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Prevalence Of Parasitic Infections In Relation To CD4$^+$ And Antiretroviral (ART) Usage Of HIV Sero-Positive Patients Attending Irrua Specialist Teaching Hospital (ISTH) Irrua, Edo State, Nigeria

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Abstract:** Intestinal parasitic infections are globally endemic and constitute the greatest single worldwide cause of illness and disease. This study was designed to determine the Prevalence of parasitic infections in relation to CD4$^+$ and Antiretroviral Usage of HIV sero-positive patients Attending Irrua Specialist Teaching Hospital (ISTH) Irrua, Edo State, Nigeria. A total of two hundred (200) subjects from Irrua Specialist Teaching Hospital (ISTH), Irrua, were recruited for this study. They were categorised into two groups based on their HIV/AIDS clinical and laboratory test results. Based on this, 170(85.0%) were HIV sero-positive and 30 (15.0%) were HIV sero-negative subjects and were between the age ranges of 18-89 years and 18-59 years respectively. The parasites isolated were hookworms, Strongyloides stercolaris, Trichuris trichuria, Ascaris lumbricoides and Enterobrius vermicularis with a prevalence of 17(10.0%),7(4.1%), 4(2.4%), 16(9.4%) and 7(4.1%) respectively for the HIV sero-positive subjects while HIV sero-negative had a prevalence of 1(3.3%), 0(0.0%), 0(0.0%), 10(33.3%), 0(0.0%) for the same parasites respectively and in addition 1(3.3%) of Schistosoma mansoni was found respectively. The mean and standard deviation for the age of subjects were 36.4±15.92 and 30.1±10.26 for HIV sero-positive and HIV sero-negative subjects respectively. Parasitic infections were higher within the age range 30-44 for HIV sero-positive and 18-29 age range for HIV sero-negative. The prevalence of parasitic infections among the HIV sero-positive subjects was 44.7%, as compared to 40.0% of HIV sero-negative. A significant difference (p<0.05) was observed in the prevalence of opportunistic parasitic infections, between CD4$^+$ count, ART use and age group.

***Keyword:** Parasites, Infections, CD4, Antiretroviral, Therapy*

I. INTRODUCTION

Intestinal parasitic infections caused by protozoans and helminths have been reported to be globally endemic and constitutes the greatest single worldwide causes of illness and disease (Chan, 1997; Pillai and Kain, 2003). In fact about one third of the world, more than two billion people are infected with intestinal parasites (Chan, 1997). These intestinal parasites cause intestinal obstruction, malnutrition, iron deficiency anaemia, diarrhoea, malabsorption and other damage to the hosts (Buchini *et al.*, 2007). It is estimated that as much as 60% of the World's population is infected with intestinal parasites which may play a significant role in morbidity due to intestinal infections (WHO, 1987). The rate of infection is also remarkably high in Sub-Saharan Africa, where the majority of Human Immune Deficiency Virus (HIV) /Acquired Immunodeficiency Syndrome (AIDS) cases are concentrated where factors including poverty and malnutrition could promote transmission of both infections in the region (UNAIDS/WHO, 2002). Intestinal parasitic infections have a worldwide distribution with high prevalence found in people with low socio-economic status and poor living conditions as well as people in over-crowded areas with poor environmental sanitation, improper garbage disposal, unsafe water supply and unhygienic personal habits (Adamu *et al*., 2006; Noor *et al*., 2007). These factors are the causes of a major proportion of the burden of disease and death in developing countries (Adamu *et al*., 2006). Intestinal protozoa and faeco-oral transmitted helminths (STH) constitute major health problems, especially in the tropical and sub-tropical regions (Savioli *et al*., 1992). They can be the cause of a wide spectrum of clinical problems ranging from apparently symptomless infections to life-threatening conditions such as intestinal obstruction as seen in Ascaris infestation (Savioli *et al*., 1992; Glickman *et al*., 1999).

