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Intestinal Parasitosis in Relation to CD4+T Cells Levels and Anemia among HAART Initiated and Non-HAART Initiated Pediatric HIV Patients in Zewditu Memorial Hospital, Addis Ababa, Ethiopia

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This is to certify that the thesis prepared by Hylemariam Mihiretie, entitled: *Intestinal Parasitosis in Relation to CD4+T Cells Levels and Anemia among HAART Initiated and Non-HAART Initiated Pediatric HIV Patients in Zewditu Memorial Hospital, Addis Ababa, Ethiopia* and submitted in partial fulfillment of the requirements for the degree of Master of Science in Clinical Laboratory Sciences (Diagnostic and Public Health Microbiology Specialty) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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Abbreviations/ Acronyms

3TC= Lamivudine

AAU=Addis Ababa University

ABC= Abacavir

AIDS=Acquired Immunodeficiency Syndrome

ART=Anti-Retroviral Therapy

AZT= Zidovudine

CD4=Cluster of Differentiation 4

d4T= Stavudine

DDI= Didanosine

EFV= Efavirenz

EPHI= Ethiopian Health and Nutrition Research Institute

FMOH= Federal Ministry of Health

HAART=Highly Active Antiretroviral Therapy

HIV=Human Immonodeficiency virus

IP/s= Intestinal Parasite/s

IPI=Intestinal Parasitic Infections

IRB=Institutional Review Board

LPV/r= Lopinavir

NVP= Neverapine

Spp= Species

WHO=World Health Organization

ZMH= Zewditu Memorial Hospital

Z-N=Ziehl-Neelson

Abstract

Background: Intestinal parasites (IPs) are major concerns in most developing countries where HIV/AIDS cases are concentrated and almost 80% of AIDS patients die of AIDS-related infections. In the absence of HAART, HIV/AIDS patients in developing countries unfortunately continue to suffer from the consequences of opportunistic and other intestinal parasites.

Methods: A comparative cross-sectional study was conducted among HAART initiated and non-HAART initiated pediatric HIV/AIDS patients of Zewditu Memorial Hospital (ZMH) between August 05, 2013 and September 25, 2013. A total of 180 (79 HAART initiated and 101 non-HAART initiated) children were selected by using consecutive sampling. Stool specimen was collected and processed using direct wet mount, formol-ether concentration and modified Ziehl-Neelson staining techniques. A structured questionnaire was used to collect data on Socio-demographic and associated risk factors. Data were entered and analyzed using SPSS version 16 software and logistic regressions were applied to assess any association between explanatory factors and outcome variables. P values < 0.05 were taken as statistically significant.

Results: The overall prevalence of IPs was 37.8% where 27.8% of HAART initiated and 45.5% of non-HAART initiated pediatric HIV/AIDS patients were infected with IPs ($p < 0.05$). *Cryptosporidium* species, *E. histolytica/dispar*, *Hook worm* and *Taenia* species were IPs associated with lower CD4+ T cell counts <350 cells/μL or 25% in non-HAART patients. The overall prevalence of anemia was 10% in HAART and 31.7% in non-HAART groups. Intestinal parasitic infection (IPI) was significantly associated with anemia in non-HAART patients [AOR, 95% CI: 4.5(1.3, 15.2), P< 0.05]. *Hook worm*, *S. stercoralis* and *H. nana* were helminthes associated with anemia in non-HAART patients. The prevalence of IPs in non-HAART patients was significantly associated with Eating unwashed/raw fruit [AOR, 95%CI: 6.3(1.2, 25.6), P<0.05], open field defecation [AOR, 95%CI: 9.3(1.6, 53.6), P<0.05] and diarrhea [AOR, 95%CI: 5.2(1.3, 21.3), P<0.05]. IPs significantly increased in rural residents [AOR, 95%CI: 0.4(0.1, 0.9)] and in those with abdominal pain [AOR, 95%CI: 21(3.7, 121)] in both groups.

Conclusion: The overall prevalence of intestinal parasites (IPs) differed by HAART status and *cryptosporidium* species were found only in HAART naïve patients with low CD4+ T cell counts. Anemia was also more prevalent and associated with IPs in non-HAART patients. This study identified some environmental and associated risk factors for intestinal parasitic infections. Therefore, Public health measures should continue to emphasize the importance of environmental and personal hygiene to protect infections with intestinal parasites and maximize the benefits of highly active anti-retroviral therapy (HAART).

1. Introduction

1.1. Background

Intestinal parasitic infection is a serious public health problem throughout the world particularly in developing countries. *Amoebiasis, Ascariasis, Hookworm* infection and *Trichuriasis* are among the ten most common infections in the world [1].

In children, intestinal parasitic infections, particularly geo- helminthiasis is the cause of common health problems in tropical countries. Several factors like climatic conditions, poor sanitation, unsafe drinking water, and lack of toilet facilities are the main contributors to the high prevalence of intestinal parasites in the tropical and sub-tropical countries. Further, lack of awareness about mode of transmission of parasitic infections increases the risk of infection [2].

Studies have shown higher rates of parasitic infections occur in HIV/AIDS positive than negative subjects. HIV/AIDS compromises the immune system of the body thus patients are threatened by a great number of diseases caused by different kinds of biological agents including bacterial, fungal, viral, protozoan and intestinal parasitic pathogens. In such patients, opportunistic parasitic gastrointestinal infections presents in the form of severe diarrhea which profoundly compromises the absorptive function of the small intestine, leading to significant mortality [3].

On the other hand, intestinal parasitic infections also cause chronic immune activation which has been shown to increase host susceptibility, thereby promoting HIV/AIDS infection and disease progression. Infections with the major human gastrointestinal parasites have been shown to be rampant in most parts of Africa and the prevalence of these infections varies from country to country [4-6].

With impaired immunity especially in patients with CD4+ T cell counts < 200 cells/mm^3, infections with opportunistic intestinal parasites result in diarrheal symptoms. A study has shown that patients with CD4+T cell count of > 180 cells/mm^3 usually have self-limiting infections, whereas most patients with counts < 140 cells/mm^3 develop severe and persistent infections. With the introduction of HAART which partially restores the immune function, the incidence of opportunistic parasitic infections such as cryptosporidiosis has declined [5].

Anemia, which can be mild, moderate or severe, is one of the several complications associated with parasitic infections. Anemia is also a frequent complication of HIV/AIDS which contributes to reduced quality of life and increased morbidity and mortality [6].

Although, anemia and intestinal parasitic infections have been reported as comorbidities in HIV infected patients [6], there is paucity of information in Ethiopia if these triple burdens (HIV, intestinal parasitic infections and anemia) coexist and associate with CD4+ T cell levels in HIV infected children.

1.2. Statement of the Problem

Intestinal parasitic infections are among the most common infections worldwide, the majority being children [7]. The rate of parasitic infection is remarkably high in sub-Saharan Africa, where the majority of Human Immunodeficiency virus (HIV) and AIDS cases are concentrated. In Ethiopia too, the ecology of intestinal parasitism is very wide [7, 8, and 9]. Intestinal parasitic infections, mostly helminthes, have been linked with an increased risk of nutritional anemia, protein-energy malnutrition and growth deficits in children [10].

Diarrhea, which is caused by opportunistic protozoa, is a cause of considerable morbidity and mortality. It occurs in almost 90% of the AIDS patients in the developing countries. In HIV infected patients, the progressive decline in their immunological responses makes them extremely susceptible to a variety of common and opportunistic infections [11].

Younger children are predisposed to heavy infections with intestinal parasites since their immune systems are not yet fully developed, and they also habitually play in fecally contaminated soil. In addition to considerable mortality and morbidity, infection with intestinal helminthes has been found to profoundly affect a child's mental development, growth and physical fitness while also predisposing children to other infectious agents [12, 13].

HIV infection has increased significance of parasitic infection especially opportunistic intestinal parasites. More importantly, with emergence of AIDS, the epidemiology as well as outcome of diseases caused by opportunistic parasites was significantly modified. But, the effect of HIV on some other parasites is not clearly understood. Overall, either backed by HIV or independently, intestinal parasitic infections have continued to be major cause of morbidity and mortality in humans [14].

Intestinal coccidian infection including *Cryptosporidium, Isospora, Cyclospora* and *Microsporidia* are increasingly becoming prevalent in AIDS patients. Intestinal infection by *Cryptosporidia* is self-limited, but leads to persistent diarrhea in the advanced stage of AIDS and there is no effective treatment available for it. *Isospora* causes chronic diarrhoea in AIDS

patient, but can be treated effectively with available antimicrobials. *S.stercoralis* is an important human parasitic infection primarily because of its potential for serious and even lethal disease in immunosuppressed patients [15].

In a study done in Zambia, the mortality rate was much higher in HIV seropositive children compared to HIV seronegatives. The 1-month mortality rates for HIV-seropositive children with parasitic infection and HIV seronegative children were 24% and 11%, respectively. Children with cryptosporidiosis also experienced higher mortality: 18 of 51 children with cryptosporidiosis died compared with 21 of 148 without [16].

In Ethiopia, intestinal parasitic diseases are among the ten top causes of morbidity nationwide. Most of the intestinal parasites are more common and their manifestations are more severe in children than adults. Infection in children is also associated with malnutrition, growth retardation and poor school performance [17].

Most of the previous comparative studies conducted in Ethiopia have focused on the prevalence and distribution of intestinal parasitic infections mainly in ART and pre-ART adults but the pediatrics population is usually untouched. This initiated us to conduct a research on intestinal parasitosis in HAART and non-HAART pediatric HIV patients in relation to anemia and CD4+ T cell levels.

1.3. Significance of the Study

Ethiopia is among the sub-Saharan countries with overlapping high rate of HIV and parasitic infections. Intestinal parasites are highly prevalent in Ethiopia due to shortage of clean water, lack of sewage system and other unhygienic factors that increase the probability of infection. However, there have been few studies to ascertain whether epidemiology of intestinal parasites take different picture as pediatric population with HIV/AIDS is growing.

Only a few studies have reported the magnitude of intestinal parasitic infections among under-five children and adults but not in those pediatric HIV patients. The present study is, therefore, aimed to determine the prevalence of intestinal parasites in association with CD4+ T cell levels and anemia in HAART initiated and non-HAART initiated pediatric HIV patients.

The Federal Ministry of Health is now working to eliminate tropically neglected diseases and minimize mothers and child mortality as a millennium development goal by the year 2020. Most of the targets are soil transmitted helminthes and other intestinal parasites. This study, therefore, will provide data for the policy makers, clinicians and other stake holders to know the prevalence of intestinal parasites in Pediatric HIV patients in relation to HAART, anemia, CD4 count and other clinical variables in the study area.

Furthermore, the data obtained from this study will serve as an input for pediatric HIV clinicians to decide about the need of further care, HAART initiation, health education and management of intestinal parasites in pediatric HIV patients under follow up care in Zewditu Memorial Hospital.

2. Literature Review

2.1. Intestinal Parasitosis, Anemia and CD4+ T cell count in HIV/AIDS Patients

Opportunistic intestinal parasitic infections cause severe diarrhea especially in infants and in immune-compromised people worldwide. A study in Jakarta, Indonesia showed that *Cryptosporidium* was identified in two children who were HIV infected with CD4 percentages < 15%. Parasites were most frequently found in preschool age children (16/23), in those with recurrent or watery diarrhea (23/24 and 14/18, respectively), and in HIV subjects not receiving antiretroviral treatment (16/22) [18].

A study done in Central Brazil showed that intestinal parasites were detected in 27% of HIV patients and 17.6% of non-HIV infected patients. In HIV patients the most frequent parasites were *Strongyloides stercoralis* (12%), with 2 cases of hyper-infection; *Isospora belli,* 7%; *Cryptosporidium* sp., 4%; with 1 asymptomatic case and hookworm, 4% [19].

Data from Nepal showed that 25% of symptomatic and 2.8% of asymptomatic patients harbored one or more intestinal parasites. Moreover 12.3% of the study population had intestinal parasites with 7.3% being infected with opportunistic parasites. The mean CD4 count of healthy subjects was 307cells/μL while those with parasites were 204 cells/μL[20].

