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Opportunistic and other intestinal parasites among HIV/AIDS patients attending Gambi higher clinic in Bahir Dar city, North West Ethiopia

Abebe Alemu, Yitayal Shiferaw*, Gebeyaw Getnet, Aregaw Yalew, Zelalem Addis

Department of Medical Laboratory Sciences, College of Medicine and Health Sciences, University of Gondar, Gondar, Ethiopia

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ABSTRACT

Objective: To determine the magnitude of opportunistic and non-opportunistic intestinal parasitic infections among HIV/AIDS patients in Bahir Dar. **Methods:** Cross-sectional study was conducted among HIV/ AIDS patients attending Gambi higher clinic from April1- May 30, 2009. Convenient sampling technique was employed to identify the study subjects and hence a total of 248 subjects were included. A pre-tested structured questionnaire was used to collect socio-demographic data of patients. Stool samples were examined by direct saline, iodine wet mount, formol-ether sedimentation concentration and modified Ziehl-Neelsen staining technique. **Results:** Out of 248 enrolled in the study, 171(69.0%) (90 males and 81 females) were infected with one or more intestinal parasites. The highest rate of intestinal parasites were observed among HIV/AIDS patients (80.3%, 151/188), and the infection rate of HIV negative individuals was 33.3% (20/60). *Cryptosporidum parvum* (43.6%), *Isospora belli* (15.5%) and *Blastocystis hominis* (10.5%) were opportunistic parasites that were found only in HIV/AIDS patients. **Conclusions:** Opportunistic parasite infections are common health problem among HIV/ AIDS patients in the study area. Therefore, early detection and treatment of these parasites are important to improve the quality of life of HIV/AIDS patients.

1. Introduction

Current estimates showed that at least more than one-quarter of the world's population is chronically infected with intestinal parasites and that most of these infected people live in developing countries. However, intestinal parasites once considered to be controllable in developed countries remain a major cause of morbidity and mortality worldwide[1-3]. Currently dramatic 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 and several other factors also contribute to the expansion and reinvasion of newly emerging intestinal parasites[4, 5].

The public health importance of intestinal parasites as a major concern in most developing countries has been pronounced with the co-occurrence of malnutrition and HIV/AIDS. With HIV/AIDS pandemic, many intestinal parasites, previously considered being sporadic or zoonotic infections have become opportunistic parasites causing uncontrollable life threatening diarrhoea[6]. Also HIV infection has been shown to predispose the patient to intracellular opportunistic intestinal parasites such as *Cryptosporidium parvum*, *Isospora belli*, *Cyclospora cayetanensis*, *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis*[7].

Some studies have also argued that mucosa dwelling parasites may benefit from HIV-induced pathological changes and the reduced immune response due to HIV infection which creates suitable environment for opportunistic intestinal parasites in HIV/AIDS patients[6,7]. Most clinical manifestations of HIV/AIDS patients results either from the reactivation of pre-existing latent pathogens, as the individuals become immunosuppressed or is caused by exposure to locally predominant pathogens. Consequently, clinical presentations of AIDS and the pathogens responsible in different geographical areas reflect the differing prevalence of opportunistic intestinal parasitic infections in a given community[6,8].

Like in many other developing countries, intestinal parasites are widely distributed in Ethiopia largely due to the low level of environmental and personal hygiene,

*Corresponding author: Mr. Yitayal Shiferaw, University of Gondar, Gondar town, North West Ethiopia, Po.BOX: ET 196, Gondar, Ethiopia.
E-mail: yitayal96@yahoo

contamination of food and drinking water that results from improper disposal of human excreta[9]. More than half a million annual visits of the out patient services of the health institutions are due to intestinal parasitic infections[10]. However, this report may be an underestimate, because most of the health institutions 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.

In Ethiopian, previous studies showed high prevalence of intestinal parasites like *Ascaris lumbricoides, Entanoeba histolytica, Giardia lamblia* and others in HIV positive[11,12]. Where as, HIV epidemic is highly distributed through out the country affecting the population severely with prevalence of approximately 4.4%[13]. However, information regarding the magnitude of opportunistic parasitic infections among HIV/AIDS patients is scarce in Ethiopia and not well studied in the study as population with HIV/AIDS is growing. Also previous studies gave due attention to distribution of IPs on different altitudes, in different community groups such as preschool children, school children and other groups confined to camps and refugees rather than HIV/AIDS patients. Therefore, the purpose of this study was to determine the magnitude of opportunistic and other intestinal parasitic infections among HIV/AIDS patients attending Gambi higher clinic at Bahir Dar city, North West Ethiopia.