Furthermore, Intestinal parasites are widely distributed in Nigeria largely due to the low level of environmental and personal hygiene, contamination of food and drinking water that results from improper disposal of human excreta (Uzairue *et al.*, 2013). Intestinal parasites as a major concern in most developing countries have been pronounced with the co-occurrence of malnutrition and HIV/AIDS. Opportunistic parasitic infections are a common feature in HIV/AIDS infections where almost 80% of AIDS patients die of AIDS-related infections including intestinal parasites rather than of the HIV infection itself which usually occur late in the course of HIV infection when Cluster of Differeation (CD4) + T-cell count has been severely depleted mostly below 200cells/mm3 (Kam, 1994; Kelly, 1998; Shah *et al.*, 2005). In the absence of Anti-Retroviral Therapy (ART) HIV/ AIDS patients in developing countries unfortunately continue to suffer the consequences of opportunistic parasites (Maggi *et al.*, 2000). Patients enrolling into ART programmes with very low CD4 cell counts have heightened risk of morbidity and mortality before ART (Lawn *et al.*, 2005). There is evidence that the control of these opportunistic parasitic infections in HIV-positive persons under Highly Active Anti-Retroviral Therapy (HAART) is also induced by the inhibition of the aspartyl protease of the parasites and by the reconstitution of the immune system of the patient (Willemot and Klein, 2004; Alfonso and Monzote, 2011). However, patients in resource limited settings typically start ART programmes with advanced symptomatic disease and very low blood CD4 cell counts which predisposes them to high rates of both clinical and subclinical opportunistic infections (Lawn *et al.*, 2005). Intestinal obstruction, anaemia, malnutrition, dysentery syndrome, fever, dehydration, vomiting and colitis are the major complications associated with soil transmitted helminths infections (Cooper, 1991).

The clinical course of infection with larger parasites, such as the helminths, can vary widely from asymptomatic to severe secondary complications and these asymptomatic hosts may experience several health effects depending on the number, type, and severity of infections present (Higgins *et al*., 1984; Checkley *et al*., 1997). Therefore, this study is set to assess the prevalence of intestinal parasites in Irrua Specialist Teaching Hospital (ISTH) Irrua,. Parasitic infections are infections or opportunistic infections that affect persons who are immune-compromised, where HIV positive subjects are highly implicated (Chan, 1997; Pillai and Kain, 2003). Therefore, it is important to determine the relationship between $CD4^+$ count, ART and parasitic infections as this will help be of immense benefit to government and healthcare policies.

II. MATERIALS AND METHODS

STUDY AREA

This study was carried out in Irrua Specialist Teaching Hospital (ISTH), Irrua, the administrative headquarters of Esan Central Local Government Area of Edo State, Nigeria. Majority of the population are civil servants, traders, businessmen/women, transporters, farmers, teachers/lecturers and students. The samples were examined in the Research Diagnostic Laboratory, of the Department of Medical Laboratory Science, College of Medical Sciences, Ambrose Alli University, Ekpoma.

STUDY POPULATION

Two hundred (200) stool samples comprising one hundred and seventy (170) samples from HIV Sero-positive individuals and thirty (30) samples from HIV sero negative individuals were recruited from the study area. HIV sero positive individuals with other underlying health and who did not give consent were excluded from this study. HIV sero negative individuals with any health condition, exhibiting signs and symptoms of any illness, and who did not gieve consent were excluded from the study. These informations were obtained from the medical records of these subjects.

ETHICAL APPROVAL

This was obtained from the Ethics and Research Committee of Irrua Specialist Teaching Hospital (ISTH), Irrua. Also, informed consent was sought from the subjects while explaining the aim and objectives, economic importance and Health benefits of the study to the subjects.