According to a study in Vietnam, the prevalence of anemia in children was 25% and mean hemoglobin concentration was 120 ± 8.3 g/L. Children infected with *Hookworm* and *Trichuris* showed a lower hemoglobin concentration and a higher prevalence of anemia [21].

Two and a halve times higher prevalence of intestinal parasites was reported by another study from Nigeria among the HIV seropositive patients than the seronegative ones. Patients with CD4+ T cell count <200/μL had more coccidian parasites in their stool and also had higher prevalence of intestinal poly-parasitism ranging from 2 to 4 different species per stool sample. The rate of detection of parasites that were AIDS defining was also higher among patients whose CD4+ T cell count was less than 200cells/μL. About 50% of all the cases of isolated *Isospora belli* and 62% each of *Cryptosporidium* and *Cyclospora* spp were from these severely

immunocompromised hosts. So were 53% and 56% of *Strongyloides stercoralis* and *Giardia lamblia,* respectively [22].

In line with the report from Brazil [19], a study done in Cameroon revealed 27.8% overall intestinal parasites prevalence in HIV patients. Seventeen (45.9%) out of 37 patients with diarrhea and 38 (23.2%) out of 164 without diarrhea were parasitized. The most frequent parasites were *Cryptosporidium spp*. (7.4%) and *Entamoeba histolytica/ dispar* (3%). The highest parasite counts ($p=0.035$) and diarrhea ($p<0.0001$) were found in patients with CD4 < 200 cells/µL [23].

Different degrees of intestinal parasitosis at the different CD4 categories were also demonstrated in a study conducted in Malaysia. The number of patients infected with intestinal parasites ranged from 49% (48 of 98) in the category of CD4 counts of < 50 cells/mm3 group, 18.3% (18 of 98) and 17.3% (17 of 98) in patients with CD4 counts of 51-100 cells/mm3 and 101-200 cells/mm3 respectively, whilst 11.2% (11 of 98) in CD4 counts of 201-400 cells/mm3 and only 3.0% (3 of 98) in CD4 > 400 cells/mm3. It was observed that *E. histolytica/dispar, A. lumbricoides, Cryptospordium* spp. and *Isospora* spp. were more common as compared to other parasites. All in all the maximum parasitic isolation was in the group of patients who had CD4 counts below 200 cells/mm^3 [24].

According to a research done in China, the overall prevalence of intestinal helminthic infections among HIV positives was 4.3% (13/302). The prevalence of protozoa infections among HIV positives was 23.2% while the rate was 25.8% among HIV negatives. The species-specific prevalences among HIV positives were as follows: 3.6% for hookworm, 0.7% for *Trichuris trichiura*, zero for *Ascaris lumbricoides*, 0.3% for *Clonorchis sinensis*, 1.3% for *Giardia intestinalis*, 16.2% for *Blastocystis hominis*, 1.7% for *Entamoeba spp*. and 8.3% for *Cryptosporidium spp*. *Cryptosporidium spp*. infections were significantly more prevalent among HIV positives (8.3%) compared to the HIV negative group (3.0%; $P < 0.05$) [25].

Higher prevalence rates of intestinal parasites in both HIV infected and non-infected individuals were reported from Northeast Iran. In this study, the overall prevalence of intestinal parasites among HIV positive population was 67.7% and in control group was 55% without significant

difference between the two groups. More specifically, the following parasites were identified in the HIV infected group: *Giardia lamblia* 22.6%, *Blastocystis hominis* 22.6%, *Chilomastics mesnili* 22.6%, *Entamoeba coli* 9.7%, and *Entromonas* 3.2%. In the control group *Entromonas* (45%), *B. hominis* (15%), *E. coli* (10%), *G. lamblia* (5%), and *Hymenolepis nana* (10%). However, the prevalence of *G. lamblia*, *B. hominis and C. mesnili* was greater for HIV positive patients ($p<0.05$) [26].

In another study in South West Ethiopia, regardless of diarrhea status, the general prevalence of intestinal parasites in HIV infected children was 44.8%. Among these, *C. parvum, I.belli and C.cayatenesis* were detected in HIV patients with chronic diarrhea. Majority of *S.stercolaris, S.mansoni, G.lamblia and E.histolytica* were found in acute diarrheic HIV patients [8].

2.2. Intestinal Parasitosis and HAART in HIV/AIDS patients

Several studies demonstrated a lower prevalence of intestinal parasitosis in HAART taking compared to non-HAART taking patients. A study in Brazil showed that intestinal parasites were detected in 63.9% of the patients of pre-HAART group , while in HAART group this prevalence was 24% ($p<0.0001$). A significant reduction in the prevalence of the helminthes: *Strongyloides stercoralis*, *Ascaris lumbricoides*, hookworms and *Trichuris trichiura* was observed in the HAART era in comparison with the pre- HAART era ($p<0.0001$). Cryptosporidium was observed in 8.1% of non-HAART but not detected in HAART group [27].

With respect to intestinal protozoans, a statistically significant reduction was seen in *Giardia duodenalis* ($p = 0.0076$) and *Cryptosporidium sp*. ($p=0.0007$) between the HAART and pre-HAART era; the latter had a prevalence of 8.1% in pre- HAART group but was not detected in HAART Group. *Strongyloides stercoralis* was the most frequent intestinal parasite in the two eras, with a prevalence of 30.1% and 11%, respectively, occurring more often in men (32.41%) than in women (19.04%) of the pre-HAART era ($p = 0.018$) [27].

The difference in the occurrence rate of intestinal parasitic infection between the HIV infected children who received antiretroviral (2/4) and those who did not (14/18) were observed with a 0.56 prevalence ratio (95%CI 0.2-0.63) in Indonesia [18].

In non- HAART HIV patients in Nigeria, 93.3% of HIV infected study subjects had anemia and 18% of them had parasitic infections. *Isospora belli* and *Cryptosporidium* species were the only intestinal parasite associated with CD4 count <200cells/μL. *Cryptosporidium* species, *Ascaris lumbricoides*, hookworm and *Taenia* species were the intestinal parasites associated with anaemia. The prevalence of intestinal parasites were *Entamoeba histolytica* (3.33%), *Giardia intestinalis* (0.56%), *Isospora belli* (7.78%), *Cryptosporidium* species (22.22%), *Ascaris lumbricoides* (33.06%), Hookworm (20.56%), *Strongyloides stercoralis* (6.39%), *Trichuris trichuria* (5.00%) and *Taenia* species (1.11%) [6].

According to a research done in Nigeria, CD4 count < 200 cells/mm^3 and diarrhea were significant risk factors for acquiring intestinal parasitic infections in HIV-positive HAART initiated patients. Anemia was significantly associated with intestinal parasitic infections. *Ascaris lumbricoides, hookworm and Strongyloides stercoralis* were the only intestinal parasites recovered and it showed a low prevalence (5.3%) of intestinal parasitic infections among HAART patients, whereas opportunistic coccidia were not detected [28].

On the other hand, according to a study in Gondar, although a higher rates of intestinal parasites (43.5%) were detected among the pre-ART group compared to those who started ART, 24.3% ($p<0.01$), no statistically significant difference was noted in terms of individual parasite prevalence between the two groups ($p>0.05$) [29].

The prevalence of opportunistic intestinal parasites was significantly higher in pre-ART diarrheal subjects than that on-ART ($P<0.05$) in Nekemte. In addition, a large number of opportunistic intestinal parasites were detected in diarrheal subjects when compared to non-diarrheal ones. The prevalence of Cryptosporidium was 25.5% and 8.4% in ART and pre-ART HIV patients, respectively [30].

In a study done in Adama, Afar and Dire Dawa, most (60%) of the study participants were on antiretroviral therapy (ART). Out of those, only two (1.5%) were diagnosed with an opportunistic parasite, and 96 (48%) of the non-ART study participants were infected with at least one other intestinal parasite species. Significant association was observed between lower

CD4+ T cell count (<200 cells/μL) and the prevalence of *Cryptosporidium* spp, *Isospora belli*, and *Blastocystis hominis* in non-HAART patients [31].

According to a study done in Dessie Hospital, the overall prevalence of IP in pre-ART and on-ART was 39% and 17.6%, respectively with significant decrease of intestinal parasite in the ART era ($p < 0.001$). Cryptosporidiasis were found in the pre-ART patients and significantly associated for lower CD4 <200cells/mm^3. The study also reported, absence of toilet (AOR = 7.57; 95% CI = 1.3, 44.22), source of water (AOR = 6.03; 95% CI = 1.14, 31.98), living condition (AOR = 13.29, 95% CI = 5.14, 34.35); WHO stage (AOR = 6.06; 95% CI = 2.49, 14.74) and ART status (AOR = 7.55; 95% CI = 3.24, 17.59) have significant association with prevalence of intestinal parasites [32].

A cross sectional study in Jimma health center showed that the overall prevalence of intestinal parasitic infections in HAART initiated patients was found to be 39.56%. Eight types of intestinal parasites was identified, the most dominant being *Ascaris lumbricoides* 21.67%, *Entamoeba histolytica* 15% and *Cryptosporidium parvum* 13.33% [33].

A study done in Zewditu Memorial Hospital showed that the prevalence of intestinal parasites at HAART was 34.3%. The most common parasites detected were *Strongyloides stercolaris* (14.8%), followed by *Ascaris lumbricoides* (8.3%), *Entamoeba histolytica/dispar* (7.5%) and *Giardia lamblia* (2.8%). The opportunistic intestinal parasite *Isosbora belli* was detected in one patient [33].

Taken together, the majority of the studies particularly in relation to anemia and CD4 count involve adult patients showing a significant association between advanced stage of HIV disease (low CD4 count) and anemia with intestinal parasitosis which shows improvement after initiating HAART. However, to our knowledge no published study addressed the issue in Pediatric patients in Ethiopia.

3. Objectives

3.1. General Objective

- To determine the prevalence of intestinal parasites in relation to CD4+ T cells levels and anemia among HAART initiated and non-HAART initiated pediatric HIV patients in Zewditu Memorial Hospital, Addis Ababa, Ethiopia.

3.2. Specific Objectives

- To determine the prevalence of intestinal parasites in relation to CD4+ T cell levels among HAART initiated and non-HAART initiated pediatric HIV patients
- To determine the prevalence of intestinal parasites in relation to anemia among HAART initiated and non-HAART initiated pediatric HIV patients
- To assess risk factors associated with intestinal parasites among HAART initiated and non-HAART initiated pediatric HIV patients

4. Materials and Methods

4.1. Study Area and Period

The study was conducted in Zewditu Memorial Hospital which is one of the Hospitals under Addis Ababa City administration Health Bureau. This hospital is selected because there are large numbers of pediatric HIV patients under follow up care and it is an ART model hospital as well. The hospital provides many health care services including pediatric HIV testing, counseling and ART. Currently, there are more than 1250 pediatric HIV patients registered in the hospital of which around 550 patients are on HAART and the rest are at pre-HAART stage. The study was conducted between August 05, 2013 and September 25, 2013.

4.2 Population

4.2.1 Source Population

The source population was all pediatric HIV patients who came to the hospital‟s ART clinic during the study period.

4.2.2 Study Population

The study population was all pediatric HIV patients who gave assent/whose guardians give permission during the study period.

4.3 Study Design

A comparative cross sectional study was conducted.

4.4 Sample Size and Sampling Technique

Since there are two populations (HAART initiated and non-HAART initiated) and the study aims to compare intestinal parasitosis between the two populations, the sample size was calculated as follows.

$$let\ P = \frac{P1 + rP2}{1 + r}$$

$$n1 = \frac{[Z\frac{a}{2}\sqrt{P(1-P)(1+\frac{1}{r})} + Zb\sqrt{\mathrm{P1}(1-\mathrm{P1})\frac{P2(1-P2)}{r}}]2}{(P1-P2)^2}$$

[35].

Where;

P1 is the prevalence of parasites in HAART initiated patients

P2 is prevalence of parasites in non-HAART initiated patients

P is the average prevalence proportion in the two groups (P=P1+rP2/1+r) =0.65

According to a research done in Indonesia, P1 is 0.50 and P2 is 0.77 [18]. With a

Precision of 5% and power of 80%, n1 is 72; n2 is *r*n1, where *r* is the ratio of N2 to N1 (700/550=1.3). Thus n2 is 94.

