ORIGINAL ARTICLE

Prevalence of Opportunistic Intestinal Parasites and Associated Factors among HIV Patients while Receiving ART at Arba Minch Hospital in Southern Ethiopia: A Cross-sectional Study

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ABSTRACT

BACKGROUND: Opportunistic Intestinal Parasites (OIPs) commonly cause morbidity in HIV/AIDS patients due to the decline of CD4$^+$T-cells. The burden of Opportunistic Intestinal Parasitic Infections (OIPIs) in Ethiopia is expected to be high due to poor sanitation and co-pandemicity of HIV/AIDs. Therefore, frequent assessment of the magnitude and associated factors for intestinal parasitosis is essential for the management of HIV/AIDS patients.
METHODS: A hospital based cross-sectional study was conducted among patients attending Arba Minch Hospital Antiretroviral Therapy (ART) Clinic from March to June 2016. Stool specimens were processed for parasitological examination using direct wet mount, formol-ether sedimentation and modified Ziehl-Neelsen staining techniques. CD4+ T-cell count data were taken from patients' medical records. A structured questionnaire was used to collect data on socio-demographic characteristics and possible associated factors for OIPIs. All the data were analyzed using SPSS version 20.
RESULTS: Two hundred and twenty ART patients participated in the study. The overall prevalence of intestinal parasitic infections was 28.18% while that of OIPIs alone was 17.72%. Among identified intestinal parasites, Cryptosporidium species accounts for the highest frequency (19/220, 8.63%), followed by Cyclospora species (13/220, 5.90%). Presence of domestic animals (AOR=2.07, 95%CI:1.07-8.40, P= 0.032) and CD4+ T-cell count <500cell/µl (AOR=4.66, 95%CI:1.17-5.35, P= 0.001) were significantly associated with OIPIs.
CONCLUSION: The study indicated that co-infection rate of OIPs is high among ART patients. It also found that contact with domestic animals and having CD4+ count <500 cell/µl predict for the presence of OIPs.
KEYWORDS: Opportunistic intestinal parasites, ART patients, CD4 status

INTRODUCTION

Human Immuno Deficiency Virus (HIV) infection is a major public health problem. Its emergence and pandemic spread currently poses the greatest challenge to the globe. According to the World Health Organization (WHO) report, there were 2.1 million new HIV infections worldwide and a total of 36.7 million people were living with the virus in 2015. Eastern and Southern Africa, where Ethiopia is located, is one of the most affected regions where there were 19 million cases in 2015 (1).

The hallmark of HIV infection is depletion of CD4+ T lymphocytes resulting in overall impairment of the immune response (2,3). Therefore, HIV/AIDS patients are more likely to acquire other infections. They are prone to more severe morbidiry once the infection is established. They also have disseminated rather than localized infection. Moreover, they are unable to effectively clear infecting micro-organisms. All these lead to greater morbidity and mortality, mainly because of opportunistic infections (4). Opportunistic infections can be of viral, bacterial, fungal or parasitic origins which usually affect the gastro-intestinal, nervous and respiratory systems (5,6). About 80% of deaths of AIDS patients are related to opportunistic infections rather than the virus itself, and of these, more than 47% happen due to OIPs (7).

Opportunistic protozoan parasites such as *Cryptosporidium species (spp), Cyclospora cayetanensis, Isospora belli* and *Microsporidia spp* have been identified in individuals living with HIV/AIDS (8,9). OIPIs have been a major source of morbidity in tropical countries where HIV/AIDS is endemic (10). *S.stercoralis* causes a severe human parasitic infection primarily because of its potential for serious and even lethal diseases in immunosuppressed patients (11). The sign and symptoms include severe chronic watery diarrhea with frequent and explosive bowel movements accompanied by loss of appetite, weight loss, abdominal cramp, nausea, fever, headache and vomiting (12,13). However, the intensity of the harm depends on parasite species, nature and course of infection, nature of interactions between the parasite and concurrent infections, nutritional and immunological status of the host and numerous socioeconomic factors (7,14).

Although there has been an improvement with the introduction of ART for individuals in HIV/AIDS, the presence of OIPIs create serious challenge in reducing associated morbidity and mortality. Studies have also evidenced that the prevalence of OIPs among those patients is high (15). Nevertheless, with the introduction of combination antiretroviral therapy and also more effective prophylaxis against these 'classic' opportunistic infections, the rate has decreased. However, OIPIs still represent a frequent cause of morbidity and mortality in most developing countries (16,17).