2.Materials and methods

2.1.Study design and area

A cross-sectional study was conducted at Gambi higher clinic situated in Bahir Dar City, North west Ethiopia from April1- May 30, 2009. Bahir Dar is a city in north western Ethiopia, and the capital of the Amhara Region. It is situated on the southern shore of Lake Tana, the source of the Blue Nile (or Abay), in what was previously the Gojjam province. The city is located approximately 578 km north-northwest of Addis Ababa, having a latitude and longitude of 11°36′N 37°23′E, 11.6°N 37.383°E and an elevation of 1 840 meters above sea level. Based on the 2007 Census conducted by the Central Statistical Agency of Ethiopia (CSA), this city has a total population of 221 991. According to the city health office report HIV, malaria, TB and IP are the common health problems in the study area. Bahir Dar had one general clinic, one health center, 15 clinics, and 12 private pharmacies.

2.2. Sampling techniques and study subjects

A convenience sampling technique was used to determine the magnitude of opportunistic and other intestinal parasite in among HIV screening patients attending Gambi higher clinic during study period.

2.3. Socio-demographic data

A pre-designed structured questionnaire was utilized to collect socio-demographic characteristics of the patients.

2.4. HIV serology

The presence of HIV-1 antibodies in the serum was determined using rapid HIV-1 diagnostic test kits following manufacturers' instructions. Results were then interpreted following the current national algorithm for screening of sera for HIV-1 infection that was adopted from WHO. In brief, the sera were first tested with KHB HIV-1/2. If the result was found to be negative it will be taken as negative. If not, it was further being tested with STATPACK HIV-1/2. If the result of STAT PACK was found positive , then the serum was considered as positive for HIV-1 antibodies, if not it will be tested for a third time with a tiebreaker, Unigold HIV1/ 2 and it was reported as negative or positive depending on the result.

2.5. Stool sample collection and direct wet mount

A single stool sample was obtained in labelled caps from all consenting patients selected for the study. 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. A small portion of the stool samples were also preserved in 10% formalin for repeating the tests whenever required.

2.6. Formol ether concentration method

A portion of each fresh stool sample was taken and processed. Briefly, 1 g of stool was placed in a clear 15 mL conical centrifuge tube containing 7 mL formalin saline by using applicator stick. 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 form 3 minutes. The supernatant was poured away and the tube was replaced in its track. Finally smear was prepared from the sediment and observed under light microscope with a magnification of 100× and 400×.

2.7. 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. Thin smear was prepared directly from sediment of concentrated stool and allowed to air dry. The slides were then fixed with methanol for 5 minutes and stained with carbol fuchsine for 30 minutes. After washing the slides in tap water, they were decolorized with acid alcohol (99 mL of 96% ethanol and 1 mL HCl) for 1-3 minutes and

counterstained 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 ×.

2.8. Quality control, data entry and analysis

After data collection process, the data were checked for completeness and any incomplete or misfiled questionnaires filed again. Then the result of laboratory examination was recorded on well prepared format carefully and finally was attached with the questionnaire. Data were double entered and analyzed by using SPSS-15 database program.

2.9. Ethical Consideration

Ethical clearance was obtained from University of Gondar College of medicine and health sciences department of medical laboratory sciences. Additionally, after explaining the importance of the study briefly an informed written consent was obtained from study participants. Anyone not willing to take part in the study had full right to do so and confidentiality of the study participants was also maintained. Finally patient results were reported to the appropriate physician at Gambi higher clinic in order to manage and treat patients appropriately.

3. Results

3.1.Socio-demography of study subjects

A total of 248 patients (132 males and 116 females) were included in the study. The age of study subject range from 19-34 years with median of 26 years. The majorities (87.6%) of the study subjects were Amahara by ethnicity and Orthodox Christianity was the major religion in the area accounting for 93.1%. More than half of study subjects were educated or literate (52.8%) and 47.1% of them were married. Among 248 patients tested for HIV, 188 (75.8%) were HIV positive and the remaining 60 were HIV negative. From infected individuals 96(51%) were females and 92 (49%) were males with 1： 1.04 male to female ratio.