N1 is the number of HAART initiated pediatric HIV patients currently found in the hospital (550) and N2 is the number of non- HAART initiated pediatric HIV patients currently found in the hospital (700). So taking 10% contingency, n1 was 80 and n2 was 104 and a total of 184 study participants with 4 non respondents (180 completed) were enrolled in the study using consecutive sampling technique.

4.5 Measurement

4.5.1 Dependent Variables

- Prevalence of intestinal parasites

4.5.2 Independent Variables

- CD4+ T cell count and Anemia
- HAART status and Socio-demographic characteristics
- Environmental and clinical variables (diarrhea, abdominal pain, life style, WHO stage)

4.5.3. Inclusion and Exclusion Criteria

Inclusion Criteria

- Above 6 months and below 18 years of age
- months and above on HAART
- Patients whose guardians gave permission
- Non-HAART (for the non-HAART group)

Exclusion Criteria

- Those taking anti-parasitic medications within the past two weeks except Cotrimoxazole
- Those with any other acute/chronic disease causing immunosuppression and/or anemia

4.6 Data Collection

4.6.1. Data Collection Procedure

Site assessment and pre-test was done prior to data collection and adjustment made accordingly. After patients and guardians were informed about the objective of the study, volunteers were checked for the exclusion criteria by interviewing and observing their clinical data and stool specimens were collected from each pediatric patient whose guardians give permission patient by the assigned nurse who received onsite training. Structured questionnaires were used to assess independent variables. Complete blood count and CD4+ T cell count, from EDTA whole blood, using Cell-Dyn 1800 and FACScalibur, respectively, are routinely performed for patients on follow up visits in ZMH. Thus CD4+ T cell count and hemoglobin level were taken simultaneously with stool specimen collection. Therefore, no blood specimen was collected for the purpose of this study.

4.6.2. Specimen Collection and Processing

Stool specimen collection and direct wet mount: A single stool specimen was collected from each patient. It was obtained from all assenting/permitting 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 100X and 400X magnifications. A small portion of the stool specimen was also preserved in 10% formalin for repeating the tests whenever required and further analysis [36].

Formol –Ether concentration method: A portion of each preserved stool specimen was taken and processed. Briefly, 1g of stool was placed in a clean conical centrifuge tube containing 7 mL 10% formol water by using applicator stick and shacked gently. The resulting suspension was filtered through a sieve into another conical tube. After adding 3-4 ml of diethyl ether to the formalin solution, the content was centrifuged at 3000 rpm for 1 minute. The supernatant was poured away and the tube was replaced in its rack. Finally smear was prepared from the sediment and observed under light microscope with a magnification of 100X and 400X [36].

Modified Z-N method: A small portion of the concentrated stool specimen was processed for detection of opportunistic parasites using the Modified Ziehl Neelsen method. Thin smear was prepared directly from sediment of concentrated stool and allowed to air dry. The slides were fixed with methanol for 1 minute and stained with carbol fuchsine for 30 minutes. After washing the slides in tap water, they were decolorized with 1% acid alcohol for 1-2 minutes and counterstained in 0.3% methylene blue for 60 seconds. Then the slides were washed in tap water and observed under light microscope with a magnification of 1000X after air dry [37]. The positive slides for oocysts were cross checked by experienced laboratory professionals in ZMH and EHNRI/EPHI.

4.7. Data Processing and Analysis

After the data were cleaned and coded it was entered and analyzed using SPSS version16 software. Binary logistic regression was used to determine the association between intestinal parasites and demographic and clinical variables like diarrhea, regimen type, abdominal symptoms, HIV stage and lifestyle. Multiple logistic regressions were used to control the confounding factors. P values less than 0.05 were taken as statistically significant and results were presented using figures and tables.

4.8. Data Quality Assurance

All laboratory analyses were carried out using standard operating procedures

Pre-analytical: Adequate stool specimen (40 gram formed stool and 10 ml diarrheic stool) was collected using carefully labeled, dry, leak proof, grease free transparent stool caps. The specimen was kept free of contamination from water, soil, and urine. Specimens contaminated with water, urine and soil were rejected.

Analytical: Direct stool examination was performed within 30 minutes of collection and appropriate amount of stool sample was used to make a good smear devoid of air bubbles. After checking the contrast using 10X objective, the ova, larva, cyst and trophozoites of helminthes and protozoa were diagnosed by 40X objective. The oocysts of coccidian parasites were examined by 100X immersion oil from modified Z-N stained film. Each stool smears were examined for at least 10-15 minutes.

Post-analytical: All microscopic findings were encoded and reported appropriately. CD4+ T cell categorization and anemia definition were based on WHO criteria.

4.9. Ethical Considerations

The study was conducted after it was ethically reviewed and approved by the Research and Ethical Committee of Addis Ababa University, Department of Medical Laboratory, Addis Ababa University. Ethical clearance was also obtained from Addis Ababa Health Bureau. Then a letter informing the Hospital administrators was written from the Health Bureau and Permission obtained from Zewditu Memorial Hospital. All the information obtained from the study participants was coded to maintain confidentiality and data was collected after informed consent/parental permission signed. The positive results were timely reported to the clinicians for appropriate intervention.

4.10. Operational Definition of Terms

Mild Anemia: Hemoglobin level between 10-10.9 g/dl for under 5 and between 11-11.9 g/dl for under 18 aged [38].

Moderate Anemia: Hemoglobin level between 7.0-9.9 g/dl for under 5 and between 8.0-10.9 g/dl for under 18 aged [38].

Severe Anemia: Hemoglobin level <7.0 g/dl for under 5 and < 8.0 g/dl for under 18 aged [38].

HAART initiated: Those HIV patients who are using a combination of three antiretroviral drugs.

Mild Immunosuppression: CD4+T lymphocyte counts between 350-499 cells/μL for under 18 children or between 25-35% for under 5 children [39].

Advanced Immunosuppression: CD4+T lymphocyte counts between 200-349 cells/μL for under 18 children or between 15-25% for under 5 children [39].

Severe Immunosuppression: CD4+ T cell count < 200 cells/ μL L for under 18 children or less than 15% for under 5 children [39].

Pediatrics: age less than 18 years

Intestinal Parasitosis: Prevalence of intestinal parasites

5. Results

5.1. Socio-demographic Characteristics

A total of 180, 79(43.9%) on HAART and 101(56.1%) non-HAART, study participants were enrolled. Comparatively, in the HAART and non-HAART groups the distribution by sex revealed a slightly higher male case that were 44 (55.7%) and 54 (53.5%), respectively. The majority of HAART (97.5%) and non-HAART (95%) study participants were in the age range of 5-17 years with median age of 12 years (range 4-17 years) and 11 years (range 0.3-17 years), respectively. Majority of HAART and non-HAART study participants 76 (96.2%) and 81 (80.2%) were urban residents, respectively. The assessment of educational status of HIV/AIDS patients involved in the study showed 68(86%) of HAART and 83 (82%) of non-HAART were at primary school level. In binary logistic regression analysis; age group, educational status and gender did not show association with intestinal parasitic infection (IPI) in both groups (P>0.05), but the presence of infection with IPs showed a significance decrease in urban residents in both HAART experienced and HAART naïve pediatric HIV patients [AOR, 95% CI: 0.4(0.1, 0.9) P< 0.05] (Table 1).

Among all HAART initiated patients, only 1(1.3%) was taking second line regimen i.e. 5a (ABC+DDI+ LPV/r) while 78 (98.7%) were on first line ART therapy (4a-4g). Majority of HAART initiated pediatric HIV patients 45 (57%) were on 4c (AZT+3TC+NVP) regimen followed by 4a (d4T+3TC+NVP) 20 (25.3%), 4d (AZT+3TC+EFV) 10 (12.7%), 4g (ABC+3TC+AZT) 2 (2.5%), 4b (d4T+3TC+EFV) 1 (1.3%) and 5a (ABC+DDI+LPV/r) 1 (1.3%). None of the regimen types showed a statistically significant association with prevalence of IPs in HAART initiated HIV/AIDS patients in binary logistic regression analysis.

Table 1: Associations of socio-demographic factors with prevalence of intestinal parasites by HAART status using binary and multiple logistic regressions in pediatric HIV/AIDS patients attending ZMH from August 5, 2013 to September 25, 2013, Addis Ababa, Ethiopia (N=180).

		Prevalence of intestinal parasites							
Variables		**HAART (n=79)**		**Non-HAART (n=101)**		**Total (N=180)**		**COR (95%CI)**	**AOR (95% CI)**
		Pos n(%)	**Neg n(%)**	**Pos n(%)**	**Neg n(%)**	**Pos n(%)**	**Neg n(%)**		
Age group (years)	< 2	0(0)	0(0)	1(33.3)	2(66.7)	1(33.3)	2(66.7)	1(1,1)	
	2-5	1(50)	1(50)	1(33.3)	2(66.7)	2(40)	3(60)	0.6(.04,9.8)	
	6-11	9(28)	23(72)	19(36.5)	33(63.5)	28(33.3)	56(66.7)	1.2(0.2,6.8)	
	12-18	12(26.7)	33(73.3)	24(54.5)	19(45.5)	36(40.4)	52(59.6)	1	
Gender	Male	9(20.5)	35(79.5)	25(46.3)	29(53.7)	34(34.7)	64(65.3)	1.3(0.7,2.4)	
	Female	13(37)	22(63)	20(42.6)	27(57.4)	34(41.5)	48(58.5)	1	
Residence	Urban	23(30)	54(70)	32(39.5)	49(60.5)	55(34.8)	103(65.2)	1	1
	Rural	0(0)	3(100)	13(68.4)	6(31.6)	13(59)	9(41)	2.7(1.1,6.7) *	0.4(0.1,0.9) *
Educational status	Not begin	0(0)	1(100)	1(33.3)	2(66.7)	1(25)	3(75)	1	
	KG	3(42.8)	4(57.2)	1(20)	4(80)	4(33.3)	8(66.7)	3.5(0.3,48)	
	Primary	17(25)	51(75)	38(45.8)	45(54.2)	56(37)	95(63)	2.3(0.4,11)	
	Secondary	2(66.7)	1(33.3)	5(50)	5(50)	7(53.8)	6(46.2)	1.9(0.6,6)	

* Significant at P value < 0.05, AOR: adjusted odds ratio, COR: crude odds ratio, KG: Kindergarten

5.2. Prevalence of intestinal parasites among HAART and non-HAART groups

From all the 180 pediatric HIV/AIDS patients participated in this study, intestinal parasites (IPs) were detected in 68(37.8%) of them. Among the 79 HAART initiated pediatric HIV patients, 22(27.8%) were infected with intestinal parasites. There were no opportunistic intestinal parasites found in this group. The highest prevalent intestinal parasite was *E. histolytica/dispar* accounting for 10% followed by *G. lamblia* and *A. lumbricoides* 6.3% each, *S. stercoralis* 2.5%, *Hook worm* and *H. nana* 1.3% each (Figure 2).

The overall prevalence of intestinal parasites in the non-HAART arm was 46(45.5%). The most prevalent intestinal parasite was *G. lamblia* (10.9%) followed by *E. histolytica/dispar* (7.9%), *Hookworm* and *S. stercoralis* (6.9% each), *Ascaris lumbricoides* and *Cryptosporidium species* (3.96% each), *Taenia species* (1.9%), and *H. nana* (2.97%) (Figure 1).