In Ethiopia, OIPIs among HIV patients are estimated to be high due to poor level of environmental sanitation and personal hygiene plus contamination of food and drinking water. This happens because of improper disposal of human and animal excreta. Patients are not also frequently been screened for these pathogens on regular follow-up at monitoring centers/clinics (18). Hence, informationa bout the co-infection rate of OIPs among patients attending ART is very limited. In this regard, the present study was initiated to assess the magnitude of OIPIs and associated factors among ART following patients at Arba Minch Hospital, Southern Ethiopia.

MATERIALS AND METHODS

Study design and area: A hospital based cross-sectional study was conducted in Arba Minch Hospital ART Clinic from March to May, 2016. Arba Minch is located in the southern part of the country about 454km far from Addis Ababa, the capital of Ethiopia. Its population is around 70,000 (19). Arba Minch Hospital is serving a population of more than 1.5 million. It provides different health services such as outpatients, inpatients, pharmacy and laboratory with full service, especially for ART purpose. The hospital has been providing counseling and testing service for HIV since the early 1990s. It also serves as a referral center for all other health institutions in Gamo Gofa Zone. Screening as well as treatment and follow-up of HIV/AIDS patients is one of the routine services provided in the hospital.

Sample size and sampling technique: The study participants consist of randomly selected ART following patients who came to Arba Minch Hospital ART Clinic during the data collection period. Sample size was calculated using single population proportion formula with the following assumptions: margin of error=5% at 95% confidence level, p=0.1538 from similar study conducted in Jimma (20). Hence, the total sample size was 230 after adding 15% for non-respondents. Systematic random selection method was followed by calculating k value (interval), where N=1600, n=230 and K= 1600/230=7. The first subject was recruited by lottery method and then every 7th ART following patient was included in the study. Those ART attending patients whose age was ≥ 12 years old who were willing to participate in the study were included while severely ill patients who were unable to respond to questions and those < 12 years of age were excluded.

Data collection: A pre-tested structured questionnaire administered through face-to-face interview was used to collect data regarding socio-demographic characteristics and associated factors for OIPIs among patients. CD4+ count and ART status were taken from patients' medical records . which was done during the enrollment of patients in the study period. For the purpose of parasitological examination, each study participant was informed about sample collection and supplied with labeled plastic stool cup, toilet paper and applicator stick and instructed to collect around 3-5 grams of stool. Direct saline wet mount smear was examined immediately to detect the presence of motile parasite stages while the rest of the sample was preserved with 10% formalin. About 1 gram from the preserved stool was concentrated and examined by the formol-ether concentration techniques for the detection of ova, cyst and larvae of different intestinal parasites following standard procedure as described elsewhere (21), and modified ziehl-Neelsen technique was followed to detect oocysts of *Cryptosporidium spp, Isospora belli*, and *Cyclospora spp* (22). The direct wet mount and formol-ether concentrated preparations were examined under 10x and 40x objectives while the modified ziehl-Neelsen stained slides were inspected under 100x objectives.

Quality control: All laboratory materials and reagents were checked for expiry and stored appropriately by experienced laboratory professionals. Standard operating procedures were strictly followed for stool examination. Specimens were also checked for serial number, quality and procedures of collection. To minimize missed parasite identification and discrepancy, each slide was examined by the two trained professionals. In addition, intestinal coccidian parasite identifications were checked using colored atlas.

Statistical analysis: Data was entered and analyzed using SPSS version 20.0. Descriptive statistics were calculated to describe the the characteristics of the study population. Bivariate logistic regression was used to assess associations between dependent and independent variables. Multivariate regression analysis was then applied for variables with p≤ 0.25 in the bivariate analysis. Association between variables was considered statistically significant only if P-value ≤ 0.05 at 95% confidence level.

Ethics: Ethical approval for the research was granted by the Review Board of Arba Minch University. Official permission letter was also obtained from Arba Minch Hospital. All the study participants were informed about the purpose of the study and their right to refuse or accept. Written informed consent/assent was obtained prior to the interview. All results of infected ART patients were reported to healthcare providers with their codes so that they could get prompt treatment.

RESULTS

Socio-demographic characteristics of the study participants: A total of 220 patients (91 males and 129 females) participated in the study with a response rate of 95.65%. Ten patients were excluded due to extremely outlier CD4+ results and incompleteness of records and inabllity to bring stool sample. About 56.36%(124/220) and 43.64% (96/220) were urban and rural dwellers respectively. The majority (76.82%) were aged in between 26-49 (Table 1).

