent variable, whereas age, sex, educational status, hand washing habit, source of drinking water, presence of toilet, ART status,CD4 status and diarrheal status were used as independent variables.

Sample size and sampling techniques: The prevalence of intestinal parasites among ART-naïve and on-ART groups of HIV-positive individuals was taken as 39% and 17.6%, respectively, from a previous study(17) and to detect a difference of 15% between the two groups with the assumption of 95% confidence interval(CI), power of 80%, and ratio of 2:1 between on-ART and ART-naïve groups. Double proportion formula was applied to calculate the sample size, which was 150; (102 on ART and 48 ART naïve).

A systematic random sampling technique was used to enroll study subjects. By considering an average ART service 70(20 for ART-naïve and 50 on-ART) adults per day at the University of Gondar Hospital ART clinic. Since the sample collection period for this study was one month, the total number of adult ART-naïve and on-ART HIV patients that came to the hospital for service was 600 (N1) and 1,500 (N2), respectively. To determine K, the following formula was used, K1 (for pre-ART) = N1/n1 = 600/48 = 12, so every 12th ART-naïve adult HIV patient that came to the ART clinic from December to January was included in the sample until the required sample was achieved. Similarly, K2(for on-ART) = N2/n2 = 1,500/102 = 15 for on-ART adult HIV patients, so every 15th on-ART adult HIV patient that came to the ART clinic from December to January was included in the sample until the required sample was achieved.

Operational definitions: ART-naïve patients: People who are HIV positive but are not eligible for ART.
ART patients: People who are HIV positive, and who eligible for and have started ART.
Opportunistic infections: Infections that frequently occur in individuals with a damaged or weakened immune system (e.g., due to HIV/AIDS, various forms of cancer or other causes).
Diarrhea: The passage of three or more loose or liquid stools per day, or more frequently than the normal bowel habit.

Data collection and laboratory methods: A pre-tested structured questionnaire was utilized to collect socio-demographic characteristics, clinical information and other risk factors. A single fresh stool was collected with a labeled stool cup from all study participants following standard procedures by laboratory technicians who work at University of Gondar Hospital. A direct saline and iodine wet mount of each sample was used to detect intestinal parasites microscopically. The wet mounts were examined under light microscope at ×100 and ×400 magnifications.

Formol-ether concentration method: A portion of each fresh stool sample was taken and processed. Briefly, 1g of stool was placed using an applicator stick in a clear 15ml conical centrifuge tube containing 7ml of formalin saline. The resulting suspension was filtered through a sieve into another conical tube. After adding 3ml of diethyl ether to the formalin solution, the content was centrifuged at 3,200 rpm for three minutes. The supernatant was poured away and the tube was replaced in its track. Finally, a smear was prepared from the sediment and observed under a light microscope with magnifications of ×100 and ×400(18).

Modified Ziehl-Neelsen staining method: A small portion of the fresh stool sample was processed for detection of opportunistic parasites using the Ziehl-Neelsen method. A thin smear was prepared directly from sediment of the concentrated stool and allowed to air dry. The slides were then fixed with methanol for five minutes and stained with carbol fuchs in for 30 minutes. After washing the slides in tap water, they were decolorized with acid alcohol for one to three minutes and stained in methylene blue for one minute. The slides were then washed in tap water and observed under light microscope with a magnification of×1,000(18).

Automated flow cytometry analyzer: Five milliliters of EDTA blood was collected from each study participant to measure the CD4 cell count. The CD4 T lymphocyte count was determined using the Becton Dickinson (BD FACSCount) system (19).

Quality control and data analysis: Data collectors were trained and the questionnaire was pre-tested before the actual data collection. After the data collection process, data were checked for completeness. Then, the result of each laboratory examination was recorded carefully on a well-prepared format and finally attached to the questionnaire. Data were double-entered and analyzed using the SPSS20 database software program. Data were summarized using frequency tables. The frequency distribution of both dependent and independent variables was worked out and the association between the independent and dependent variables was measured and tested using OR and 95% CI. The relative contribution of each selected variable to the outcome of interest was assessed using logistic regression, and p-values<0.05 were considered statistically significant.