3.2. Intestinal parasites infection

From a total of 248 stool examined for intestinal parasites 171(69.0%) (90 males and 81 females) were infected with one or more intestinal parasites (Table 1). Among 188 HIV patients, 151 had infection (80.3%), while 20(33.3%) HIV negative persons were infected with one or more intestinal parasites and there was statistically significant difference between them (*OR*=8.2, *P*<0.001, 95% *CI*=4.3, 15.6). Infected individuals were found to harbor one and up to four types of parasites. There were 70 HIV positive patients with one parasite infection (83.3%), 81 with multiple infection (93.1%); 14 HIV negative patients with one infection (16.7%) and 6 with multiple infection (6.9%). Multiple infections (polyparasitism) were observed in 66.1% (164/248) of the total examined participants and in 95.9% (164/171) of those with parasitic infection. There was statistically significant difference in multiple infections between HIV positive and HIV negative individuals (*OR*=6.81, *P*=0.001, 95% *CI*=2.8, 16.6), but no significant difference in one infection (*OR*=1.95, *P*=0.047).

In HIV patients diarrhea was common clinical finding 152(80.9%) had acute (54.3%), chronic (21.2% and intermittent diarrhea (5.3%). Out of 188 HIV positive patients 131(69.7%) were infected with one or more intestinal opportunistic parasites and there was statistical significant association with HIV negative individual (*P*=0.000, 95% *CI*=0.405, 0.587). *Cryptosporidium parvum* was the most common among opportunistic parasites (62.6%, 82/131) detected followed by *Isospora belli* (22.1%, 29/131) and *Blastocystis hominis*(15.3%, 20/131)(Table 1).

A total of 248 stool examined for intestinal parasites 40(16.1%) were infected with one or more non opportunistic intestinal parasites. The most frequent Non opportunistic intestinal parasites diagnosed were *Entamoeba histolytica/dispar* (20.0%, 8/40) followed by *Ascaris lumbricoides*, Hook worms, *Strongyloides stercoralis*, *Schistosoma mansoni, Trichuris trichiura, Giardia lamblia* and *Tinea* species. The rate of the over all prevalence of these non-opportunistic intestinal parasites in the HIV negative patients (33.3%, 20/60) was significantly higher (*OR*=1.62, *P*<0.001, 95% *CI*=0.117, 0.484) than in HIV positive (10.6%, 20/88)(Table 1).

Table 1
Distribution of different intestinal parasites among HIV positive and negative subjects, Gambi higher clinic, Bahir Dar, Ethiopia, 2009 [*n*(%)].

	Type of parasites	HIV positive	HIV negative	Total	*P*- value
Opportunistic parasites	*Cryptosporidum parvum*	82(43.6)	0(0.0)	82(33.1)	-
	Isospora belli	29(15.4)	0(0.0)	29(11.7)	-
	Blastocystis	20(10.6)	0(0.0)	20(8.1)	-
Non-opportunistic parasites	*Entanoeba histolytica/dispar*	8(4.3)	4(6.7)	12(4.8)	0.448
	Giardia lamblia	2(1.1)	0(0.0)	2(0.8)	
	Ascaris lumbricoides	4(2.1)	5(8.3)	9(3.6)	0.025
	S.stercoralis	3(1.6)	1(1.7)	4(1.6)	0.969
	S.mansoni	0(0.0)	3(5.0)	3(1.2)	-
	T. trichiura	1(0.5)	2(3.3)	3(1.2)	0.084
	Hookworm species	2(1.1)	3(5.0)	5(2.0)	0.053
	Tinea species	0(0.0)	2(3.3)	2(0.8)	-

4.Discussion

HIV/AIDS pandemic has brought about a great change in intestinal parasite fauna. As the spectrum of immunodeficiency progresses, HIV infected individuals become susceptible to a variety of opportunistic parasite infections that occur with greater frequency and severity. Almost 80% of AIDS patients die from AIDS-related infections including intestinal parasites rather than HIV infection itself[4]. Several intestinal parasites previously considered non pathogenic or with transient pathogenic potential in immuno competent individuals are opportunistically becoming aggressive and causing debilitating illness in HIV/AIDS patients. Most of these infections are caused by organisms that do not normally affect immuno competent individuals[5].

In this study the high prevalence of opportunistic intestinal parasites *Cryptosporidium parvum, Cyclospora, Isospora belli* and *Blastocystis*, that are consistently found in HIV/AIDS patients with diarrhoea has been reported as the most likely causes of diarrhoea from elsewhere[14-16]. In HIV negatives, these opportunistic intestinal parasites were not found which is in agreement with what was reported by others and appears to be predominantly infected by the common intestinal parasites such as *Ascaris lumbericoides*, *Taenia* species and *Entamoeba histolytica/dispar*[5,6,17]. It has been suggested that HIV infection may have caused some change in the gut structure that may not be suitable for the common intestinal parasites[5,6,18].