COLLECTION OF SAMPLES/ DATA COLLECTION

Specimen containers (Wide Mouthed Screw Capped Plain Plastic Universal Containers), which were clean, dry and leak proof (Cheesbrough, 2006), and properly labelled with subjects' names, sex, age, and serial number were used for sample collection. The subjects were properly educated on how to collect the sample, the part of the stool sample to be collected and as well as the quantity needed. Early morning stool was advised to be taken. The samples were received the next morning with each subjects name, age, sex and serial number entered into the record book. The samples were then transported immediately to the laboratory for examination. Samples that could not be examined early enough were preserved with 10% formol saline (1g of stool to 3ml with 10% formol saline). Data of ART use and $CD4^+$ count of the subjects were obtained from their respective registers in the

Hospital. Stool samples were collected from those who are positive to HIV sero-test, after informed consent was obtained from them and they equally completed a structured questionnaire.

EXAMINATION AND ANALYSIS OF SAMPLES

The samples were examined using the Macroscopic and Microscopic methods. Samples were examined macroscopically for colour, consistency and constituents such as mucus, blood, pus, adult worms such as *Enterobius vermicularis* or *Ascaris lumbricoides* and tape worm segments. The consistency was used as a guide as to whether the trophozoites or the cyst stage, egg or worm of the parasite is likely to be present (Cheesbrough, 2006). The microscopic methods included the use of wet preparations (Saline and Lugol's iodine) and concentration methods. Iodinated formol-ether concentration method was employed as described by WHO, (1991) and Cheesbrough, (2006). Also, modified cold Ziehl-Neelsen's stain (for Cryptosporidum species) was employed as described by Current (1990). Lymphocyte subset, CD4+ count was analyzed using FACScan flow cytometry (Becton Dickinson Immunocytometry system, and Jose, CA., USA).

STATISTICAL ANALYSIS

The percentage prevalence was calculated in each case and comparative analysis of the results was done using Chi-square. A p-value less than 0.05 ($p<0.05$) was considered statistically significant.

III. RESULTS

A total of 200 subjects from Irrua Specialist Teaching Hospital (ISTH), Irrua were recruited for this study. They were categorised into two group based on their HIV/AIDS clinical and laboratory test results. Based on this, 170(85.0%) were HIV sero positive and 30(15.0%) were HIV sero-negative where the age ranges between 18-89 years for HIV sero positive and 18-59 years for HIV sero-negative. The results of the socio-demographic data revealed that the mean age of the subjects were 36.4±15.92 and 30.1±10.26 for HIV sero-positive and HIV sero-negative respectively. The majority of the subjects 112(56.0 %) were female and 84(44.0%) were male (Table 1).

Also, the results showed the age and sex distribution of hiv sero-positive and sero-negative of the study subjects (table 2)

The results on the parasites encountered among HIV sero positive and HIV sero negative subjects revealed that Hookworms with 17(10.0%) had a higher prevalence for helminthic infections while cryptosporidium species with a prevalence of 25(14.7%) was the only protozoan recorded for HIV sero-positive subjects. Also, *Ascaris lumbricoides* with a prevalence of 10(33.3%) was the most occurring helminthic infection in HIV sero negative subjects and there was no protozoan isolated. The overall prevalence for parasitic infections for both HIV sero-positive and negative subjects were 88(44.0%). The overall prevalence of parasitic infections for HIV sero-positive was 76(44.7%) while HIV sero-negative was 12(40.0%) (Table 3).

The results on the parasites encountered, as it relate with HIV status, ART status and $CD4^+$count revealed that the prevalence of helminthic and protozoan infections for HIV sero positive subjects were 51(81.0%), and 25(100.0%) respectively while HIV sero negative subjects recorded prevalence of 12(19.0%) and 0(0.0%) for helminthic and protozoan infections respectively. HIV sero positive subjects on ART recorded a prevalence of 22 (43.1%) for helminths and 9(36.0%) for protozoa infection while those not on ART recorded a prevalence of 29(56.9%) for helminthic infections and 16 (64.0%) for protozoa infection. As it relates to $CD4^+$ count, the helminthic infection was 32(62.7%),11(21.6%) and 8(15.7%) for <200 cells/µL, 200-499cells/µL and ≥500cells/µL $CD4^+$ count respectively while the protozoan infection was 18(72.0%), 5(20.0%) and 2(8.0%) for <200 cells/µL, 200-499cells/µL and ≥500cells/µL $CD4^+$ counts respectively (Table 4).