Ethical considerations: Ethical clearance was obtained from the Ethical Review Committee, College of Medicine and Health Sciences, University of Gondar. Official permission and written informed consent were obtained from University of Gondar Hospital administration office and from each study participant, respectively. To ensure the confidentiality of participants' information, anonymous typing was used, whereby names of participants and any other identifiers were excluded from the questionnaire. For further purposes of privacy, participants were interviewed separately. Any study participant who tested positive for intestinal parasites was referred to a physician for treatment, according to the national standard for clinical management.

Results

Socio-demographic characteristics of study participants: A total of 150 study participants (48 ART-naïve and 102 on ART) were included in the study. The age of study subjects ranged from 5-60 years (34.61 and ±11.1 years, mean and SD, respectively). Of these, 90/150(60%) were females. The majority of ART-naïve (32%) and on-ART (68%) study participants were in the age group 30-44 years.

Of the study participants, 120/150 (80%) lived in urban areas, of whom 68/150 (45.3%) were civil servants. More than half of study participants 77/150(51.3%) were educated and 97/150 (64.7%) were married (Table1).

Table1: **Socio-demographic characteristics of HIV-positive patients enrolled in the study at University of Gondar Hospital, Ethiopia, 2016**

Variables	**ART status**		
	Pre-ART No. (%)	**On-ART No. (%)**	**Total No. (%)**
Sex			
Female	24 (16)	66(44)	90 (60)
Male	24(16)	36(24)	60(40)
Age			
$\leq$ 14	0(0)	8(5.3)	8 (5.3)
15-29	14 (9.3)	40 (26.7)	54(36)
30-44	24 (16)	74 (49.3)	98(65.3)
$\geq$ 45	10 (6.6)	28 (18.7)	38 (25.3)
Residence			
Urban	40 (26.7)	80 (53.3)	120 (80)
Rural	8 (5.3)	22 (14.6)	30 (20)
Marital status			
Single	15 (10)	38 (25.3)	53 (35.3)
Married	25 (16.6)	72 (48)	97 (64.6)
Occupation			
Civil servant	28 (18.7)	40 (26.7)	68 (45.4)
Merchant	7 (4.6)	18 (12)	25 (16.6)
Farmer	0(0)	8 (5.3)	8 (5.3)
Housewife	5(3.3)	9 (6)	14 (12.3)
Student	0(0)	5 (3.3)	5 (3.3)
Daily laborer	10 (6.6)	20 (13.3)	30 (20)
Educational status			
Literate	22 (14.6)	55 (36.6)	77 (51.2)
Unable to read and write	26 (17.3)	47 (31.3)	

No. = number

Prevalence of opportunistic and non-opportunistic intestinal parasites in ART-naïve and on-ART HIV patients: A total of 150 stool samples from both groups were examined for intestinal parasitic infections. Of these, 68/150 (45.3%) (95% CI: 37.1, 52.1) were infected with one or more intestinal parasite (opportunistic and non-opportunistic).

The overall prevalence of opportunistic intestinal parasitic infections among HIV-infected patients enrolled in the study was 12/150 (8%).The prevalence of both opportunistic and non-opportunistic intestinal parasitic infections among HIV patients in relation to ART status was 38/150 (25.3%) and 30/150 (20%), ARTnaïve and onART, respectively.

Various types of non-opportunistic and opportunistic intestinal parasites, including coccidian, protozoa, trematodes, cestodes and nematodes, were detected. The most frequent non-opportunistic intestinal parasites diagnosed were: *Entamoeba histolytica/dispar*31/50 (45.5%), followed by *Ascarislumbricoides*6/68(12%),*S.stercoralis* 5/68(10%), *Giardia lamblia* 5/68(10%), *S.mansoni*2/68(4%) and *H. worm*2/68(4%). Opportunistic protozoa included: *Cryptosporidium* species (spp.)7/68(14%) and *Isospora belli*2/68(4%). Mixed infections comprised *Entamoeba histolytica/dispar*10/60 (16.5%) and *S.stercoralis*7/60 (11.6%); *Entamoeba histolytica/dispar* and *Cryptosporidium*spp.1/60 (1.6%); and *Cryptosporidium* spp. and *Isospora belli*2/60 (3.3%).

The overall prevalence of intestinal parasites in the ART-naïve group was 33/48 (68.75%), which is higher compared to on-ART groups 35/102(34.31%), indicating a statistically significant lower incidence of intestinal parasites in ART patients ($p < 0.007$) (Table 2).