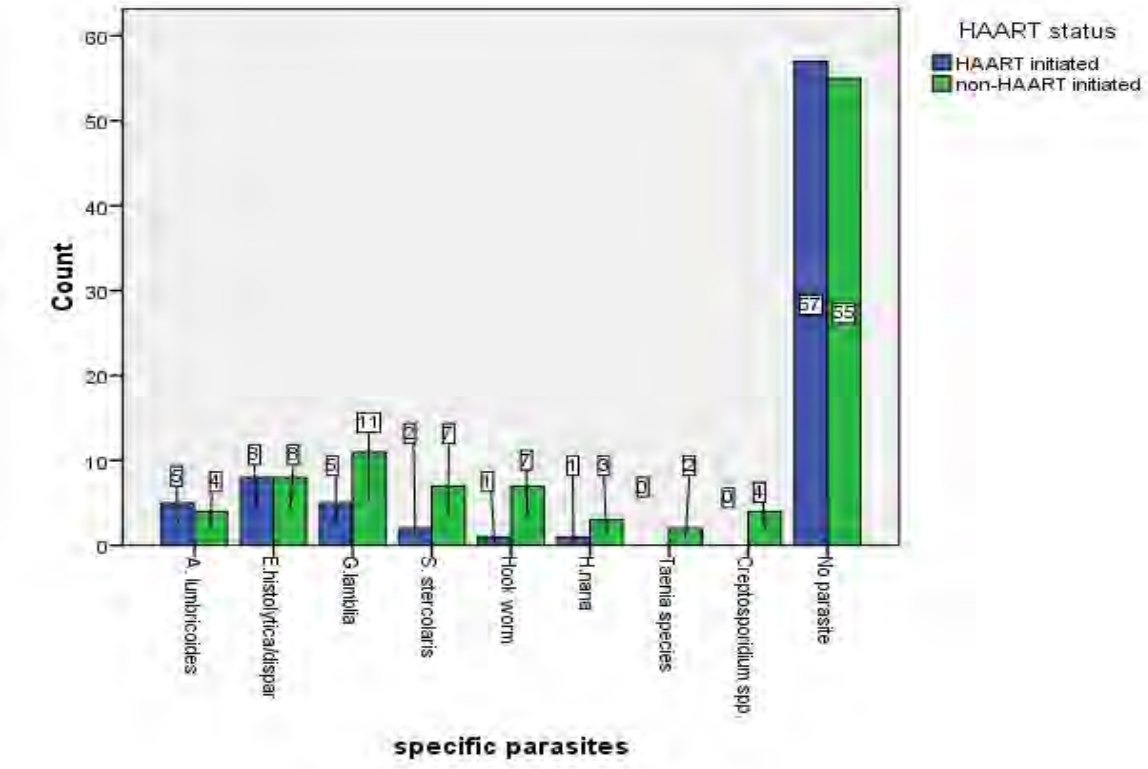

Figure 1: Distribution of species specific IPs among HAART initiated and non-HAART initiated pediatric HIV patients in ZMH from August 05, 2013 to September 25, 2013, Addis Ababa, Ethiopia

There was no statistically significant difference in the prevalence of species specific IPs in both groups in binary logistic regression analysis (P>0.05). But the overall prevalence of IPs is higher in non-HAART (2.2 times higher in odds IPI than HAART) which is 45.5% compared to HAART (27.8%) indicating statistically significant decrease of IPs as a result of HAART use [OR, 95%CI: 2.2(1.2, 4.0), P<0.05] (Table 2)

Table 2: Relationship between HAART status and infection with intestinal parasites among pediatric HIV/AIDS patients in ZMH from August 5, 2013 to September 25, 2013, Addis Ababa, Ethiopia (N=180).

Variable		Infection with IPs		OR (95% CI)	P-value
		Yes n (%)	No n (%)		
HAART Status	On HAART (n=79)	22(27.8%)	57(72.2%)	1	
	Non-HAART (n=101)	46(45.5%)	55(54.5%)	2.2(1.2,4.0)	0.016*

* Significant at P value< 0.05, OR: Odds ratio, IPs: Intestinal Parasites

5.3. Prevalence of intestinal parasites in relation to CD4+ T cell levels among HAART initiated and non-HAART initiated pediatric HIV patients

The mean CD4+ T cell count of HAART patients was 591±272 cells/µL. About 42 (53%) of the participants were not immunosuppressed (CD4 T cell levels > 500 cells/µL or >35%). Mild immunosuppression (CD4+ T cell levels 350-500 cells/µL or 25-35%), advanced immunosuppression (CD4+ T cell levels 200-350 cells/µL or 15-35%), and sever immunosuppression (CD4+ T cell levels < 200 cells/µL or <15%) account for 24(30.5%), 11(14%), and 2(2.5%), respectively.

In non-HAART groups the mean CD4+ T cell count was 486±221 cells/µL. Forty (39.6%) of the participants were not immunosuppressed (CD4+ T cell levels > 500 cells/µL or 35%). Mild immunosuppression (CD4+ T cell levels 350-500 cells/µL or 25-35%), advanced immunosuppression (CD4+ T cell levels 200-350 cells/µL/15-25%), and sever immunosuppression (CD4+ T cell levels < 200 cells/µL or 15%) account for 27(26.7%), 31(30.7%), and 3(2.97%), respectively.

The study participants who were on HAART consisted of 66 patients (83.5%) with CD4+ T cell count > 350 cells/µL or 25% and 13 patients (16.5%) with CD4+ T cell count < 350 cells/µL or 25%. Among the 13 HAART patients with CD4+ T cell count < 350 cells /µL or 25%, parasites were identified in 4 (30.8%) patients while 18 (27.3%) of those with CD4+ T cell count>350 cells /µL or 25% had IPs, showing no statistically significant association with CD4+ T cell levels [AOR, 95% CI: 1.2(0.3, 4.3), P=0.8) (Table 3).

From all the study participants who were not on HAART, 66 patients (65.3%) had CD4+ T cell count > 350 cells/µL or 25% while 35 patients (34.7%) had CD4+ T cell count < 350 cells/µL or 25%. Among the 35 non-HAART patients with CD4+ T cell count < 350 cells /µL or 25%, parasites were identified in 28 (80%) patients much higher compared to those with CD4+ T cell levels above 350 cells/µL or 25% which was 25.8%. Those having CD4+ T cell counts below 350 cells/µL or 25% were about 7.3 times higher in odds of getting infection with intestinal parasites than those having CD4+ T cell counts above 350 cells/µL or 25% [AOR, 95%CI: 7.3(1.5, 35), P< 0.05) (Table 3).

Table 3: The effect of CD4+ T cell count on intestinal parasites among pediatric HIV/AIDS patients in ZMH from August 5, 2013 to September 25, 2013, Addis Ababa, Ethiopia (N=180)

Variables			Intestinal Parasites		AOR (95%CI)	P value
			Present n(%)	Absent n(%)		
HAART	CD4+ T cell count category(cells/μL)	<350	4(30.8)	9(69.2)	1.2(0.3,4.3)	0.8
		> 350	18(27.3)	48(72.7)	1	
Non-HAART	CD4+ T cell count category (cells/μL)	<350	28(80)	7(20)	7.3(1.5,35)	0.013*
		>350	17(25.8)	49(74.2)	1	

* Significant at P< 0.05, AOR: Adjusted odds ratio

Among all HAART initiated pediatric HIV patients, there was no specific parasite identified to be significantly associated with CD4+ T cell levels < 350 cells/μL or 25% in binary logistic regression analysis (Table 4).

Table 4: Binary logistic regression analysis of specific intestinal parasites in relation to CD4+ T cell levels among HAART initiated pediatric HIV patients in ZMH from August 5, 2013 to September 25, 2013, Addis Ababa, Ethiopia (N=79)

Specific parasites			COR	95% CI	P- value
A.lumbricoides	CD4+ T cell count (cells/μL)	< 350	0.00	1,1	0.1
		>350	1		
E. histolytica/dispar	CD4+ T cell count (cells/μL)	< 350	0.8	0.1,7.9	0.9
		>350	1		
G. lamblia	CD4+ T cell count (cells/μL)	< 350	2.73	0.5,19	0.23
		>350	1		
S. stercoralis	CD4+ T cell count (cells/μL)	< 350	1.0	0, 1	1.0
		>350	1		
Hook worm	CD4+ T cell count (cells/μL)	< 350	1.0	0,1	1.0
		>350	1		
H. nana	CD4+ T cell count (cells/mm^3)	< 350	1.0	0,1	1.0
		>350	1		

COR: crude odds ratio

But in case of non-HAART pediatric HIV patients, *E. histolytica/dispar* [AOR, 95%CI: 9.33(1.7, 50.7), P<0.05], *Hook worm* [AOR, 95%CI: 9(1.7, 50), P<0.05], and *Taenia species* [AOR, 95%CI: 14(1.1, 175), P<0.05] were statistically significantly associated with CD4+ T cell levels < 350 cells/μL or 25%. *Cryptosporidium species* was highly significantly associated in non-HAART patients with CD4+ T cell levels < 350 cells/μL or 25% [AOR, 95%CI: 13(10.5, 97.6), P<0.01] (Table 5).

Table 5: Binary and Multiple logistic regression analysis of specific intestinal parasites in relation to CD4+ T cell levels among non-HAART initiated pediatric HIV patients in ZMH from August 5, 2013 to September 25, 2013, Addis Ababa, Ethiopia (N=101)

Specific parasites			COR 95% CI	P-value	AOR 95% CI	P- value
A.lumbricoides	CD4+ T cell count (cells/mm^3)	< 350	1.5(0.2,4.3)	0.67		
		>350	1			
E. histolytica/dispar	CD4+ T cell count (cells/mm^3)	< 350	3.8(1.8,9.2)	0.046*	9.33(1.7,50.7)	0.010*
		>350	1		1	
G. lamblia	CD4+ T cell count (cells/mm^3)	< 350	0.5(0.1,0.9)	0.034*	2.73(1,17)	0.06
		>350	1		1	
S. stercoralis	CD4+ T cell count (cells/mm^3)	< 350	1.2(0.7,4.3)			
		>350	1			
Hook worm	CD4+ T cell count (cells/mm^3)	< 350	7.9(1.1,12)		9.33(1.7,50.7)	0.010*
		>350	1		1	
H. nana	CD4+ T cell count (cells/mm^3)	< 350	7(0.3,167)			
		>350	1			
Taenia species	CD4+ T cell count (cells/mm^3)	< 350	3.2(1.4,7.6)	0.044*	14.0(1.1,75.5)	0.041*
		>350	1		1	
Cryptosporidium species	CD4+ T cell count (cells/mm^3)	< 350	8.9(1.6,33)		13.2(10.5,76.2)	0.000**
		>350	1		1	

COR: crude odds ratio, AOR: adjusted odds ratio, *significant at P value <0.05, **highly significant at P<0.01

5.4. Prevalence of intestinal parasites in relation to anemia among HAART initiated and non-HAART initiated pediatric HIV patients

From the total of 79 HAART initiated pediatric HIV patients, 8(10%) were anemic (mean hemoglobin level 13.36±1.35g/dl) of which 4(50%) were infected with IPs and 4(50%) were moderately anemic (hemoglobin level 8.0-10.9 g/dl for above 5 children or 7.0-9.9g/dl for under five children). Mild anemia (hemoglobin level 11.0-11.9 g/dl for above 5 children or 10.0-10.9g/dl for under five children) and severe anemia (hemoglobin level <8.0 g/dl for above 5 children or <7.0 g/dl for under five children) account for 3(37.5%) and 1(12.5%), respectively. Most of anemic patients use 4c (AZT+3TC+NVP) 4(44.4%) and 4d (AZT+3TC+EFV) 2(22.3%) drug regimens.

Unlike HAART initiated pediatric HIV patients, 32(31.7%) of non-HAART pediatric HIV patients were anemic (mean hemoglobin level 12.89±1.9 g/dl) of which 24(75%) were infected with IPs and 18(56.3%) have mild anemia (hemoglobin level 11.0-11.9 g/dl for above 5 children or 10.0-10.9g/dl for under five children). Moderate anemia (hemoglobin level 8.0-10.9 g/dl for above 5 children or 7.0-10.9g/dl for under five children) and severe anemia (hemoglobin level <8.0 g/dl for above 5 children or <7.0 g/dl for under five children) account for 13(40.7%) and 1(3.0%), respectively.

The presence of intestinal parasites was not statistically significantly associated with anemia in HAART initiated patients (COR, 95% CI=0.34(0.08, 1.5), P=0.15) but presence of IPs was statistically significantly associated with the presence of anemia in non-HAART patients i.e. infected HAART naïve patients had 4.5 times likelihood of being anemic in comparison to their counterparts [AOR, 95% CI: 4.5(1.3, 15.2), P< 0.05] (Table 6).