The results on the parasites encountered in relation to ART status showed that the prevalence of parasitic infections were 31(40.8%) for those on ART and 45(59.2%) for those not on ART (Table 5).

The results on the magnitude of parasitic infection as related to HIV sero positive and HIV sero negative subjects revealed that the HIV sero-positive and HIV sero-negative subjects had prevalence of 63(88.7%) and 8(11.3%) for light infections respectively,8(72.7%) and 3(27.3%) for medium infections respectively, 5(83.3%) and 1(16.7%) for heavy infections respectively, while 7(87.5%) and 1(12.5%) for mixed parasitic infections respectively. From the results it was observed that the individuals who were HIV sero-positive had the highest parasitic infection as compared to those of the HIV sero-negative subjects (Table 6).

The results on the prevalence of parasitic infections in relation to ART use and $CD4^+$ Counts showed that those subjects with $CD4^+$ count <200 had the highest prevalence with 50(65.8%) with prevalence of 16(21.1%) for $CD4^+$ count of 200-499 and 10(13.1%) for $CD4^+$ count of ≥500 (Table 7).

Table 1: Socio-Demographic Characteristic of the Study Subjects

	HIV^+n(%)	HIV^-n(%)	Total n(%)	X^2 p value
Sex				**0.229(0.6322)**
Male	76(44.7)	12(40.0)	88(44.0)	
Female	94(55.3)	18(60.0)	112(56.0)	
Age(Mean ± SD) years	36.4±15.92	30.1±10.26	35.3±15.21	
Residence				**7.38(0.0066)**
Urban	122(71.8)	14(46.7)	136(68.0)	
Rural	48(28.2)	16(53.3)	64(32.0)	
Marital Status				**3.58(0.058)**
Married	105(61.8)	13(43.3)	118(59.0)	
Not married	65(38.2)	17(56.7)	82(41.0)	
Occupation				**19.067(0.0079)**
Bankers	6(3.5)	0(0.0)	6(3.0)	
Traders	32(18.8)	8(26.7)	40(20.0)	
Civil Servants	39(22.9)	1(3.3)	40(20.0)	
House Wives	14(8.2)	2(6.7)	16(20.0)	
Students	47(27.7)	18(60.0)	65(32.5)	
Farmers	13(7.7)	1(3.3)	14(7.0)	
Drivers	13(7.7)	0(0.0)	13(6.5)	
Health Workers	6(3.5)	0(0.0)	6(3.0)	

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Table 2: Age and Sex Distribution of HIV Sero-Positive and Sero-Negative Subjects

Age (years)	Sex	Clinical Status of HIV$^+$n(%)	Study SubjectsHIV$^-$n(%)
0-14	Male	7	3
	Female	10	5
15-29	Male	21	5
	Female	22	2
30-44	Male	23	6
	Female	34	3
45-59	Male	16	2
	Female	19	4
60-74	Male	6	0
	Female	7	0
75-89	Male	3	0
	Female	2	0
Total		170(85.0)	30(15.0)

Table 3: Parasites isolated among HIV Sero-Positive and Negative Subjects

Parasite identified	HIV$^+$ n= 170 (%)	HIV$^-$ n= 30(%)	Total n= 200(%)	X^2 (p value)
Helminths				**27.27(0.00011)**
Hookworms	17(10.0)	1(3.3)	18(9.0)	
Strongloidesstercolaris	7(4.1)	0(0.0)	7(3.5)	
Trichuristrichura	4(2.4)	0(0.0)	4(2.0)	
Ascarislumbricoides	16(9.4)	10(33.3)	26(13.0)	
EnterobiumVermicularis	7(4.1)	0(0.0)	7(3.5)	
Schisostomamansoni	0(0.0)	1(3.3)	1(0.5)	
Protozoans				
Crytosporidium species	25(14.7)	0(0.0)	25(12.5)	
Total	**76(44.7)**	**12(40.0)**	**88(44.0)**	