Table 2: **The prevalence of opportunistic and non-opportunistic intestinal parasites in relation to ART status among HIV-positive patients enrolled in the study at University of Gondar Hospital, Ethiopia, 2016**

Parasites	**ART status**					
	Pre-ART		**On-ART**		**X^2**	**P-value**
	Positive No. (%)	**Negative No. (%)**	**Positive No. (%)**	**Negative No. (%)**		
E,histolytica	10(6.6)	38(25.3)	21(14)	81 (54)	0.001	0.97
G.lamblia	2(1.3)	46(30.6)	4(2.6)	98 (65.3)	0.005	0.94
S.stercoralis	5(3.3)	43(28.6)	0(0)	102 (68)	11	0.001*
A,lumbricoides	2(1.3)	46(30.6)	4(2.6)	98 (65.3)	0.005	0.94
S.mansoni	0(0)	48(32)	2(1.3)	100 (66.6)	0.95	0.33
H.worm	2(1.3)	46(30.6)	0(0)	102 (68)	4.3	0.038*
C,parvum	5(3.3)	43(28.6)	3(2)	99 (66)	3.6	0.05*
I.belli	1(0.7)	47(31.3)	1(0.7)	101 (67.3)	0.3	0.58
C.parvum and Cystoisospora	2(1.3)	46(30.6)	0(0)	102 (68)	4.31	0.038*
Amoebiasis and I, belli	1(0.7)	47(31.3)	0(0)	102 (68)	2.14	0.14
Amoebiasis and giardiasis	3(2)	45(30)	4(2.6)	98 (65.3)	0.39	0.52
Amoebiasis and Strongyloides	8(5.3)	40(26.6)	3(2)	99 (66)	9	0.003*
Total					**36.001**	**0.0001***

* $P \leq 0.05$ (significance level); X^2=Chi-square; No. = number

Association of opportunistic intestinal parasites with status of CD4 counts: The study participants who were pre-ART consisted of 27 (54%) with CD4 counts<200 cells/mm^3 and 10(28.4%) with CD4 counts of 200–500 cells/mm^3, while patients who were on ART consisted of 14 (46%) with CD4 counts<200 cells/mm^3, 22 (71.6%) with CD4 counts of 200–500 cells/mm^{3}and 26 (100%) with CD4 counts of>500 cells/mm^3. Almost all the opportunistic parasites were found in ART-naïve patients with CD4 counts<200 cells/mm^3. CD4 T-cell counts from all these *Cryptosporidium* infections were significantly associated in ART-naïve patients withCD4 T-cell counts<200 cells/mm^3 (p = 0.037) (Table 3).

Table 3: Prevalence of opportunistic intestinal parasites among ART-naïve and on-ART HIV-positive individuals in relation to their CD4 counts at University of Gondar Hospital, Ethiopia, 2016

Variables	**ART status**							
	Pre-ART CD4 category				**On-ART CD4 category**			
	<200 cells/ul No. (%)	200-500 cells/ul No.(%)	>500 cells/ul No. (5)	P-value	<200 cells/ul No.(%)	200-500 cells/ul No. (%)	>500cells/ul No. (%)	P-Value
Cryptosporidium	5(100)	0(0)	0(0)	0.037*	3(100)	0(0)	0(0)	0.055
Cystoisospora	1(100)	0(0)	0(0)	0.37	1(100)	0(0)	0(0)	0.63
Cryptosporidium and I,belli	2(100)	0(0)	0(0)	0.20	0(0)	0(0)	0(0)	ND
S.stercoralis	3(60)	2(40)	0(0)	0.85	0(0)	0(0)	0(0)	ND
E,histolytica	6(60)	4(40)	0(0)	0.78	9(42.9)	11 (52.4))	1(4.8)	0.01*
G,lamblia	1(50)	1(50)	0(0)	0.85	1 (25)	3(75)	0(0)	0.47
S.stercoralis and E,histolytica	7(87.5)	1(12.5)	0(0)	0.05*	0(0)	2(66.7)	1(33.3)	0.63
H.worm	2(100)	0(0)	0(0)	0.20	0(0)	0(0)	0(0)	ND
A,lumbricoides	0(0)	2(100)	0(0)	0.10	0(0)	4(100)	0(0)	0.14
S,mansoni	0(0)	0(0)	0(0)	ND	0(0)	2 (100)	0(0)	0.38