The prevalence of *Cryptosporidum parvum* in the current study, which was 43.6%, was quite high when compared with a study conducted in different countries in Africa including Ethiopia. A study conducted in Jimma and Addis Ababa was reported prevalence of *Cryptosporidium parvum* was 17%[19]. Also it was quite higher when compared with a report from Ivory Coast (7.5%) and Zaire (8%)[20,21]. Generally the variation in the prevalence of cryptosporidial infections in diarrhoeal HIV patients could be related to the immune status of the HIV/AIDS patients examined, the sensitivity of the diagnostic techniques used and the experience of the technicians. Moreover, oocyst excretion is usually variable[22,23]. In addition, single stool specimen processing might underestimate the prevalence of cryptosporidial infection[23].

Compared to other tropical and subtropical countries, the prevalence of *Isospora belli* (15.5%) infection observed in this study was high. It was identified in approximately 15%-20% patients with chronic diarrhoea and AIDS; low prevalence was recorded in other African countries including Uganda (1.1%), Zaire (12%) and Tanzania (11.6%)[5,8,20]. It was also quite high with a report from Gondar[24] and from Addis Ababa[19] where a 2.4% and 1.4% prevalence rate of *Isospora belli* among HIV/AIDS patients with diarrhoea were reported. This may be because that the patients observed in the present study were free from antiretroviral treatment or they were HIV naive. However, this 15.5% prevalence of *Isospora belli* was lower as compared with a study from India, Ivory Coast and Zaire in which a high rate 16.6%, 17.9% and 19% of *Isospora belli* among HIV patients have been reported[17,20,21]. This might be due to high prevalence of this parasite in those target populations and geographic areas due to different factor like sanitation condition and water source of the study area.

With regard to helminths, *Strongyloides stercoralis* which considered as opportunistic parasite was less frequently (1.6%) detected from HIV positive patients. The difference observed in infection prevalence determinations between the present study and earlier reports among HIV/AIDS patients from Ethiopia[26-28] may be a reflection of the difference in the diagnostic method used, the endemicity and variation in infective dose of the parasite in certain locality and possibly the immune status of the patients.

Strongyloides stercoralis is one of the most difficult intestinal parasitic infections to diagnose with conventional stool examinations having low sensitivity even when examined several times. This is because the parasite load is low in the majority of the infected individuals and the larval output is minimal and irregular[29]. Serological methods have also their own limitations in endemic areas[30,31]. In the present study, direct and concentration methods were applied whereas the Baermann method could not be used, since the majority of the stool samples were watery diarrhoea and the debris could not be retained by sieving gauze. However, as described by others, the direct and concentration methods used in this study were the easiest and quite specific but extremely insensitive[32]. In rare occasion of hyperinfection leading to disseminated cases, the rhabditi from larvae (L_1) may be easily detected by these methods as lots of swarming larvae are known to occur.

A study conducted on randomly selected cohort participants at Wonji showed about 59% of *Blastocystis hominis* infection. This is in agreement with the rate recorded in developing countries which showed more than 50%[33]. However, in this study, 10.3% of HIV patients with diarrhoea harbouring *Blastocystis hominis* were too low. The observed difference among HIV/AIDS patients reported so far from Ethiopia could be due to difference in the experience of the technicians and/ or the clinical condition of the patients examined.

According to our finding diarrhea was one of the clinical manifestations in HIV patients. This might be caused by various etiological agents. Among those ethological agents coccidian parasites like *Isospora belli* and *Cryptosporidum* are the causative agent of life threatening chronic watery diarrhoea, weight loss and malabsorption. Since HIV/AIDS is a major problem in Ethiopia, it is recommended to extend the application of different diagnostic methods to clinic level and to referral peripheral laboratories for early detection of opportunistic intestinal parasites for better understanding and management of diarrhoeal illness in HIV/AIDS patients.

The general trend in prevalence and species of intestinal parasites fauna is dramatically changed with the HIV/AIDS epidemic in Ethiopia. In this study, intestinal protozoan parasites were found to be dominant as compared to helminthic infections in HIV patients. This finding calls for establishment of specific diagnostic tests in all health laboratories and train health professionals, with special attention to intestinal protozoan parasites. Again the majority of HIV/AIDS patients with diarrhoea had opportunistic intestinal parasites. Therefore, for proper management and

treatment of HIV/AIDS patients with chronic and intermittent diarrhoea, adequate diagnosis of opportunistic intestinal parasites must be carried out.

Conflict of interest statement

We declare that we have no conflict of interest.

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