Table 1: Socio-demographic, economic and clinical characteristics of patients attending Arba Minch Hospital ART Clinic from March to May, 2016 (N=220).

Variables		Number (%)
Sex	Males	91 (41.36)
	Females	129 (58.64)
Age group	12-25	31 (14.09)
	26-49	169 (76.82)
	>50	20 (9.09)
	Unable to read & write	48 (21.82)
Educational status	Primary/secondary	120 (54.55)
	Diploma & above	52 (23.63)
Residence	Rural	96 (43.64)
	Urban	124 (56.36)
	Married	148 (67.27)
Marital status	Unmarried	45 (20.45)
	Divorced/widowed	27 (12.28)
Use latrine	Yes	189 (85.90)
	No	31 (14.10)
Monthly income	>2000 birr	30 (13.63)
	1000-2000	109 (49.55)
	<1000 birr	81 (36.82)
Common food source	Cooked at home	184 (83.64)
	From hotel	36 (16.36)
Habit of washing fruits/vegetable	Yes	186 (84.55)
	No	34 (15.45)
Swimming habit	Yes	53 (24.09)
	No	167 (75.91)
Habit of eating raw meat	Often or always	183 (83.18)
	Never or occasionally	37 (16.82)
Hand washing habit before meal	Often or always	211 (95.91)
	Never or occasionally	9 (4.09)
Hand washing habit after defecation	Often or always	198 (90.0)
	Never or occasionally	22 (10.0)
Water source for drinking	Pipe water	206 (93.64)
	River/stream/lake	14 (6.36)
Domestic animals in the compound	Yes	98 (44.55)
	No	122 (55.45)
History of diarrheal in < 3 months	Yes	21 (9.55)
	No	199 (90.45)
ART starting time	>5 years ago	48 (21.82)
	2-5 years ago	101 (45.91)
	<2 years ago	71 (32.27)
CD4 status	>500cell/µl	79 (35.91)
	200-500cell/µl	98 (44.55)
	<200 cell/µl	43 (19.54)

Prevalence of intestinal parasites: Among the total of 220 study participants, intestinal parasites were detected in 62(28.18%) patients while the remaining 158(71.81%) were negative. Infection

by OIPs accounted for 17.72%(39/220). Ten types of intestinal parasites were identified, and *Cryptosporidium spp* were the most frequent parasites detected (19,8.63%) followed by *Cyclospora spp* (13,5.9%). Among non-opportunistic intestinal parasites, *E. histolytica* was with the highest prevalence (3.62%) followed by *S. stercoralis*, *G. lamblia* and *A. lumbricoides* (1.81 % each), *Taenia spp* (1.36%) and *Hookworm* and *H. nana* (0.91 % each) (Table 2).

Table 2: Prevalence of intestinal parasites among patients attending Arba Minch Hospital ART Clinic from March to May, 2016 (N=220).

Type of para	Frequency	%
Cryptosporidium spp	19	(8.63)
Cyclospora spp	13	(5.90)
Isospora belli	3	(1.36)
S. stercoralis	4	(1.81)
E. histolytica /dispar	8	(3.62)
A. lumbricoides	4	(1.81)
G. lamblia	4	(1.81)
Taenia spp	3	(1.36)
Hook worm	2	(0.91)
H. nana	2	(0.91)

Factors associated with opportunistic intestinal parasites: The present study shows that females (11.3%) were more affected by OIPs than males (6.3%). However, the difference was not statistically significant (AOR=0.53, 95%CI: 0.03-2.65, p=0.269). High rate of OIPIs was observed in the age group of 26-49 years (76.81%); but the difference was not significant (AOR=2.44, 95% CI: 0.92-6.03, p=0.263). In multivariate logistic regression analysis, patients who had contact with domestic animals in their compound were more likely to acquire OIPIs than patients who had no contact with domestic animals (AOR=2.07, 95%CI: 1.07-8.40, P= 0.032). Patients who stayed <2 years duration on ART had more chance to be infected with OIPs than patients who took ART for >2 years (AOR=1.27, 95%CI: 1.71-3.57, P= 0.041). Moreover, patients whose CD4 T-cell count was <500cell/µl were more likely to be infected by an OIPs (AOR=4.66, 95% CI:1.17-5.35, P= 0.001) (Table 3).

Among OIPs, *Cryptosporidium spp* had the highest prevalence (8.63%), followed by

Cyclospora spp (5.9%). *Cryptosporidium spp* infection was higher among patients who had CD4+ T - cell count <200 cells/µl than patients who had a CD4+ T-cell count above 500 cells/µl (x^2; 9.34, p = 0.001) (Table 4).