* $P \leq 0.05$ (significance level); ND = not determined

In terms of the selected environmental and clinical variables as determinant factors for intestinal parasite infection, absence of toilet, those who are not on ART, using stream water for drinking, individuals who have experience diarrhea and having CD4 counts of <200cells/mm^3 were significantly associated with intestinal parasitic infections, as shown in (see Table 4). HIV patients who did not have toilets in their homes were almost five times more likely to have intestinal parasites than those who have toilets [AOR=5.21, 95% CI: 1.82, 16.03]. Adult HIV patients whose source of water was stream are almost six times [AOR = 2.8, 95% CI: 1.05, 7.72] more likely to be infected by intestinal parasites than those whose source of water was tap water. Concerning diarrheal status, those HIV/AIDS patients who had reported diarrhea are 11.4 times [AOR = 11.38, 95% CI: 4.69, 15.61] more likely to have parasites than those who have no diarrhea. Moreover, WHO Stage III [AOR =5.3, 95% CI: 2.47, 11.56] patients are five times more likely to have internal parasites than stage I patients. With respect to ART status, pre-ART patients were 4.2 times [AOR = 4.2, 95% CI: 2.02, 8.77] more likely to have intestinal parasites than on ART patients (Table 4).

Table 4: **Associations of selected environmental and clinical findings of HIV-positive patients with prevalence of intestinal parasites by ART status using binary and multiple logistic regression at University of Gondar Hospital, Ethiopia, 2016**

Variables	**ART status**					
	Pre-ART		**On-ART**			
	Intestinal parasite		**Intestinal parasite**			
	Positive No. (%)	**Negative No. (%)**	**Positive No. (%)**	**Negative No. (%)**	**AOR (95%Cl)**	**P-value**
Sex						
Female	17 (70.8)	7 (29.2)	19 (28.8)	47 (71.2)	0.98(0.57, 1.7)	0.78
Male	16 (66.7)	8 (33.3)	16 (44.4)	20 (55.6)		
Age						
<14	0 (0)	0 (0)	5 (62.5)	3 (37.5)	2.33(0.52,13.69)	0.99
15-29	9 (64.3)	5 (35.7)	10 (38.5)	16 (61.5)	1.45(0.16,13.23)	0.062
30-44	18 (75)	6 (25)	14 (28)	36 (72)	1.53 (0.24,8.3)	0.088
>45	6 (60)	4(40)	6 (33.3)	12 (66.7)		
Educational status						
Unable to read and write	19 (73.18)	7 (26.9)	16 (34)	31(66)	0.3 (0.05,2.4)	0.602
Literate	14 (63.6)	8 (36.4)	19 (34.5)	36 (65.5)		
Hand washing habit after latrine						
Yes	3(75)	1 (25)	15 (41.7)	21(58.3)		
No	30 (68.2)	14(31.8)	20 (30.3)	46(69.7)	0.83 (0.10,0.57)	0.25
Latrine used						
No	21 (63.6)	7 (46.7)	19(54.3)	19 (28.4)	5.21 (1.82,16.03)	0.005*
Yes	12 (36.4)	8(53.4)	16 (45.7)	48 (71.6)		
Source of water						
Pipe	20 (66.7)	10(33.3)	22 (29.7)	52 (70.3)		
Well	11 (73.3)	4 (26.7)	11 (45.8)	13 (54.2)	2.39(0.15,15.12)	
Stream	1 (50)	1 (50)	1 (33.3)	2 (66.7)	2.8 (1.05,7.72)	*0.007
River	1 (100)	0 (0)	1 (100)	0(0)	1.16(0.86,11.01)	
Diarrheal status						
No	5 (31.2)	11(68.8)	5 (8.9)	51(91.1)		
Yes	28 (87.5)	4 (12.5)	30 (65.2)	16(34.8)	11.38(4.69,15.61)	0.000
CD4 category						
<200 cell/mm^3	24 (88.9)	3(11.1)	11(47.8)	12 (52.2)	3.2 (0.07, 0.33)	0.001*
200-500 cell/mm^3	9 (42.9)	12(57.1)	23 (43.4)	30 (56.6)	3.2 (0.07, 0.33)	0.99
>500 cell/mm^3	0 (0)	0(0)	1 (3.8)	25 (96.2)	1	1
ART status						
Pre-ART	33 (68.8)	15(31.2)	0 (0)	0(0)	4.2(2.02,8.77)	0.000*
On-ART	35 (34.3)	67(65.7)	0 (0)	0(0)		
WHO stage						
Stage I	10 (37.03)	17 (263.07)	25 (41.6)	35 (59.4)		
Stage II	3 (33.3)	6 (66.7)	11 (35.5)	20 (64.5)	1.31(0.56, 3.06)	0.99
Stage III	6 (60)	4 (40)	3 (30)	7 (70)	5.3 (2.47,11.56)	0.002*
Stage IV	2 (100)	0 (0)	1 (100)	0 (0)	ND	ND