Table 6: Relationship between anemia and intestinal parasites among pediatric HIV/AIDS patients in ZMH from August 5, 2013 to September 25, 2013, Addis Ababa, Ethiopia (N=180)

Variables			Intestinal Parasites		AOR (95%CI)	P value
			Present n(%)	Absent n(%)		
HAART	Anemia	Present	4(50)	4(50)	1.2(0.5,3.4)	0.87
		Absent	18(25.4)	53(74.6)	1	
Non-HAART	Anemia	Present	24(75)	8(25)	4.5(1.3,15.2)	0.016*
		Absent	21(30.4)	48(69.6)	1	

* Significant at P< 0.05, **highly significant at P<0.01, COR: Crude odds ratio, AOR: Adjusted odds ratio

In non-HAART patients, *Hookworm*, *S. stercoralis* and *H. nana* were highly statistically significantly associated with different status of anemia [AOR, 95% CI: 2.3 (1.01, 5.61), P<0.01] (Table 7)

Table 7: Association between specific intestinal parasites and status of anemia (mild, moderate, sever) among non-HAART initiated pediatric HIV patients in ZMH from August 5, 2013 to September 25, 2013, Addis Ababa, Ethiopia (N=101).

Specific parasites	Anemia status					
	Mild n(%)	P value	Moderate n(%)	P value	Sever n(%)	P value
A.lumbricoides	0(0)	0.6	0(0)	0.8	0(0)	0.98
E. histolytica/dispar	2(66.7)	0.2	1(33.3)	0.18	0(0)	0.96
G. lamblia	3(75)	0.17	1(25)	0.34	0(0)	0.95
S. stercoralis	1(14.3)	0.08	6(85.7)	0.003**	0(0)	0.90
Hook worm	4(57)	0.000**	2(28.6)	1	1(14.4)	0.99
H. nana	0(0)	0.83	1(100)	0.003**	0(0)	0.98
Taenia species	1(100)	0.33	0(0)	1	0(0)	0.99
Cryptosporidium species	0(0)	0.67	1(100)	0.13	0(0)	0.99

**highly significant at P<0.01

5.5. Assessment of risk factors associated with intestinal parasites among HAART initiated and non-HAART initiated pediatric HIV patients

Table 8 shows that among the 79 HAART initiated pediatric HIV/AIDS patients, diarrhea present in 17(21.5%) of which 8(47%) were infected with intestinal parasites. Comparatively, the number of diarrheic patients in non-HAART initiated pediatric HIV/AIDS patients was high; 35.6% (36/101). From the 36 diarrheic non-HAART initiated patients, 29(80.6%) were infected with intestinal parasites. The prevalence of intestinal parasites was significantly associated with the presence of abdominal pain symptoms in both HAART and non-HAART groups [AOR, 95%CI: 21(3.7, 121), P<0.01].

In binary logistic regression; hand washing after meal and toilet, playing with soil, eating raw meat, source of drinking water and finger nail trim were not significantly associated with IPI in both groups (P>0.05). On the other hand, the prevalence of intestinal parasites in non-HAART group was significantly associated with eating unwashed/raw fruit [AOR, 95%CI: 6.3(1.2, 25.6), P<0.05], open field defecation [AOR, 95%CI: 9.3(1.6, 53.6), P<0.05] and diarrhea [AOR, 95%CI: 5.2(1.3, 21.3), P<0.05]. So those non-HAART patients who were diarrheic, eat unwashed/raw fruit, and defecate openly had 5.2, 6.3, and 9.3 times likelihood of getting infection with intestinal parasites, respectively (Table 8).

Table 8: Risk factors associated with intestinal parasites among HAART and non-HAART initiated pediatric HIV patients with prevalence of intestinal parasites (N=180) in ZMH from August 5, 2013 to September 25, 2013, Addis Ababa, Ethiopia

Variables		Presence of parasite				COR 95% CI	P value	AOR 95% CI	P value
		HAART Status							
		On HAART (n=79)		Non-HAART (n=101)					
		Pos n(%)	Neg n(%)	Pos n(%)	Neg n(%)				
Hand washing after meal	Yes	18(25)	54(75)	42(44.2)	53(55.8)	1			
	No	4(57)	3(43)	1(50)	1(50)	1.3(0.1,20)	0.87		
Eating raw/unwashed fruit	Yes	12(22.6)	41(77.4)	36(50.7)	35(49.3)	0.4(0.1,0.9)	0.04*	6.3(1.2,31)	0.023*
	No	10(38.5)	16(61.5)	7(27)	19(73)	1		1	
Eating raw meat	Yes	8(29.6)	19(70.4)	14(53.8)	12(46.2)	0.6(0.2,1.5)	0.26		
	No	14(28)	36(72)	29(40.8)	42(59.2)	1			
Open field defecation	Yes	21(29)	51(71)	39(55.7)	31(44.3)	0.2(0.1,0.4)	0.001**	9.3(1.6,53.6)	0.012*
	No	1(14.3)	6(85.7)	4(14.8)	23(85.2)	1		1	
Hand washing after toilet	Yes	19(26.8)	52(73.2)	38(42.7)	51(57.3)	1			
	No	3(37.5)	5(62.5)	5(62.5)	3(37.5)	2.2(0.5,10)	0.3		

Finger nail trim	Yes	16(25.8)	46(74.2)	26(41.3)	37(58.7)	1			
	No	6(35.3)	11(64.7)	17(50)	17(50)	1.4(0.6,3.3)	0.4		
Wearing closed shoe	Always	20(27)	54(73)	31(38.3)	50(61.7)	1		1	
	Some times	2(40)	3(60)	12(70.6)	5(29.4)	3.8(1.2,12)	0.019*	0.8(0.1, 4.5)	0.8
Playing with soil	Yes	14(32.6)	29(67.4)	25(46.3)	29(53.7)	0.8(0.4,1.8)	0.6		
	No	8(22.2)	28(77.8)	18(41)	26(59)	1			
Diarrhea	Present	8(47)	9(53)	29(80.6)	7(19.4)	0.1(0.03,0.2)	0.000**	5.2(1.3,21.3)	0.022*
	Absent	14(22.6)	48(77.4)	16(24.6)	49(75.4)	1		1	
WHO stage	I	4(30.8)	9(69.2)	5(27.7)	17(72.3)	1		1	
	II	17(28.8)	42(71.2)	28(43.8)	36(56.2)	13.6(2.7,68.1)	0.001*	0.2(0.02, 1.3)	0.09
	III	1(14.3)	6(85.7)	12(80)	3(20)	5.1(1.3,20)	0.018*	0.4(0.03, 6.4)	0.56
	IV	0(0)	2(100)	0(0)	0(0)				

AOR: adjusted odds ratio, *significant at P value <0.05, **highly significant at P<0.01, COR: crude odds ratio

6. Discussion

HIV infection has increased significance of parasitic infection especially due to opportunistic intestinal parasites. More importantly, with emergence of AIDS, the epidemiology as well as outcome of diseases caused by opportunistic parasites was significantly modified. But, the effect of HIV on some other parasites is not clearly understood. Overall, either backed by HIV or independently, intestinal parasitic infections have continued to be major cause of morbidity and mortality in humans [14]. Children are more vulnerable to these dual morbidities. Thus, in this study, intestinal parasitosis in relation to anemia and CD4+ T cell count was assessed in pediatric HIV infected children with and without HAART in Zewditu Memorial Hospital.

The significantly higher prevalence of common and opportunistic intestinal parasites observed in the non-HAART initiated children in this study (45.5% versus 27.8%) signifies the value of HAART in reducing intestinal parasites through improving the immune response as also demonstrated by the lower prevalence in those with higher CD4+ T cell counts. Moreover, the observed 45.5% prevalence of intestinal parasite among non-HAART initiated patients in this study was in comparable with reports from South west Ethiopia (44.8%) and Gondar (43.5%) [8, 29]. However, the finding was lower compared to studies from Indonesia (77%), Brazil (63.9%), from selected ART centers of Adama, Afar and Dire-Dawa (52%) [18, 27, 31]. This low prevalence in this study might be due to geographic difference in sampling and residence (more than one study area for most reports and include rural areas), and time gap where those studies were done some years ago but nowadays there is a better awareness of the patients about intestinal parasite infection and their cause.

On the other hand, the 27.8% prevalence of IPs detected among HAART initiated children in this study was higher than that reported in Brazil (24%), Dessie (17.6%) [27, 32], which might be due to small sample size and specimen collected during rainy season in this study, but lower than from studies from Indonesia (50%), in selected ART centers of Adama, Afar and Dire-Dawa (48%), Jimma (39.6%), and Addis Ababa (34.3%), [18, 31, 33, 34]. This lower prevalence might be due to better life style (toilet facilities and water source), better awareness of the patients/guardians in adopting prevention and treatment measures against IPs, and better

adherence to anti-helminthics and HAART may decrease detection of IPs in our study area due to urban life.

This study revealed statistically significant reduction of intestinal parasite in the HAART era ($p < 0.05$).This finding was in line with previous studies done in Indonesia, Brazil, and Dessie where those studies indicated significant decrease of intestinal parasites in the HAART experienced HIV/AIDS patients [18, 27, 32].

Strogyloides stercoralis is an important human parasitic infection primarily because of its potential for serious and even lethal disease in immunosuppressed patients. The prevalence of *Strongyloides stercoralis* is higher in non-HAART groups (6.9%) than HAART groups (2.5%) in the present study which is in agreement with a study in Nigeria (6.39%) but higher than studies in Malaysia (0%), China (0%), Dessie (0.7%) [6, 24, 25, 32]. The possible reasons might be due to geographical differences, sample collected during rainy season in our case (increase transmission of IPs), comparatively small sample sizes in this study and age differences i.e. pediatric patients (targets in this study) have more exposure to soil than adults because pediatric age groups usually play football in bare foot, mostly wear open shoes and play with soil.

This study showed no statistically significant difference in the prevalence of species specific intestinal parasites between the two groups ($P>0.05$) and this result is in agreement with a study done in Gondar where authors reported no statistically significant difference of species specific intestinal parasite between HAART initiated and non-initiated groups [29].

Intestinal coccidian parasites including *Cryptosporidiumspecie, Isospora, Cyclospora* and *Microsporidia* are increasingly becoming prevalent in AIDS patients. Intestinal infection by *Cryptosporidia* is self-limited, but leads to persistent diarrhea in the advanced stage of AIDS and there is no effective treatment available for it [15].

The prevalence of *cryptosporidium species* (3.96%) in non-HAART patients in this study is much lower than the prevalence reported in Brazil (8.1%), Gondar (8.7%), Nekemte (25.5%), and selected ART centers of Adama, Afar and Dire-Dawa (8%) and Jimma (13.3%) [27, 29, 30, 31, 33] but higher from Dessie (1.5%) [32]. The existence of such variation might be due to the difference in geographic location, general hygiene (mainly water and food sanitation) of the

population in Addis Ababa may be better than the other regions, only single specimen collected, and moreover study participants were recruited without considering their diarrhea status in this study.

In case of non-HAART Pediatric HIV patients, *E. histolytica/dispar* [AOR, 95%CI: 9.33(1, 50), P<0.05], *Hookworm* [AOR, 95%CI: 9(1.7, 50), P<0.05], and *Taenia species* [AOR, 95%CI: 14(1, 175), P<0.05] were non opportunistic IPs significantly associated with CD4+ T cell levels < 350 cells/μL or 25% in this study. This result is similar with a study in Malaysia [24] and Dessie (for only *E. histolytica/dispar*) [32].

This study indicated that infection with *cryptosporidium species* and other intestinal parasite infections among non-HAART patients were found significantly associated with having CD4+ T cell count <350 cells/μL or 25% when compared to the HAART experienced patients without *Cryptosporidium species* infection in any of the CD4+ T cell categories. This may be due to the fact that opportunistic parasites are known to resolve spontaneously with immune restoration among HIV/AIDS patients on HAART [5]. The association of these parasites for lower CD4+ T cell count <350 cells/μL or 25% was in agreement with that of findings in Nigeria, Nekemte, in selected ART centers of Adama, Afar and Dire-Dawa, and Dessie [28, 30,31, 32].