DISCUSSION

In this study on HIV positive patients who were taking antiretroviral treatment, the prevalence of intestinal parasites was 28.18 %. It is similar with the result of a study conducted in India (28.2%) (23). The overall intestinal parasite prevalence was lower than previous studies reported from Jimma (62%) (20) and Thailand (50%) (24).

Among opportunistic intestinal parasites, *Cryptosporidium* spp was the most prevalent and known causative agent for the majority of enteritis and diarrhea. Its prevalence was higher ,19(8.63%), than findings from Burkina Faso (5.2%) (26). However, it was inconsistent with a similar study from India (28.7%) (23). This difference might be due to variations in sample size, diagnostic approaches followed and treatment to prevent OIPs in ART patients. The second most prevalent opportunistic intestinal parasite was *Cyclospora spp* (5.9%) which goes in line with a similar study conducted in Thailand (5%) (24). However, it is lower than the findings from Jimma (10%) and Northern India (31%) (20,23). The prevalence of *I.belli* infection observed in this study was low (1.36%) as compared to findings from Tanzania (11.6%), Zaire (2.4%) and Gondar (2.4%) (25-27). However, it was consistent with reports from Addis Ababa (1.4%) (28).

Table 3: Bivariate and multivariable logistic regression analysis of opportunistic intestinal parasitic infection with associated factors among ART following patients in Arba Minch Hospital, 2016 (N=220).

Risk factors	Category	Number examined	Rate of OIP Infection N(%)	Crude OR (95% CI)	P value	Adjusted OR* (95% CI)	P value
Sex	Males	91	14(15.38%)	1.00			
	Females	129	25(19.38%)	1.06[0.87-1.37]	0.220	0.53[0.03-2.65]	0.269
Age group	12-25	31	13(41.93%)	1.53[0.87-1.99]	0.351		
	26-49	169	19(11.24%)	2.44[0.92-6.03]	0.263		
	>50	20	7(35.0%)	1.00			
	Unable to read & write	48	13(27.08%)	0.32[0.04-4.3]	0.410		
Educational status	Primary/secondary	120	20(16.67%)	0.55[0.94-4.6] 1.00	0.540		
	Diploma & above	52	6(11.53%)				
Residence	Rural	96	16(16.67%)	0.99[0.49-2.01]	0.300		
	Urban	124	23(18.55%)	1.00			
	Married	148	26(17.57%)	0.61[0.14-1.58]	0.260		
Marital status	Unmarried	45	8(17.78%)	0.47[0.21-1.46] 1.00	0.280		
	Divorced/widowed	27	5(18.52%)				
Use latrine	Yes	189	28(14.81%)				
	No	31	11(35.45%)	0.63(0.26-1.48)	0.125	0.8(0.6-7.11)	0.174
Monthly income	>2000 birr	30	1(3.33%)	1.00			
	1000-2000	109	17(15.60%)	0.96[0.92-3.54]	0.061	0.82[0.52-3.82]	0.075
	<1000 birr	81	21(25.92%)	1.28[0.01-4.11]	0.097	0.64[0.12-2.38]	0.421
Common food source	Cooked at home	184	37(20.11%)	1.27[0.41-3.92] 1.00	0.073	0.93[0.54-2.36]	0.673
	From hotel	36	2(5.56%)				
Habit of washing fruits/vegetable	Yes	186	36(19.35%)	2.35[1.03-4.37] 1.00	0.445		
	No	34	3(8.82%)				
Swimming habit	Yes	53	13(24.53%)	0.64[0.94-4.01]	0.058	0.36[0.12-2.38]	0.491
	No	167	26(15.57%)	1.00			
Habit of eating raw meat	Often or always	183	30(16.39%)	1.00			
	Never or occasionally	37	9(24.32%)	0.99(0.43-2.27)	0.989		

Table 3. Continued….

