

Prevalence of Intestinal Parasites in HIV-Infected adult Patients in Southwestern Ethiopia.

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Abstract

Background: Parasitic infection of the intestinal tract is a major source of disease in patients with HIV particularly in the tropics, where diarrhea is a common complaint with variable severity and specific pathogens are identified in more than half of the HIV/AIDS patients with persistent diarrhea.

Objective: The objective of this study was to determine the prevalence of intestinal parasites and their association with diarrhea of HIV infected patients.

Methods: A cross-sectional study was conducted on 372 consecutive patients from Feb.-July, 2001, on HIV infected and non-infected patients which were confirmed by Wellcozyme ELISA (Murex, UK). Chronic diarrhea was defined as three or more loose stools passed daily for more than two weeks. Parasite infections were diagnosed by examination of single stool specimen which were examined as fresh wet mounts, formol-ether concentration technique and Modified acid fast stain.

Result: Diarrhea was more prevalent in HIV infected 99(51.1%) than in HIV non infected patients 53(29.5%). Regardless of their diarrhea status, the general prevalence of intestinal parasites in HIV infected and HIV non-infected were 44.8% and 34.4% respectively. Among the 192 HIV infected patients 54 (28.1%) and 45(23.4%) of them had chronic and acute diarrhea respectively. The prevalence of intestinal parasites were 28(51.9%) in patients with chronic and 17(37.8%) in those acute with diarrhea. *C. parvum*, *I. belli*, and *C. catyensis* oocyst were detected only in HIV infected patients with chronic diarrhea ($P<0.001$, $P<0.01$, $P<0.01$ respectively), whereas the majority of (60%-100%) *S. stercoralis*, *S. mansoni*, *E. histolytica*, and *G. lamblia* were detected in diarrheic stool samples of HIV infected patients ($P<0.05$).

Conclusion: This study re-affirms the previously held view that in more of HIV patients with chronic diarrhea etiologic agent can be identified. Diarrhea and intestinal parasites prevalence were higher in HIV infected than HIV non-infected patients. Intestinal coccidian are opportunistic infections which are found exclusively in HIV patients with chronic diarrhea. [*Ethiop.J.Health Dev.* 2003;17(1):71-78]

Introduction

Gastrointestinal involvement in HIV/AIDS is almost universal, and significant disease occurs in 50-96% of patients. Diarrhea can be a presenting manifestation or a life threatening complication of infection with HIV sometimes during the course of the disease. Infectious causes of diarrhea have been found in 30-80% of patients depending on the extent of the study

and patient characteristics. Such pathogens include opportunistic agents that consistently cause severe, chronic, or frequent gastrointestinal disease and non-opportunistic agents that usually cause acute, treatable diarrhea illness (1-5). The etiology for such diarrhea could be either parasitic, bacterial, fungal, enteric virus or HIV itself may contribute to the diarrhea. In addition to microbes, other factors such as medication, immune deregulation, autonomic dysfunction and nutritional supplementation play substantial role in diarrhea of HIV/AIDS patients (4,6,7). Several species of protozoa have been associated with acute and chronic diarrhea in

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HIV diseases. These include: *Cryptosporidium parvum*, *Isospora belli*, *Microsporidia* species, *Giardia intestinalis*, *Entamoeba histolytica*, *Cyclospora species*, *Blastocystis hominis*, and *Dientamoeba fragilis*, but convincing evidence is lacking as to the causality of last two protozoans. Besides this, the nematode *Strongyloides stercoralis* a ubiquitous parasite in tropical and subtropical areas, can cause diarrhea and overwhelming infestation (hyperinfection syndrome) in patients with variety of immunosuppressive disorders, including HIV/AIDS (7-10). Studies on HIV infected patients show that no etiological agent is found in 15-50% of patients with chronic diarrhea. The two most commonly (up to 60%) diagnosed pathogens were *C. parvum* and *I. belli*. *G. intestinalis* and *E. histolytica*, organisms often associated with diarrhea in the tropics, were very uncommon in AIDS patients except the former organism in Buenos Aires and, the latter in Maryland and Ivory Coast, which account for 36%, 25% and 21% of patients respectively. In addition to this, Strongyloidiasis is also found in higher proportion (17%) in Ivory Coast (5,7-17). *Cyclospora*, a newly recognized human protozoan parasite that causes prolonged watery diarrhea in traveler and HIV/AIDS patients, has limited epidemiological relevance especially in association with HIV infection. A study done in Thailand shows a prevalence of 2.2% in HIV infected patients (17-18).