Table 4: Parasite isolated as it relates with HIV Status, ART Status and CD4$^+$Count

	Type of parasitic intestinal infection		
Characteristics	**Helminthic infection**	**Protozoanic infection**	**X^2 (p value)**
HIV Status			**5.51(0.019)**
HIV$^+$	51(81.0)	25(100.0)	
HIV-	12(19.0)	0(0.0)	
ART Status			**0.35(0.552)**
ON ART (31)	22(43.1)	9(36.0)	
NOT ART(45)	29(56.9)	16(64.0)	
CD4$^+$ count (170) (Seropositive Only)			**0.99(0.607)**
<200 cells/µL	32(62.7)	18(72.0)	
200-499 cells/µL	11(21.6)	5(20.0)	
>500 cells/µL	8(15.7)	2(8.0)	

Table 5: Parasites isolated in relation to ART Status

Parasite identified	On ART	Not on ART	X^2 (p value)
Helminths			**1.497(0.91)**
Hookworms	7	10	
Strongloidesstercolaris	3	4	
Trichuristrichura	1	3	
Ascarislumbricoides	7	9	
EnterobiumVermicularis	4	**3**	
Protozoans			
Crytosporidium species	9	16	
Total	**31(40.8)**	**45(59.2)**	

Table 6: Magnitude of Parasitic infection as related to Sero-Positive and Negative Subjects

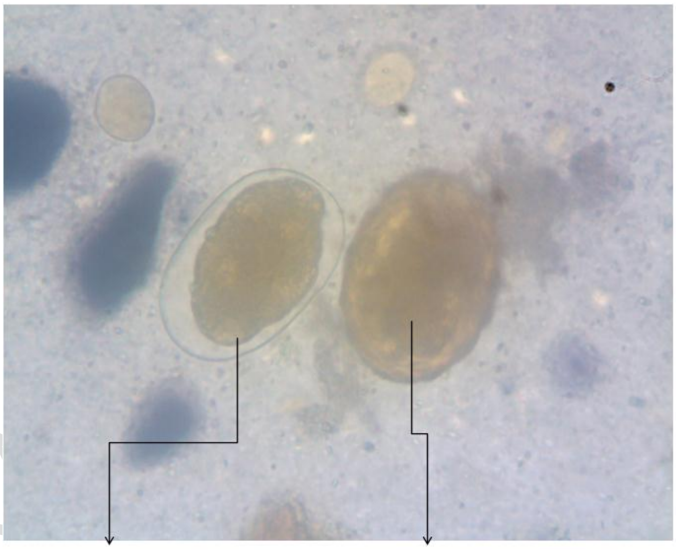
Clinical status of	**Magnitude of Infection**			
	Light	**Medium**	**Heavy**	**Mixed**
HIV$^+$ n=76	63(88.7)	8(72.7)	5(83.3)	7(87.5)
HIV$^-$ n=12	8(11.3)	3(27.3)	1(16.7)	1(12.5)
Total=88	**71(74.0)**	**11(11.4)**	**6(6.3)**	**8(8.3)**

X^2 = 2.143, degree of freedom=3, p-value=0.54

Table 7: Prevalence of Parasitic Infection in Relation to ART use and CD4$^+$ Counts