Anemia, which can be mild to severe, acute or chronic, is one of the multitudes of complications associated with parasitic infections [6]. Studies show that the prevalence of anemia in HIV positive people has been estimated to be 63% to 95% in different study settings and its prevalence in HAART naïve persons is higher than HAART experienced persons [40] which strengthen the case in this study.

The prevalence of anemia in non-HAART initiated patients in this study was 31.7% which is lower than studies in Nigeria (93.3%) [6]. This might be due to differences in age groups (factors other than HIV, and intestinal parasites also contribute to anemia in children) and unknown HAART status in the previous study.

In this study, *Hookworm, S. stercoralis*, and *H. nana* were helminthes associated with anemia in non-HAART which is in agreement with a study in Veitnam [21] where *Hookworm* and

Trichuris trichuira associated with anemia, but different (except for *Hookworm*) from a study in Nigeria where *Cryptosporidiasis, Ascariasis, Hook worm* and *Taeniasis* were associated with anemia [6]. These differences might be due to low number of children with *Cryptosporidiasis, Taeniasis* and *Ascariasis* in this study.

In the present study, only 8 (10%) of HAART initiated pediatric patients were anemic from which 4(50%) are infected with IPs. However, the presence of intestinal parasites was not statistically significant associated with anemia in HAART initiated patients (COR, 95% CI=2.9(0.7, 13), P=0.15) which is different from a study in Nigeria where the presence of IPs was associated with anemia [28]. This difference might be due to geographical difference, age difference, small sample size and difference in duration of HAART where they included 3-6 months of HAART duration unlike this study where only children with HAART for at least 6 months were recruited.

In children, intestinal parasitic infections, particularly soil-transmitted helminthiasis is the cause of common health problems in tropical countries [2]. Diarrhea remains one of the most common illnesses in children, and one of the major causes of infant and childhood mortality in developing countries, as reported by the WHO [12].

The total prevalence of diarrhea detected in the present study (29.4%) is consistent with a study in Nekemte (27%) [30], but lower than a study in South Western Ethiopia (51.1%), Cameroon (48.6%), in selected ART centers of Adama, Afar and Dire-Dawa (37.5%) [8, 23, 31]. The possible explanation might be due to better life style in urban settings (like Addis Ababa), and partly could be due to low prevalence of opportunistic intestinal parasites in our study setting.

Non-HAART initiated patients who were diarrheic, ate unwashed/raw fruit, and defecate openly have 5.2, 6.3, and 9.3 times likelihood of getting infection with intestinal parasites, respectively. This result is different from a study in Dessie where absence of toilet, source of water and living condition were significantly associated with the presence of IPs [32]. This difference might be due to; majority of the study participants had toilet and used pipe water for drinking in case of this study.

7. Limitations of the Study

Main limitations are as follows:

- Due to financial constraints sensitive techniques like PCR was not used to identify the species of *Cryptosporidium* and differentiate *Entamoeba histolytic*a from *Entamoeba dispar*
- We have not used water-ether sedimentation method for *Microsporidia* and other methods like Molecular techniques and immunoflourescent techniques sensitive for parasites
- No HIV negative controls were used
- Lack of similar literatures in pediatric HIV population: we face difficulty to discuss our results because of the absence of similar literatures and thus obliged to compare pediatric results with adult results.

8. Conclusion

The prevalence of intestinal parasites differed by HAART status and found to be significantly higher in HAART naïve than HAART experienced patients. High proportions of intestinal parasites were significantly associated with lower CD4+ T cell counts in non-HAART initiated patients. Infections with opportunistic intestinal parasite were present and significantly associated with lower CD4+ T cell counts in HAART naïve patients only. Increasing the immune status of HIV infected patients with antiretroviral therapy may help to reduce acquisition of parasites. Moreover, the prevalence of anemia was higher in non-HAART patients where helminthes like *Hookworm*, *Strogyloides stercoralis* and *Hymenolopis nana* were IPs associated with anemia. Open field defecation, eating raw/unwashed fruit, diarrhea and being non-HAART significantly increased the prevalence of intestinal parasites in the study area.

9. Recommendations

Based on our results we recommend that;

- HAART initiation for all eligible pediatric HIV patients with low CD4+ T cell counts to help them in reducing opportunistic and other intestinal parasites should be encouraged.
- Determination and monitoring of hematologic profiles like hemoglobin level should be strengthened during follow up visits to make the quality of HIV/AIDS patients‟ life better by reducing anemia.
- Public health measures should continue to emphasize the importance of environmental and personal hygiene like avoiding open field defecation and not consuming raw/unwashed fruit aiming to obtain a better quality of life for those patients.
- Stool examination including Modified Z-N staining should be routinely performed in the follow-up of pediatric patients with HIV/AIDS attending ART clinic in order to improve treatment and other preventive measures.
- Additionally large scale longitudinal study is needed to determine the effect of HAART for both opportunistic and non-opportunistic intestinal parasites.

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11. Annexes

Annex I: English and Amharic Versions of Questionnaires

Addis Ababa University, College of Health Sciences, Department of Medical Laboratory Sciences.

Questionnaire for data collection on the prevalence of Intestinal Parasites in Relation to CD4 Count and Anemia among HAART initiated and non-HAART initiated Pediatric HIV Patients in Zewditu Memorial Hospital, Addis Ababa, Ethiopia

My name is Hylemariam Mihiretie. I am a post graduate student of AAU, College of Health Sciences, and Department of Medical Laboratory Sciences. Your participation is voluntary and you are not obliged to answer any questions that you do not want to answer. Please take a few moments to complete this questionnaire so that we can determine the prevalence of IPIs. Thank you in advance in anticipation of your cooperation. If you are not comfortable with the interview, please feel free to stop it any time you like. Participation or withdrawal from this study will have not any effect on your service at Zewditu Memorial Hospital.

A) For children above 7 years of age or information to be given by guardians					
I) Socio-demographic data (for all age groups)					
Participant code:_____					
101	Age				
102	Sex	Male	Female		
		1	2		
103	Residence	Urban	Rural		
		1	2		
104	Educational Status	Not yet begin	Kindergarten	Primary	Secondary
		1	2	3	4
II) Life Style (for age groups 5 years and above)					
105	Practice of hand washing before and after eating	Yes	No		
		1	2		

106	Practice of eating raw and/or unwashed fruit	Yes		No	
		1		2	
107	Practice of eating raw meat	Yes		No	
		1		2	
108	Water source of drinking	Pipe		River	Unprotected well
		1		2	3
109	Practice of open field defecation	Yes		No	
		1		2	
110	Practice of hand washing after toilet/defecation	Yes		No	
		1		2	
111	Presence of toilet	Yes		No	
		1		2	
112	Practice of finger nail trim	Yes		No	
		1		2	
113	Practice of wearing closed shoe	Always	Sometimes	Rarely	Never
		1	2	3	4
114	Practice of playing with soil	Yes		No	
		1		2	
	III) Clinical data (for all age groups)				
115	Diarrhea	Present		Absent	
		1		2	
116	Diarrhea condition	Chronic (4 weeks)		Acute (1 week)	
		1		2	
117	Abdominal pain symptoms	Present		Absent	
		1		2	
118	Stool characteristics	Formed	Soft	Loose	Diarrhea
		1	2	3	4
b) Immunological variables to be filled by data collectors (for all age groups)					

119	HAART initiation	Yes				No			
		1				2			
120	When you start HARRT	Date		Month		Year			
121	Current WHO HIV stage	I	II	III		IV	None		
		1	2	3		4	5		
122	Type of ARV drug	First line		Second line					
		1		2					
123	Regimen type and duration								
124	Current CD4 count (Cells/μL or %)								
125	CD4 category	<350 (25%)		>350 (25%)					
		1		2					
126	Immunosuppression	No	Mild		Advanced		Sever		
		1	2		3		4		
127	Current Hemoglobin level (g/dl)								
128	Anemia	Present		Absent					
		1		2					
129	Anemia status	Mild(11-11.9)		Moderate(8-11		Sever <8			
		1		2		3			
130	Parasites	Present		Absent					
		1		2					

Stool microscopy result

Direct Wet Mount:_____

Concentration:_____

Modified Z-N:_____

መጠይቅ

አዲስ አበባ ዩኒቨርሲቲ፣ አላይድ ጤና ሳይንስ ኮሌጅ፣ የህክምና ላቦራቶሪ ሳይንስ ት/ት ክፍል፣ በዘዉዲቱ መታሰቢያ ሆስፒታል በኤች.አይ.ቪ በሽተኛ ህጻናት ላይ ለሚደረገዉ የሆድ ትላትሎች ጥናት መረጃ መሰብሰቢያ መጠይቅ፡፡

እኔ ኃ/ማርያም ምህረቴ እባላለሁ፡፡ በአዲስ አበባ ዩኒቨርሲቲ፣ አላይድ ጤና ሳይንስ ኮሌጅ፣ የህክምና ላቦራቶሪ ሳይንስ ት/ት ክፍል የሁለተኛ ዲግሪ ተማሪ ነኝ፡፡ የእርስዎ ተሳትፎ በፍቃደኝነት ላይ የተመሰረተ በመሆኑ መረጃ መስጠት ካልፈለጉ አይገደዱም፡፡ ካለዎት ጠባብ ጊዜ ወስደዉ መጠይቁን በመሙላትዎ በቅድሚያ እያመሰገንኩ መጠይቁ ካልተመቸዎት በማንኛዉም ሰዓት እንዲያቆሙት በትህትና እገልፃለሁ፡፡

ከሰላምታ ጋር

ዕድሜያቸዉ ከ7 ዓመት በላይ ለሆኑ ወይም በተንከባካቢዎች የሚሞላ					
ሀ) ማህበራዊ መረጃ (ለሁሉም የእድሜ መደቦች)					
የተሳታፊ ኮድ:_____					
101	እድሜ				
102	ፆታ	ወንድ	ሴት		
		1	2		
103	የመኖሪያ አድራሻ፤	ከተማ	ገጠር		
		1	2		
104	የት/ት ደረጃ	ለት/ት ያልደረሰ/ች	ኪንደርጋርተን	የመጀመሪያ ደረጃ ት/ት	የሁለተኛ ደረጃ ት/ት
		1	2	3	4

ለ) የአኗኗር ዘዴ (5 ዓመትና ከዚያ በላይ ለሆኑ)				
105	ከምግብ በፊትና በኃላ እጅ የመታጠብ ልምድ	አለ	የለም	
		1	2	
106	ጥሬ/ያልታጠበ ፍራፍሬ የመመገብ ልምድ	አለ	የለም	
		1	2	
107	ጥሬ ስጋ የመመገብ ልምድ	አለ	የለም	
		1	2	
108	የመጠጥ ዉሃ መገኛ	ቧንቧ	ወንዝ	ያልተከለለ ጉድጓድ
		1	2	3
109	ሜዳ ላይ የመፀዳዳት ልምድ	አለ	የለም	
		1	2	
110	ከሽንት ቤት መልስ ወይም ከተፀዳዱ በኋላ እጅ የመታጠብ ልምድ	አለ	የለም	
		1	2	
111	ሽንት ቤት	አለ	የለም	
		1	2	
112	የእጅ ጥፍር ቶሎ ቶሎ የመቁረጥ ልምድ	አለ	የለም	
		1	2	