Infective causes of chronic diarrhea are perhaps the most satisfying to diagnose because with the exception of cryptosporidiosis and HIV related enteropathy one can expect good response to treatment. Unfortunately, all etiologic agents can not be diagnosed in Africa on routine basis because of limited diagnostic facilities and trained personnel (18). Since the pathogens responsible in different geographic area are not similar, laboratory diagnostic evaluations are needed to determine disease prevalence in a specific population so that it can provide guidelines for empirical treatment

for treatable etiologic agents and necessary data for planning and evaluation of HIV/AIDS care. No similar studies have been done in Jimma Zone. Hence, this parasitological investigation was designed to determine the prevalence of intestinal parasites and their association with diarrhea of HIV infected patients.

Methods

Study design: A cross-sectional study was conducted from Feb.-July, 2001 on HIV infected and non-infected patients in order to determine the prevalence of intestinal parasites and their association with diarrhea of HIV infected patients. Serological testing for HIV antibodies was done using Wellcozyme ELISA (Murex, UK) in the Jimma Zone Regional Blood Bank Laboratory. Following verbal consent, socio-demographic and clinical information were obtained from the study subjects using interview by pre-structured questionnaire. All stool samples for parasitological investigation were processed in the Microbiology and Parasitology Laboratory of Jimma University, without prior knowledge of the study subjects' HIV status. Hence, each stool sample had almost equal emphasis during the laboratory investigations.

Patients selection: Consecutive patients during the study requested to have serological detection of their HIV status for their various clinical background were included. From each patient a single sufficient stool sample was collected for parasitological investigation. Chronic diarrhea was defined as three or more loose stool passed daily for more than two weeks (4).

Stool examination: All stool specimens were examined for parasite. Parasitic infections were diagnosed by examination of single stool specimens as fresh wet mounts, formol-ether concentration technique and Modified acid fast stain (AFS). Fresh stool specimens were examined as saline wet mount to detect motile trophozoites. Formol-ether concentration was

subsequently performed and the sediment examined as iodine wet mounts to detect ova, larva and cysts. Air dried smears from fresh stools samples were stained by a modified AFS (Fixed in methanol 3 min, stained by carbol fuschine 10min, decolorized in 1% HCl in methanol 3min, washed in running water, counter stained 0.25% methylene blue 30 sec, washed in running water and dried) to detect *Cryptosporidium*, *Isospora* and *Cyclospora* species.

Statistical methods: Statistical analysis were done using Chi-square to evaluate any association between HIV, diarrhea and parasitic infections. Observed differences in data were considered significant and noted in the text if $P < 0.05$ was obtained.

Results

During the 6 months of study period, 372 consecutive patients were examined for intestinal parasites. Among these patients, 192 were HIV infected and 180 were HIV non-infected patients.

The age and sex distribution of the study subjects is presented in table 1. HIV infected and HIV non-infected in the age ranges of 25 to 34 years of both sexes were the predominant age group (37.6% and 39.9%) respectively.

Diarrhea is more prevalent in HIV infected 99(51.1%) than HIV non-infected patients 53(29.5%). Regardless of their diarrhea status, the general prevalence of intestinal parasites in HIV infected and HIV non-infected were 44.8% and 34.4% respectively.

Among the 192 HIV infected patients 54 (28.1%) and 45(23.4%) of them had chronic and acute diarrhea respectively. Intestinal parasites were detected 28(51.9%) in patients with chronic diarrhea and 17(37.8%) of patients with acute diarrhea. Helmenthic infections were more common than protozoan in both HIV infected and HIV non-infected (65.1% and 62% respectively).