Hand washing habit before meal	Often or always	211	35(16.59%)	1.00			
	Never or occasionally	9	4(44.44%)	0.38(0.09-1.49)	0.167	0.76(0.09-6.71)	0.460
Hand washing habit after defecation	Often or always	198	27(13.63%)	1.00			
	Never or occasionally	22	12(54.54%)	0.62(0.06-7.15)	0.719		
Water source for drinking	Pipe water	206	34(16.50%)	1.00			
	River/stream/lake	14	5(35.71%)	0.43(0.13-1.42)	0.388		
Domestic animals in the compound	Yes	98	24(24.49%)	2.17[1.05-5.46]	0.023	2.07[1.07-8.40]	0.032
	No	122	15(12.30%)	1.00			
Diarrheal history in < 3 months	Yes	21	16(76.19%)	2.14[0.76-5.32]	0.089	0.03[0.63-2.82]	0.073
	No	199	23(11.56%)	1.00			
ART starting time	>5 years ago	48	9(18.75%)	1.00			
	2-5 years ago	101	12(11.88%)	1.38[0.52-3.61]	0.031	2.079 [0.71- 6.01]	0.058
	<2 years ago	71	18(25.35%)	2.29[2.93-5.65]	0.002	1.27[1.71-3.57]	0.041
CD4 status	≥500cell/μl	79	5(6.33%)	1.00			
	<500 cell/μl	141	34(24.11%)	7.99[3.04-18.01]	0.000	4.66[1.17-5.35]	0.001

***adjusted ratio only computed for p≤ 0.25 in the bivariate analysis

Table 4: Association of Opportunistic intestinal parasites with CD4+ T-cell count status of ART patients attending Arba Minch Hospital ART Clinic from March to May, 2016 (N=220).

Types of Parasites Prevalence	CD4+ >500 cells/μl (N = 79)	CD4+ 500-200 cells/μl (N = 98)	CD4+ <200 cells/μl (N = 43)	x^2	p-value
Cryptosporidium spp	2	8	9	9.34	0.001
Cyclosporaspp	3	5	5	2.95	0.024
I. belli	0	2	1		
S. stercoralis	0	3	1		
Total	5	18(8.18%)	16(7.27%)		

S. stercoralis was less frequently detected (1.8%). It is one of the most difficult intestinal parasites to diagnose because conventional stool examination has low sensitivity even when examined several times. This is because the parasite load is low in the majority of infected individuals, and the larval output is minimum and irregular (29). The difference observed in infection rate between the present study and earlier reports (13.6%) might be due to the difference in the diagnostic methods used (30).

Based on this finding, the prevalences of _Cryptosporidium and cyclospora_ spps were higher among opportunistic intestinal parasites and the prevalence of _G. lamblia_ and _E.histolytica_ were higher among non-opportunistic parasites. This is in line with findings from Jimma and India (20,23). In this study, the prevalence of OIPs has a clear statistically significant association with the presence of domestic animal, CD4 status, ART status and stool consistency.

Consistent with a previous study (31), patients who had contact with domestic animals had more chance to acquire OIPIs. _Cryptosporidium_ and _Cyclospora_s _spp_ were significantly (P < 0.05) associated with the presence of domestic animals in their compounds. The prevalence of both species was lower than other studies in Ethiopia (17). In agreement with other studies in Ethiopia (32), a lower rate of cryptosporidiosis observed in this study might be due to ART treatment as it elicits immunological responses (33).

Contact with domestic animals (P=0.001) and CD4+ count <200 cell/µl (P=0.001) were also found to be significantly associated with the presence of _Cryptosporidium_ spp infection. Consistent with another study, cryptosporidiosis occurs in AIDS patients when the CD4+ T- cell count is < 200 cells/µl(34). _Cyclospora_ spp was significantly associated with a history of diarrhea < 3 months (P=0.031) and CD4 status < 200cells/µl (P=0.04). Similarly, this result also showed a higher prevalence of these OIPIs related to CD4+ T-cells counts of < 200 cells/µl(35).

Many researchers have used CD4+ T - cell count as the marker for monitoring disease progress and initiating therapy in HIV patients (36). Our results show that patients whose CD4+ T-cell count < 200/µL had an association with OIPIs (AOR=4.66, 95%CI:1.17-5.35, P= 0.001) compared to patients who had CD4+ T-cell count > 500/µl. Several other studies have corroborated that CD4+ T cell count is lower among co-infected patients as compared to HIV-alone infected ones, and severe immune suppression is seen in those with CD4+ T cell count < 200 cells/µl(20, 23, 25).

Some parasites were not detected because of the non-availability of reagents and the non-applicability of all techniques, such as the water-ether sedimentation method for _Microsporidia_ and molecular methods to differentiate some species of the parasites. Therefore, the prevalence of intestinal parasites among the study participants might have been underestimated. In conclusion, the results of the present study indicated that the prevalence of OIPIs is high among ART patients. This study also found that contact with domestic animals and a CD4+ count <500 cell/µl are predicting factors for the presence of OIPs. Therefore, regular screening, monitoring and appropriate treatment should be given to prevent OIPIs among all HIV/AIDS patients.

ACKNOWLEDGEMENTS

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