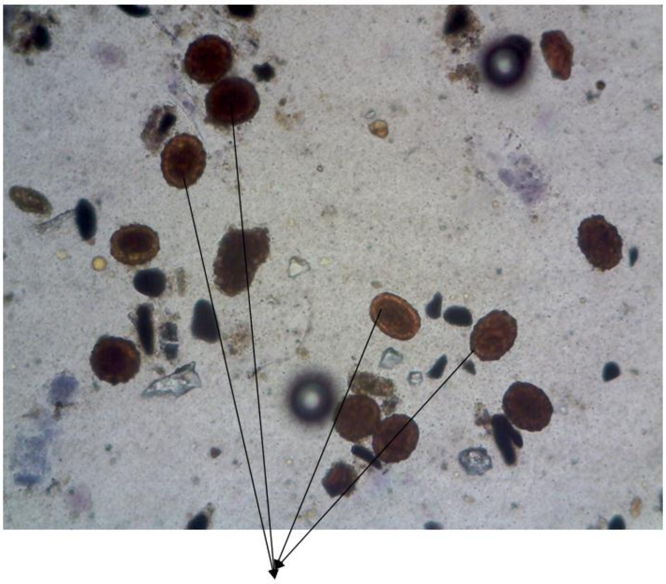
CD4+ cell/mm3	On ART n(%)	No ART n(%)	Total n(%)	X^2 (p value)
< 200	31(100.0)	19(42.2)	50(65.8)	
200-499	0(0.0)	16(35.6)	16(21.1)	**27.23(0.0000012)**
≥500	0(0.0)	10(22.2)	10(13.1)	
Total=170	**31(40.8)**	**45(59.2)**	**76(44.7)**	

CDC (1995) CD4+ T-cell counts classification



Egg of Hookworm *Ascaris lumbiociodes*

Figure 1: Mixed infection of Hookworm and Ascaris lumbiociodes (X40 Objectives)



Multiple Ova of *Acaris lumbiociodes*

Figure 2: Multiple infection of Acaris lumbiociodes (X10 Objectives)

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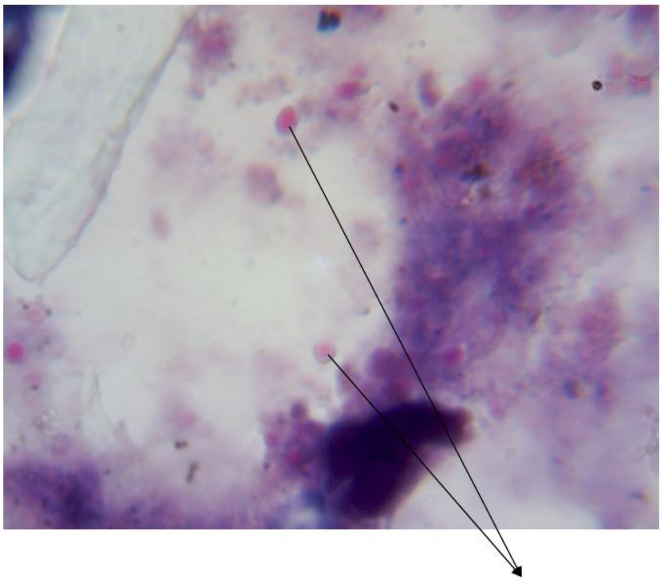

Figure 3: Multiple infections with Cryptosporidium species(X10 Objectives)

IV. DISCUSSION

Human Immune-deficiency Virus (HIV)/ AIDS pandemic has brought about a great change in intestinal fauna (Uzairue *et al.*, 2013). As the spectrum of immune-deficiency processes, HIV infected individuals become susceptible to a variety of opportunistic parasitic infection that occur with greater frequency and severity. Kumie, (2005) recorded that almost 80% of AIDS patients die from AIDS-related infections. Several intestinal parasites previously considered non-pathogenic in immune-competent individuals are opportunistically becoming aggressive and causing debilitating illness in HIV/AIDS patients (Gomez *et al.*, 1995). In this present study, the majority of HIV sero-positive subjects were found in the age range 30-44 years, the most productive and reproductive group of the society, 76(44.7%) and 94(55.3%) of the study participants were males and females respectively, showing high HIV exposure rate in both sexes.