All opportunistic coccidian parasites (i.e., *C. parvum*, *I. belli*, and *C. catyensis*) oocyst were detected only in HIV infected patients with chronic diarrhea ($P < 0.001$, $P < 0.01$, $P < 0.01$ respectively). *Ascaris*, *Trichuris* and Hook worm species (*Ancylostoma duodenale* and *Necator americanus*) were detected more in non-diarrheic stool samples of HIV infected ($P < 0.05$), whereas the majority of (60%-100%) *S. stercoralis*, *S. mansoni*, *E. histolytica*, and *G. lamblia* were detected in diarrheic stool samples of HIV infected patients ($P < 0.05$).

Table 1: **Age and sex distribution of HIV infected and HIV non-infected in Jimma Hospital, 2001**

Age n=180 (Year)	HIV infected n=192		HIV non-infected	
	No (%)		No (%)	
	M	F	M	F
15-20	14(7.3%)	25(13.0%)	30(16.7%)	34(18.9%)
25-34	36(18.8%)	36(18.8%)	48(26.6%)	24(13.3%)
35-44	43(22.4%)	14(7.3%)	26(14.4%)	10(5.6%)
>45	17(8.9%)	7(3.7%)	4(2.2%)	4(2.2%)
Total	110(57.3%)	82(42.7%)	108(60%)	72(40%)

Besides this *S. stercoralis*, *S. mansoni*, *Trichuris trichuria*, hook worm species and *Trichostongylus colubriform* had higher frequency of detection in HIV infected than HIV non-infected ($P < 0.01$). *T. colubriform* was found only in HIV infected as shown in table 3.

However, no statistical significance differences existed in the distributions of *A. lumbricoides*, *T. trichuria*, *E. histolytica* and *G.lambliia* between HIV infected and HIV non-infected patients ($p > 0.05$).

Table 2: Prevalence of intestinal parasites among HIV infected and HIV ono-infected patients in relation to their diarrhea status, in Jimma Hospital, 2001

Status of Total Diarrhea	Cases, n (%)				HIV non-infected, n (%)	
	Parasite +	Parasite -	Parasite +	Parasite -	Parasite +	Parasite -
Acute	17(37.8%)	28(62.5%)	20(62.5%)	12(37.5%)	45	32
Chronic	28(51.9%)	26(48.1%)	8(38.1%)	13(61.9%)	54	21
No Diarrhea	41(44.1%)	52(55.9%)	34(26.8%)	93(73.2%)	93	127
Total	86(44.8%)	106(55.2%)	62(34.4%)	118(65.6%)	192	180

Parasite+ = Parasite Present

Parasite - = Parasite Absent

Multiple infections were also observed in a total of 41 stool samples, where 27 of them were from HIV infected and 14 from HIV non-infected patients. The pattern of parasite combinations in HIV infected were 2,3,4 and 5

in 16(59.3%), 7(25.9%), 3(11.1%), and 1(3.7%) samples respectively. In the HIV non-infected patients the combinations are only 2 and 3, where 12(85.7%) of them had a combinations of 2 parasites.

Table 3: Prevalence of specific intestinal parasite among HIV infected and non-infected patients in relation to their diarrhea status in Jimma Hospital, 2000.

Types of Parasite	Diarrhea-status							
	Acute		Chronic		No diarrhea		Total	
	+	-	+	-	+	-	+	-
<i>Ascaris lumbricoides</i>	5	6	6	1	12	15	23	22
<i>Trichuris trichuria</i>	5	5	7	3	16	10	28	18
Hook worms	5	2	6	3	15	7	26	12
<i>S. stercoralis</i>	4	1	6	2	5	1	15	4
<i>T. colubriform</i>	1	-	1	-	1	-	3	-
<i>Taenia species</i>	-	1	-	-	3	1	3	2
<i>Hymenolopeis nana</i>	1	-	-	-	1	-	2	-
<i>S. mansoni</i>	5	1	4	-	2	3	11	4
<i>E. histolytica</i>	6	11	9	2	7	9	22	22
<i>G.lambliia</i>	3	4	1	-	-	1	45	-
<i>C.parvum</i>	-	-	6	-	-	-	6	-
<i>I.belli</i>	-	-	4	-	-	-	4	-
<i>C. catyenesis</i>	-	-	2	-	-	-	2	-
Total	34	32	52	11	61	48	146	92

+ = HIV infected

- = HIV non-infected

Discussion

The HIV prevalence is higher in the age range of 15-34 while it was also higher in males in

the age range 25-44 years. This is similar to the HIV/AIDS profile in the general population reported by Ministry of Health (19). The

finding of this study shows that pathogenic and opportunistic intestinal parasites are common among Jimma Hospital HIV/AIDS patients with chronic diarrhea. This is consistent in two different studies done in Addis Ababa (14,15), and also in Tanzania (18), and in Uganda (20).