* $P \leq 0.05$ (significance level); ND = not determined; CI = confidence interval; AOR=adjusted odds ratio; No. =number

Discussion

The HIV/AIDS pandemic has brought about dramatic changes in intestinal parasite fauna. As the spectrum of immunodeficiency progresses, HIV-infected individuals become susceptible to a variety of opportunistic infections that occur with higher frequency and severity. More importantly, with the emergence of AIDS, the epidemiology, as well as outcome of diseases caused by opportunistic parasites, has modified significantly.

Several intestinal parasites previously considered non-pathogenic or with transient pathogenic potential in immun- competent individuals, are opportunistically becoming aggressive and causing debilitating illnesses in HIV/AIDS patients. The overall prevalence of intestinal parasites in HIV/AIDS patients in this study was 45.3%, which is in agreement with results obtained from two areas of southwest Ethiopia – 47.8%(20)and 44.8%(21) – and south Iran (48.8%)(22).The prevalence is higher than some studies conducted in Ethiopia – such as Hiwot Fana Hospital (33.7%)(23), Bahir Dar (30.6%)(24), and Butajira (35.9%)(25) – but lower than the prevalence reported in northwest Ethiopia (67.6%)(26), Eastern Tigray (68.3%)(27) and Thailand (50%)(28).The difference in the prevalence might be due to differences in geographical location, sensitivity of diagnostic techniques, study participants' immunity status, environmental hygiene, socioeconomic status, and access to safe water supply. Moreover, it may also be due to certain patients' better awareness of intestinal parasite infections and their causes.

The present study revealed the prevalence of common and opportunistic intestinal parasites among ART-naïve and on-ART HIV/AIDS patients. The prevalence of intestinal parasites among ART-naïve patients was 25.3%, which is concurrent with studies conducted in Addis Ababa (27.8%)(16) and Bahir Dar (35.7%)(24). However, it is lower than results of studies from Arbaminch, Chencha and Gideo (45%), east Ethiopia (45.5%), selected ART centers in Adama, Afar and Dire-Dawa(52%), and Brazil (64%)(23,29-31).The lower prevalence of the present study might be due to geographical differences in sample size, study period, method use, and patients' better awareness of intestinal parasite infection.

The prevalence of internal parasites among on-ART patients was 20%, which is comparable with studies conducted in Dessie Hospital in Ethiopia (17.6%), in Brazil (24%),in Congo (24.6%), in Eastern Tigray (25.5%)and in Bahir Dar (25.5%) (17,24,27,29). However, prevalence is lower than that reported in Nigeria (30%) and in selected ART centers of Adama, Afar and Dire-Dawa 48% (30,32).This might be due to better follow-up through laboratory tests, and better awareness on the part of patients in adopting prevention and treatment measures against intestinal parasites. In addition, anti-helminthes may have been given to certain groups of on-ART patients for the purpose of deworming.

In this study, *Entamoeba histolytica/dispar*, which had a prevalence of20.6%, was the most common non-opportunistic protozoa investigated. The finding is similar to a study conducted in Ethiopia at Felege Hiwot Hospital in Bahir Dar (19.3%)(24), South Africa(26%)(33) and southwest Ethiopia(23.8%)(5,34). Furthermore, the rate of infection with *S.stercoralis*, a well-known opportunistic parasite, was 3.3% in ART-naïve and 0%among on-ART subjects, which is in agreement with a study conducted in Kinshasa, Democratic Republic of Congo (35).