113	ሸፍን ጫማ የመልበስ ልምደዎ ምን ይመስላል	ሁልጊዜ	አልፎአልፎ	በጣም አልፎአልፎ	አለብስም
		1	2	3	4
114	በአፈር የመጫወት ልምድ	አለ		የለም	
		1		2	
ሐ) የጤና ሁኔታ መረጃ (ለሁሉም የእድሜ መደቦች)					
115	ተቅማጥ	አለ		የለም	
		1		2	
116	የተቅማጥ ሁኔታ	የቆየ (4 ሳምንትና ከዚያ በላይ)		አዲስ (1 ሳምንት)	
		1		2	
117	የሆድ ህመም ስሜት	አለ		የለም	
		1		2	
118	የሰገራ ሁኔታ	ጠንካራ	ለስላሳ	ቀጭን	ተቅማጥ
		1	2	3	4
መ) በመረጃ ሰብሳቢዎች የሚሞላ የክሊኒካልና የኢሚኖሎጂ መረጃ (ለሁሉም የእድሜ መደቦች)					
119	"HAART" ጀምረዋል	አዎ		አልጀመርኩም	
		1		2	
120	„ՙHAARTՙՙ መቼ ጀመሩ	ቀኑን	ወር	ዓመት	
121	የአለም ጤና ድርጅት የኤች አይ ቪ ደረጃ	I	II	III	IV	የለም
		1	2	3	4	5
122	የመድሃኒቱ አይነት (drug line)	1ኛ ደረጃ		2ኛ ደረጃ	
		1		2	
123	የመድሃኒቶች ይዘት (regimen) እና ቆይታ				
124	የ "CD4" መጠን (በህዋስ/ማክሮ ሊትር)				
125	የ "CD4" ክፍፍል	ከ350 (25%) ያነሰ		ከ350 (25%) የበለጠ	
		1		2	
126	ኢሚኖሰፕረሽን	ማይልድ	አድቫንስድ	ሲቨር	
		1	2	3	
127	የሄሞግሎቢን መጠን (ግራም/ዴ.ሊ.)				
128	ደም ማነስ	አለ		የለም	
		1		2	
129	የደም ማነስ ሁኔታ	ማይልድ		ሞደሬት	ሲቨር
		1		2	3
130	የሆድ ትላትል	አለ		የለም	
		1		2	

የላቦራቶሪ ዉጤት

ዳይሬክት፡_____

ኮንሰንትሬትድ፡_____

ዚህል-ኒልሰን፡_____

Annex II: English and Amharic Versions of Participant Information Sheet

ADDIS ABABA UNIVERSITY COLLEGE OF HEALTH SCIENCES

DEPARTMENT OF MEDICAL LABORATORY SCIENCES

You are invited to participate in a study to be conducted by MSc student at Addis Ababa University, College of Health Sciences, Department of Medical Laboratory Science. Please read the following statements and ask any unclear points before you agree to participate.

Introduction

The topic of this study is Intestinal Parasitosis in Relation to CD4+T Cells Levels and Anemia among HAART Initiated and Non-HAART Initiated Pediatric HIV Patients in Zewditu Memorial Hospital, Addis Ababa, Ethiopia. Participation in this study is exclusively voluntarily. If you are not interested to participate or if you once decide to participate and withdraw yourself at any time, there will be no consequences and you will get all the services provided in the hospital with no problems. If you decide to participate, you have to sign on the assent/parental permission template form and you may obtain a copy of this information sheet.

What is expected from me as participant of the study?

As a participant of this study, you are expected to agree that 40 grams of stool will be collected. In addition, you are expected to give answers for some questions about your health and socio-demographic conditions. You need to know that your results might be discussed with other appropriate individual out of this hospital. But your name, address and phone number will not be disclosed and rather an identification code will be used in such conditions.

How much time will I spent to participate in this study?

You will spend 15-30 minutes until the specimen is collected, the questionnaire is filled and the assent/parental permission form is signed.

What are the risks of participating in this study?

Specimen collection will have no effect and pose no pain on you and the only thing you spend is just your time to fill the questionnaire.

How my information is to be kept in secrete?

All information that you give and the results from your specimen will be used for this study only. Only limited numbers of professional will have access to the information. All the information will be encoded in a computer and saved with password protection.

What are the benefits from participation?

Since this study is MSc student research, there will not be payments for participants. But your participation is important for the assessment of intestinal parasites among HAART and Non-HAART pediatric HIV patients. You will also obtain all the results of the analysis for free and communicated to your physician for the appropriate management.

What are my rights as a participant of this study?

You have the right to withdraw yourself from the study at any time and all the services provided in the hospital will not be discontinued. You are also welcomed if you have any question for further explanations about the study. You can get the results of the analysis.

What can I do if I have a problem or a question?

Please direct any questions or problems you may encounter during this study to:

Hylemariam Mihiretie

Department of Medical Laboratory Sciences, College of Health Sciences

Addis Ababa University

Mob: +251-91-o-60-05-43

Email: hylemariam@gmail.com

For additional information, please contact Addis Ababa University, College of Health Sciences, Department of Medical Laboratory Sciences at: Telephone +251-1-12-75-51-70

Agree to participate? Yes----- No-----

በአዲስ አበባ ዩኒቨርቲ፤ የጤና ሳይንስ ኮሌጅ የሕክምና ላቦራቶሪ ት/ክፍል

በአዲስ አበባ ዩኒቨርቲ ፤የጤና ሳይንስ ኮሌጅ የሕክምና ላቦራቶሪ ት/ክፍል በማስተርስ ዲግሪ ተማሪ የመመረቂያ ጥናት ላይ እንዲሳተፉ ተናብዘዋል። እባኮዎ በዚህ ጥናት ላይ ከመስማማቶ በፊት ከዚህ ቀጥሎ የሚገኘዉን ንባብ በጥሞና ያንብቡ/ይመልሱ ግል□ያልሆነዉን ማንኛዉንም ሃሳብ ይጠጥቁ።

መግቢያ

የጥናቱ ርዕስ **ኤች አይቪ በደማው ውስጥ ያሉ ህጻናት ላይ ያለዉን የሆድ ዉስጥ ትላትሎች ይዞታ ከ ሲዲፎር መጠን እና ደም ማነስ ጋር አያይዞ ለመዳሰስ ነዉ** ። እርሶ በዚህ ጥናት ላይ የሚኖሮት ተሳትፎ ሙሉ ለሙሉ በበጎ ፈቃደኝነት ላይ የተመሠረተ ነዉ። በዚህ ጥናት ዉስጥ ላለመሳተፍ ከወሰኑ በዚህ ሆስፒታል ዉስጥ የሚሰጠዉም አገልግሎት አይቋረጥም። በትናቱ ለመሳተፍ የሚስማሙ ከሆነ የስምምነት ቅጹ ላይ በጹሑፍ ወይም በጣት ፊርማዎ ማስቀመጥ ይጠበቅቦታል። ከፈለጉ ይህንን የመረጃ ቅጹ አንድ ቅጅ ለራሶ ሊያስቀሩ ይችላሉ።

የጥናቱ ተሳታፍ በመሆኔ የሚጠበቅብኝ ምንድነዉ

በዚህ ጥናት ለመሳተፍ ሚስማሙ ከሆነ 40 ግራም የአይነምድር ናሙና እደሚወሰድ እና ለጥናቱ እንዲዉል መስማማት ይጠበቅቦታል። ከተወሰደዉም ናሙና ላይ የሚገኙ መረጃዎች በዚህ ሆስፒታል ዉጭ ለሚገኙ ለሥራዉ አግባብነት ላላቸዉ ሰዎች ቢነገር የማቃወሙ መሆኑን መስማማት ይጠበቅቦታል። ይሁን እንጂ ይህ ዓይነቱ መረጃ የእርሶን ማንነት የማገልጹ ማረጃዎን ማለትም ስም፣ አድራሻ፣ የስልክ ቁጥር ና የመሳሰሉት መረጃዎት አይጨምርም። ይልቁንም ለዚህ ጥናት አገልግሎት ብቻ የሚዉል መለያ ቁጥር ጥቅም ላይ እንሚዉል ይደረጋል። በተጨማሪም ስለ እርሶ አጠቃላይ የጤና ሁኔታ ለሚቀርቡ አንዳንድ ተጨማሪ ጥያቄዎች መልስ ማስጠት ይጠበቅቦታል።

በዚህ ጥናት መሳተፍ ምን ያህል ጊዜ ይፈጃል

የተዘጋጀዉን መጠይቅ ለመሙላት፤ የስምምነት ቅጹ ላይ ለመፈረምና ናሙና ለመስጠት 15-30ደቂቃ ያስፈልጋል።

በዚህ ጥናት መሳተፍ የማስከትላቸዉ ችግሮች ምንድናቸዉ

ናሙና የሚሰበሰብበት ወቅት ምነም አይነት ችግር አያስከትልቦትም እንዲሁም ምንም አይነት የህመም ስሜት አያስከትልም ። ስለዚህም የሚያጡት ነገር ቢኖር መጠይቁን ለመሙልት የሚያጠፉት ጊዜ ነዉ።

የሕክምና መረጃ በሚስጥር ተጠብቆ መቆየት እንዴት ነዉ

ስለራሶ/ስለልጅዎ የሰጡት ማንኛዉም መረጃ ከተወሰደዉ ናሙና ላይ የተገኘዉ የላቦረቶሪ ዉጠቶች የሚዉለዉ ለጥናቱ አላማ ብቻ ነዉ። ይህን ማደር ሊያገኙ የሚችሉ የተወሰኑ የጥናቱ ተባባሪ ሠራተኞች ብቻ ናቸዉ። ከዚህም በላይ ስለእርሶ ያለዉን ማንኛዉንም መረጃ የተለየ የይለፍ ቃል ባለዉ የኮፒዉተር የመረጃ ማህደር ዉስጥ እንድቀመጥ ይደረጋል።

በዚህ ጥናት መሳተፍ የሚያስገኛቸዉ ጥቅሞች ምንድናቸዉ□

ይህ ጥናት የማስተርስ ዲግር መመረቂያ እንደማሆኑ መጠን ለተሳታፊዎች ገንዘብ አይሰጥም። ለወደፊት በተመሳሳይ ሁኔታ ዉስጥ ላሉ ህጻናት መረጃ ላይ የተመረተ ህክምና ለመስጠት ያግዛል። ከፈለጉ የሁሉንም የላቦራቶሪ ዉጤቶች ነፃ ያገኛሉ።

በዚህ ጥናት ተሳታፍ በመሆኔ መብቶቼ ምንድንናቸዉ

በጥናቱ ዉስጥ ያሎትን ተሳትፎ በማንኛዉም ጊዜ የማቋረጥ ሙሉ መብትዎ የተጠበቀ ከመሆኑም በላይ ራሶን ከጥናቱ በማግለሎ ምክንያት የሚቀርቦት ምንም ዓይነት የሆስፒታል አገልግሎት አይኖርም። ከዚህም በተጨማሪ ጥናቱን በተመለከተ ማንኛዉንም ዓይነት ጥያቄ የመጠቅና ገለፃ የማግኘት መብት አሎት። የላቦራቶሪ ምርመራ ዉጤቱንም በነፃ ማግኘት ይቻላል።

ጥያቄ ካለኝ ወይም ችግር ቢያጋጥመኝ ምን ማድረግ ይገባኛል

ይህን ጥናት በተመለከተ ወይም ከዚህ ጋራ በተዛመደ መልኩ ስለሚያጋጥዉ ድንገተኛ ችግሮች ወይም ጥያቄ ካሎት በሚከተለዉ አድራሻ ይተቀሙ።

ኃይለማርያም ምህረቴ

የሕክምና ላብራቶሪ ሳይንስ ት/ክፍል፤ የጤና ሳይንስ ኮሌጅ

አዲስ አበባ ዩኒቨርሲቲ

ሞባይል +251910600543

ኢ-ሜይል hylemariam@gmail.com

ለተጨማሪ መረጃ አዲስ አበባ ዩኒቨርሲቲ የሕክምና ላብራቶሪ ሳይንስ ት/ክፍል ይጠይቁ

ስልክ +251-1-12-75-51-70

እስማማለሁ----------አልስማማም--------

Annex III: English and Amharic Versions of Assent Form

This page contains an agreement signature to participate in the study entitled with „***Intestinal parasitosis in relation to CD4 T cell leve1 and anemia among HAART initiated and non-HAART initiated pediatric HIV patients in Zewditu Memorial Hospital, Addis Ababa, Ethiopia***". So please read the following points and sign your signature at the end in the space provided.

1. I understand the objective of the study in „***Intestinal parasitosis in relation to CD4 count and anemia among HAART initiated and non-HAART initiated pediatric HIV patients in Zewditu Memorial Hospital, Addis Ababa, Ethiopia***" and I can communicate with the peoples that conduct the study when I want them.