Among opportunistic protozoan parasites *C. parvum*, *I. belli*, and *C. cayenensis* oocyst were isolated from 6(11%), 4(7.4%) and 2 (3.7%) of HIV infected patients with chronic diarrhea respectively. The prevalence of *C. parvum* in this investigation (11%) agree with a similar studies done in Jordan (10%), Cuba (11.9%), and India (11%) (21,23,24). However, they are lower than in studies done in different countries including Ethiopia which reported a magnitude of (15-48%) (5,12,14,-17,20). This could probably be due to use of more sensitive detection method. Moreover, oocyst excretion is usually low and variable hence multiple examinations and/or prior concentrations of stool specimens may be necessary. Unlike the above studies, this study used a single stool specimen in one occasion only and without prior concentrations or the good immune status of the patient as a result of early HIV infection even though the patient later turned out to be sero-HIV infected (because many patients develop Cryptosporidium associated disease at advanced stages of HIV infection) or it is an indication of low prevalence in this locality, are the most probable explanations.

I. belli were detected in a higher rate (7.4%) than reported in Addis Ababa (1.4%), Egypt (0.9%), Jordan (3%) and Cuba (1.5%) (15,21-23). However, it is consistent with the study done in Zaire (12). On the other hand, it is lower than similar studies done in other African countries which report (10-15%) (4,12,13,20) and up to 27% in India (24) and 85% of AIDS patient in Haiti (10). This could be probably due to the application of poor detection

methods (i.e., inability to concentrate the stool before smear preparation for staining) or low magnitude of this parasite in the study area.

Cyclospora species were also identified in two (3.7%) HIV infected, which is an emerging pathogen. There is no sufficient literature for comparison; studies done in Thailand and Cuba reported a prevalence of 2.2% and 3.0% among HIV infected patients respectively, which is comparable with this study (17,24). Of the protozoan infections, *Cryptosporidium parvum*, *Isospora belli* and *Cyclospora cayenensis* are opportunistic infections that are consistently found in adult HIV/AIDS patients with chronic diarrhea which is also confirmed in this study. On the other hand, in immunocompetent adult *C. parvum* and *C. cayenensis* are often associated with water borne outbreaks of acute diarrheal illness (10,18). But there is no such report in our country.

The other protozoan infections (i.e., *G. lamblia* and *E. histolytica*) have not been found to be opportunistic in HIV infected patients because there is no evidence for an increased prevalence as observed also in this investigations or altered natural history of these parasitic infections in HIV patients despite the fact that important immune defenses against them may be expected to be deranged by HIV infection. Hence, exposure to *G. lamblia* and *E. histolytica* are likely to occur independently of HIV-infection, but heavier parasite loads may accumulate as well as experience delayed clearance of the parasite in individuals with concurrent HIV-induced immunosuppression. *E. histolytica* cyst carriage is common throughout the tropics and is not associated with diarrhea in patients with HIV. Even though some literature grouped these parasites in association with HIV/AIDS patients with diarrhea, they can cause diarrhea and intestinal

malabsorption regardless of the patients HIV status (2,8,16,25,26).

The laboratory diagnosis of the intestinal parasite was made by light microscope which has the disadvantages that it fails to distinguish between the cysts of the pathogenic *Entamoeba histolytica* from the recently recognized non-pathogenic *Entamoeba dispar*. New and sophisticated techniques such as Polymerase chain reaction technique, Isoenzyme analysis and Antigen detection are necessary for specific identification. Unfortunately such diagnosis is not available in developing countries like Ethiopia. It is estimated that *E. dispar* infections are approximately 10-fold more common than *E. histolytica* infections and *E. histolytica* is thought to be responsible for nearly all reported morbidity. Therefore, the role played by each amoeba in HIV/AIDS population is not determined which should be done on further study and report of *E. histolytica* cyst also includes that of *E. dispar* (26).