The findings of this study also show that the opportunistic parasites, *Cryptosporidium*spp. and *Isospora belli*, were predominantly found in the ART-naïve group. Of these, the prevalence of *Cryptosporidium*spp.in ART-naïve groups was 3.3%, which is supported by studies conducted in different settings in Ethiopia – Hiwot Fana Hospital(2.2%) and Dessie Referral Hospital (1.5%)(17,23) – but lower than in Brazil (8.1%), Nepal (31.4%) and selected ART centers in Adama, Afar and Dire-Dawa (8%) (5,9,29,30).While the prevalence of *Isospora belli* in ART-naïve patients was found to be 0.7%, which is in agreement with reports in northeast(0.7%),northwest (1.3%) and east Ethiopia (1.3%)(17,23,24), it is also lower than studies done in Brazil (4.8%), Nepal (2.9%), selected ART centers of Adama, Afar and Dire-Dawa (5%), and Bahir Dar (8.8%) (9,27,29,30). The lower prevalence in the present study is because of the fact that our study participants are in the ART care who were taking ART and treatment for opportunistic infection. Moreover, it might be due to difference in immunity, sample size variation (small size of the present study), diarrheic status, environmental and personal hygiene of the study participants.

The prevalence of intestinal parasites was highly significant among those ART-naïve patients with CD4counts of <200 cells/ cells//mm3 in this study. This is consistent with studies in east Ethiopia (23), selected ART centers in Adama, Afar and Dire-Dawa, Ethiopia(30),Cuba (14) and northeast Ethiopia (17).The present study shows that there was a significant correlation of CD4 with *Cryptosporidium*spp.and *Isospora belli*, which is also reported by other studies where patients have CD4 counts of <200 cells//mm^3(5,30).

Moreover, the prevalence of intestinal parasites was significantly higher among the ART-naïve group compared to on-ART group participants ($p < 0.001$). This is similar to studies carried out in Brazil and in different parts of Ethiopia, which show that there is a significant reduction of intestinal parasites when patients are administered ART (17, 29, 30).This might be because ART increases patients' quality of life, increases rates of survival, slows disease progression, decreases viral load, increases immunological response and decreases opportunist infections. On the other hand, there may be better clinical handling of the patients, with constant updating of protocols for treatment and prophylaxis, as well as better follow-up through laboratory tests.

The current study noted that WHO clinical stage III was significantly associated with the presence of intestinal parasites is. The finding is supported by studies done in Nekemt in west Ethiopia(36), northeast Ethiopia(17) and south Ethiopia (37). In the present study, the majority of HIV/AIDS patients were in the age range 30-44 years, the most productive and reproductive group in society. This finding is supported by other studies conducted in east Ethiopia (37). The present study also shows that being diarrheic was significantly associated with the occurrence of opportunistic and other intestinal parasitic infections among individuals living with HIV/AIDS. This association is in agreement with studies conducted in northwest Ethiopia (24), south Iran (22),east Ethiopia (23) and Nigeria (38).

The present study reveals that HIV-positive/AIDS individuals who had no toilet in their homes were significantly associated with the occurrence of opportunistic and other intestinal parasites. This is in agreement with previous studies done in northeast (17) and east Ethiopia(37).On the other hand, unprotected water sources (streams) was another high-risk factor that predisposes HIV-positive/AIDS individuals to opportunistic and other intestinal parasites. This is also in line with reports carried out in northeast Ethiopia (17).

Conclusions

The present study confirms the high prevalence of opportunistic and non-opportunistic intestinal parasites among HIV-positive individuals. The prevalence of intestinal parasites was found to be higher in AR-naïve than on-ART patients. In the study area, high proportions of intestinal parasites were associated with lower CD4 counts in both AR-naïve and on-ART patients. Using stream water/unprotected water, the absence of a toilet in the home, diarrhea, low CD4 count, being WHO stage III and being ARnaïve significantly increased the prevalence of intestinal parasites. Stool examinations should be routinely performed in the follow-up of patients with HIV/AIDS attending ART clinic in order to standardize treatment of institutional and other preventive measures. Counseling of ART-naïve HIV patients to link for ART, Make awareness on environmental and personal hygiene should also be given to HIV-infected individuals. Moreover, large-scale longitudinal studies are needed to determine the effect of ART on both opportunistic and non-opportunistic parasites.

Competing interests: The authors declare that they have no competing interests.

Authors' contributions: TG designed the study, collected and analyzed the data, and participated in the draft and final write up of the manuscript. HK participated in the writing and interpretation of results, and commented on the final manuscript prior to submission for publication. AA participated in the data analysis and interpretation of the results, and also commented on the final manuscript prior to submission for publication. All authors reviewed and approved the final manuscript.

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