2. I know that the information that I gave are going to be used for this study only.

3. I understand that, all the information given for the study and the results are confidential.

4. I understand that I will not get any money for my participation.

5. I understand that I have a right to stop from participation any time in the study.

6. All the information is explained by Mr./Mrs/Nurse.

Signature of the participant: _____

Address of the participant: _____

Date: _____

Please direct any questions or problems you may encounter during this study to:

Hylemariam Mihiretie

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Email: hylemariam@gmail.com

For additional information, please contact Addis Ababa University, College of Health Sciences, Department of Medical Laboratory Sciences at: Telephone +251-1-12-75-51-70

የተሳታፊ የስምምነት ቅጽ

ይህ ገን ‘ Intestinal Parasitosis in Relation to CD4 Count and Anemia among HAART initiated and non-HAART initiated Pediatric HIV Patients in Zewditu Memorial Hospital, Addis Ababa, Ethiopia’ **ኤች አይቪ በደማው ውስጥ ያሉ ህጻናት ላይ ያለዉን የሆድ ዉስጥ ትላትሎች ይዞታ ከ ሲዲፎር መጠን እና ደም ማነስ ጋር ተያዞ ለሚከሄድ ጥናት ስለመሳተፍ የሚያመለክት የስምምነት ቅጽ ነዉ፡፡ ስለዚህ በዚህ ጥናት ዉስጥ ለመከተት / ለመሣተፍ / እባክዎን የሚከተለዉን ቅጽ አንብበዉ በመጨረሻ በተሰጠዉ ክፍት ቦታ ፊርማዎን ያኑሩ፡፡**

1. የጥናቱን ዓላማ ተገንዝቤ አለሁ፤ ጥናቱን የሚያከሄድዉን ሰዉ ስፈልገዉ ማግኘት □□□እንደምችልም ተረድቻለሁ፡፡

2. የአይነምድር ናሙና ተወስ□ ለጥናቱ ዓላማ □□□እንደሚዉል ተረድቻለሁ፡፡

3. ለጥናቱ የሚሠጡ መረጃዎችና ከጥናቱ የሚገኙ ዉጤቶች በሚስጥር □□እንደሚጠበቁ ተረድቻለሁ፡፡

4. ከዚህ ጥናት ገንዘብ በተለየ መልኩ □እንደማላገኝ ተርድቻለሁ፡፡

5. ከዚህ ጥናት በፈለግሁ ጊዜ አቋርጬ መዉጣት □እንደምችል ተረድቻለሁ፡፡

6. ይህ መረጃ በአቶ/ ወ/ሮ/ነርስ_____ተፃል□ልኛል፡፡

የተሳታፊ ኝርማ፡ _____
የተሳታፊ አድራሻ፡_____
ቀን፡ _____

ችግቸ ካጋጠመዎት ወይም ጥያቄ ካለዎት፤

ኃይለማርያም ምህረቱ

የሕክምና ላብራቶሪ ሳይንስ ት/ክፍል፤ የጤና ሳይንስ ኮሌጅ

አዲስ አበባ ዩኒቨርሲቲ

ሞባይል +251910600543

ኢ-ሜይል፤ hylemariam@gmail.com

ለተጨማሪ መረጃ አዲስ አበባ ዩኒቨርሲቲ የሕክምና ላብራቶሪ ሳይንስ ት/ክፍል ይጠይቁ

ስልክ +251-1-12-75-51-70

Annex IV: English and Amharic Versions of Parental Permission Template

This page contains an agreement signature to give information on behalf of your child which is below 7 years of age in the study entitled with „***intestinal parasitosis in relation to CD4 T cell level and anemia among HAART initiated and non-HAART initiated pediatric HIV patients in Zewditu Memorial Hospital, Addis Ababa, Ethiopia***". So please read the following points and sign your signature at the end in the space provided.

1. I understand the objective of the study „***Intestinal parasitosis in relation to CD4 count and anemia among HAART initiated and non-HAART initiated pediatric HIV patients in Zewditu Memorial Hospital, Addis Ababa, Ethiopia***" and I can communicate with the peoples that conduct the study when I want them.

2. I know that the information that I gave are going to be used for this study only.

3. I understand that, all the information given for the study and the results are confidential.

4. I understand that I will not get any money for giving information about my child.

5. I understand that I have a right to stop from my child"s participation any time in the study.

6. All the information is explained by Mr./Mrs/Nurse.

Signature of the guardian: _____

Address of the guardian: _____

Date: _____

Please direct any questions or problems you may encounter during this study to:

Hylemariam Mihiretie
Department of Medical Laboratory Sciences, College of Health Sciences
Addis Ababa University
Mob: +251-91-o-60-05-43
Email: hylemariam@gmail.com

For additional information, please contact Addis Ababa University, College of Health Sciences, Department of Medical Laboratory Sciences at: Telephone +251-1-12-75-51-70

የተንከባካቢዎች የስምምነት ቅጽ (እድሜያቸዉ ከ7 ዓመት በታች ለሆኑ ህፃናት)

ይህ ገን ' Intestinal Parasitosis in Relation to CD4 Count and Anemia among HAART initiated and non-HAART initiated Pediatric HIV Patients in Zewditu Memorial Hospital, Addis Ababa, Ethiopia' **ኤች አይቪ በደማቸው ውስጥ ያሉ ህጻናት ላይ ያለዉን የሆድ ዉስጥ ትላትሎች ይዞታ ከ ሲዲፎር መጠን እና ደም ማነስ ጋር ተያዞ ለሚከሄድ ጥናት ለሚሳተ□ ልጆች መረጃ ለመስጠት መስማማትን የሚያመለክት የስምምነት ቅጽ ነዉ። ስለ□ህ በ□ህ ጥናት ዉስጥ ስለ ተሳታፊዉ ህፃን መረጃ በመስጠት ለመሳተፍ እባክዎን የሚከተለዉን ቅጽ አንብበ□ በተሰጠዉ ክፍት ቦታ ፊርማዎን ያኑሩ።**

1. የጥናቱን ዓላማ ተገንዝቤ አለሁ፤ ጥናቱን የሚያከሄድዉን ሰዉ ስፈልገዉ ማግኘት □□□እንደምችልም ተረድቻለሁ።

2. ከልጄ የአይነምድር ናሙና ተወስ□ ለጥናቱ ዓላማ □□□እንደሚዉል ተረድቻለሁ።

3. ለጥናቱ የሚሠጡ መረጃዎችና ከጥናቱ የሚገኙ ዉጤቶች በሚስጥር □□እንደሚጠበቁ ተረድቻለሁ።

4. ከዚህ ጥናት ገንዘብ በተለየ መልኩ □እንደማላገኝ ተርድቻለሁ።

5. ከዚህ ጥናት በፈለግሁ ጊዜ ስለ ልጄ መረጃ መስጠቴን ማቆም **□እንደምችል ተረድቻለሁ።**

6. ይህ መረጃ በአቶ/ ወ/ሮ/ነርስ_____ተፃል□ልኛል።

የተሳታፊ ወላጅ ወይም አሳዳጊ ኝርማ፡ _____
የተሳታፊ አድራሻ፡_____
ቀን፡ _____

<u>ችግር ካጋጠመዎት ወይም ጥያቄ ካለዎት፤</u>

ኃይለማርያም ምህረቴ

የሕክምና ላብራቶሪ ሳይንስ ት/ክፍል፤ የጤና ሳይንስ ኮሌጅ

አዲስ አበባ ዩኒቨርሲቲ

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ኢ-ሜይል፤ hylemariam@gmail.com

ለተጨማሪ መረጃ አዲስ አበባ ዩኒቨርሲቲ የሕክምና ላብራቶሪ ሳይንስ ት/ክፍል ይጠይቁ

ስልክ +251-1-12-75-51-70

Annex V: Stool Specimen Processing Procedures

a) Direct wet mount

Principle: Many parasites cause disease in man. Some of these parasites are excreted in stool; they are called intestinal parasites. Intestinal parasites can be identified by examination of fresh stool samples. In stool samples we can find worms (eg. Ascaris lumbricoides) and segments of worms (e.g. Taenia species) visible to the eye. By microscopic examination of fresh stool samples, we can find eggs (e.g. Hookworm) and larvae of worms (e.g. Strongyloides stercoralis). We also find protozoa trophozoites (e.g. Amoeba) and cysts (e.g. Cyclospora cayetanensis). In heavy and moderate infection, a direct smear examination with normal saline and/or iodine to stain cysts, is usually sufficient. For light infections, a concentration of the stool sample might be required to find helminth (worm) eggs and protozoa by microscopic examination [36].

Procedure

1. Place a drop of normal saline on a clean slide
2. Using a piece of stick, place a small amount of specimen, including blood and mucus in one end of the slide and cover it with a cover slide
3. First examine microscopically using 10 x objectives to give good contrast and use the 40x objective to identify trophozoites of protozoa.

Reporting: Report the name of the parasite found [36].

b) Formalin-Ether concentration technique

Principle: In the Ridley modified method, feces are emulsified in formol water, the suspension is strained to remove large fecal particles, ether or ethyl acetate is added, and the mixed suspension is centrifuged. Cysts, oocysts, eggs, and larvae are fixed and sedimented and the fecal debris is separated in a layer between the ether and the formol water. Fecal fat is dissolved in the ether [36].

Procedure

1. Using a stick, emulsify 1g of stool in 4 ml of 10% Formol water in a tube
2. Add a further 3-4 ml of 10% formol water, cap the tube and mix well by shaking
3. Sieve the emulsified feces and collect the suspension in a beaker
4. Transfer the suspension to centrifuge tube and add 3-4 ml of diethyl ether or ethyl acetate
5. Mix the tube for 1 minute centrifuge at 3000 rpm for 1 minute
6. Discard the ether, fecal debris and formol water
7. Tap the bottom of the tube and mix the sediment
8. Add a drop of normal saline in a clean slide, add a piece of specimen and cover it with cover slide
9. First examine microscopically using 10 x objectives to give good contrast and use the 40x objective to identify cysts and ova of parasites and add iodine to the smear for staining.

Formol- ether oocyst concentration technique

Follow steps from 1 to 5 of the above method and continue as follows;

6. Centrifuge immediately at low speed, at 1000 rpm for 1 minute, remove the fluid from fecal debris and ether and transfer it to a centrifuge tube
7. Add formol water to make volume up to 10-15 ml and centrifuge at 3000 rpm for 5-10 minute
8. Remove the supernatant, tap the bottom of the tube, mix the sediment and examine under 40 x objectives [36].

c) Modified Ziehl-Neelson (Z-N) staining method

Principle: This technique is useful for the identification of oocysts of the coccidian species (*Cryptosporidium*, *Cystoisospora*, and *Cyclospora*), which may be difficult to detect with routine stains such as trichrome. Unlike the routine Ziehl-Neelsen, this stain does not require the heating of reagents for staining [37].

Procedure

1. Prepare a smear from the sediment obtained by the formol ether oocyst concentration technique (see annex VI), air dry it, and fix the smear with methanol for 1 minuteand allow to dry
2. Stain with unheated carbol fuschin for 30 minutes and wash off the stain with water
3. Decolorize with 1% acid alcohol for 1-2 minutes and wash off with water
4. Counterstain with 0.3% malachite green (or methylene blue) for 60 seconds and wash off with water
5. Air dry and examine under low power to detect oocyst and under oil immersion to identify them.

Interpretation: Small, round to oval, pink red stained bodies measuring 4–6 micrometer indicate oocysts [37].

Annex VI: Declaration

Title of project: Intestinal parasitosis in relation to CD4+ T cell levels and anemia among HAART initiated and non-HAART initiated pediatric HIV patients in Zewditu Memorial hospital.

I, the undersigned, declare that this is my original work and has not been presented for a degree in this or any other university and all sources of materials used for this thesis have been acknowledged.

Principal investigator:

Name: **Hylemariam Mihiretie**

Address: Department of Medical Laboratory Sciences, AAU

Signature: _____ Date: _____

This thesis has been submitted with my approval as a **University advisor**;

Name: **Assaye Birhanu (MSc)**

Address: Department of Medical Laboratory Sciences, AAU

Signature: _____ Date: _____

Name: **Aster Tsegaye (MSc, PhD)**

Address: Department of Medical Laboratory Sciences, AAU

Signature: _____ Date: _____

Name**: Bineyam Taye (MPH)**

Address: Department of Medical Laboratory Sciences, AAU

Signature: _____ Date: _____