Although concurrent infections of helminthes and viruses are common, with the exception of *Strongyloides stercoralis*, previous report did not show any interaction between HIV and helminthic infections (16,26). In Jamaica, a strong association was shown to exist between HIV infection and parasitologically proven *S. stercoralis* (27,28). However, HIV infected of hyperinfection syndrome have been noted to be very rare (5). In this study it was observed that the prevalence of *Strongyloides* is (11.1%), higher than that reported from Addis Ababa (3.4%), Thailand (4.4%) and Haiti (4%) of HIV patients with chronic diarrhea (13,15,17). But it agrees with the study done in the Jimma Town (9%) which also reported the presence of disseminated *Stongyloidisis* in 3.3% of the population (29). Most *Strongyloides* detected in HIV/AIDS patients were found in higher load than HIV non-infected patients. In addition to this, 79% of the *Strongyloides* were detected in cases, which is about four times

compared with the prevalence of this parasite in HIV then HIV non-infected patients. Nevertheless, disseminated malignant strongyloides have been observed more frequently infected by HTLV-1 which, for unknown reasons, seems to facilitate these disseminated strongyloides more than HIV (28).

Like *S. stercoralis*, *S. mansoni* was also detected more frequently (70%-75%) in HIV infected and control. The association between schistosomiasis and *Salmonella* bacteraemia has been well documented. More recently, acute schistosomiasis has been shown to be a facilitating factor in the genesis of pyogenic liver abscesses caused by *Staphylococcus aureus*. New evidences indicate the presence of interaction between HIV/AIDS and schistosomiasis (30).

S. mansoni, *G. lamblia*, and *E. histolytica* may have a significant role in resulting diarrhea as (68%-82%) of these parasites were found in HIV patients with diarrhea. The occurrence of *Trichostrongylus colubriformis* in this study was associated with three HIV-infected individuals. *T. colubriformis* is ubiquitous parasite of small ruminants in the region. Inadequate personal hygiene or close association with the reservoir host would favour transmission of this spp. to man. This agree with our finding that all the three patients were farmers who came from rural areas around Jimma Town.

The overall prevalence of intestinal parasites were found to be significantly higher in Adult HIV infected patients than HIV non-infected ($P < 0.005$). This is probably due to easy detection of parasite in patients infected with HIV. Since parasite establishment is facilitated, it will increase the load of parasite hence they can be easily detected and identified in stool examination. As a result the intestinal parasite prevalence will be higher in HIV infected than HIV non-infected patients.

The present study indicate that HIV-infected patients are, in the main, not predisposed to infection with intestinal parasites at the back through exposure to infective stages in the environment, independent of any possible alteration host immunological status by HIV infected. Host immune responses are nevertheless likely to have effect on the establishment of patent infections as well as influence on parasite loads. Identification of these common parasites in up to 34.8 to 44% of HIV/AIDS patients and the HIV non-infected is a reflection of poor environmental hygiene.

Diarrhea and intestinal parasites prevalence were higher in HIV infected than HIV non-infected patients. This study re-affirms the previously held view that in more of HIV positive patients with chronic diarrhea etiologic agent can be identified. *C. parvum*, *I. belli*, *C. catyenesis*, *S. stercoralis*, *S. mansoni*, *E.histolytica*, and *G. iamblia* are associated in diarrhea of HIV patients. The intestinal coccidian are opportunistic protozoan infection which are found exclusively in HIV patients with chronic diarrhea. Newly recognized organisms such as *Microsporidia* species, that are found in AIDS patients with chronic diarrhea may play an important role in Africa. However, this study fails to detect these parasites due to lack of reagents. The etiology of chronic diarrhea in HIV/AIDS patient appears to be multifactorial because in high (48.1%) proportion of patients in this investigation, it was not possible to identify parasitic agent. Hence, comprehensive etiological studies should be conducted in the future including viral, fungal bacterial and parasitic causes of diarrhea not included in this study.

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