The prevalence of parasitic infections among the HIV sero-positive subjects was 44.7% and this in agreement with work of Sow *et al.*, 2012, Nigus (2010) and Zeynudin *et al.*, 2013 who reported 43.6%, 33.6% and 39.56% respectively. But it is in contrast to the work of Henry (1986), Therizol (1989), Babatunde *et al.*, (2010), and Inabo *et al.*, 2012, who reported 7.5%, 8.0%, 87.8 and 70.6% respectively. The reason while this study is in contrast to some work reported may be due to the sample size used, the advancement of technique use, distribution of parasitic infections geographically, the use of antiretroviral drugs(ART) and hygienic practices according to the report of WHO(2010) and CDC(1995).

In terms of identified parasites specific prevalence, *Cryptosporidium species* which had a prevalence of 25(14.7%) was the highest occurring parasites and the only opportunistic parasites recorded in this study, while the prevalence of hookworms, *Strongyloides stercolaris*, *Trichuris trichiura*, *Ascaris lumbricoides* and *Enterobrius vernicularis* were 17(10.0%),7(4.1%), 4(2.4%), 16(9.4%) and 7(4.1%) respectively for HIV sero-positive subjects. Also, HIV sero-negative subjects had prevalence of 1(3.3%), 0(0.0%), 0(0.0%), 10(33.3%), 0(0.0%) for hookworms, *Strongyloides stercolaris*, *Trichuris trichiura*, *Ascaris lumbricoides* and *Enterobrius vernicularis* respectively and in addition 1(3.3%) of *Schistosoma mansoni* was isolated while no *Cryptosporidium species* were found in HIV sero-negative individuals. There was statistically significance (p-value=0.00011, p< 0.05) when the parasites isolated from HIV sero positive and negative subjects were compared. This is in agreement with report of CDC (1995) were parasitic infections were found to be more significant with those who their $CD4^+$ count is <200, and in this study the mean $CD4^+$ count was less than 200 according to CDC classification.

Furthermore, parasitic infections were found among those that are not on ART with prevalemce of 29(56.9%) and 16(64.0%) of helminthic and protozoan infection respectively, while those on ART had 22(43.1%) and 9(36.0%) respectively for helminthic and protozoan infections, and this comparison was not statistically significant (p-value=0.552, p>0.05), and this is in contrast with the work of Nigus (2010) and Missage *et al.*,(2013) who reported that the difference was statistically significant with p-value (0.001) and (0.001), both p-values less than 0.05. On the account of $CD4^+$, the helminthic and protozoan infections of those subjects whom their $CD4^+$ count was <200, 200-499 and ≥500 were 32(62.7%),11(21.6%),8(15.7%) and 18(72.0%)5(20.0%), 2(8.0%) respectively and their difference in distribution was not significant (p-value= 0.607) and this result was in disagreement to the work of Akinbo *et al.*, (2011) and Nigus,(2010) who showed that there was significant difference in the $CD4^+$ count with p-values of 0.0001 and 0.005 in there independent study.

On the distributions of parasites species for HIV sero-positive subjects,on ART and Not on ART, it was found that the distributions was not statistically significantly (p-value=0.9, p>0.05). This may not be unconnected to the fact that the area of study is endemic to the parasites isolated and the fact that most of those that have not started ART and those on ART's mean $CD4^+$ count was between <200 to ≤250 cells/µL. There were more mixed parasitic infections i.e polyparasitism with HIV sero-positive than HIV sero-negative with 7(87.5%) and 1(12.5%) respectively.

In terms of magnitude of parasitic infection of HIV sero-positive and HIV sero-negative subjects, there was prevalence of 63(88.7%), 8(72.7%), 5(83.3%) and 8(11.3%), 3(27.3%), 1(16.7%) recorded for light, medium and heavy parasitic infection respectively. This is in agreement with the work of Missage *et al.*, (2013), Akinbo *et al.*, (2011), Henry(1986), Therizol (1989), Babatunde *et al.*, (2010), and Inabo *et al.*, 2012 who reported same but in contrast with work of Nigus, (2010) who reported differently, reason be that most of Nigus's HIV sero-positive subjects were on Antiretroviral(ART) drugs, which improved their immunity and fight most parasitic infections which most times are opportunistic infections. When the distribution of parasitic infections from subjects on ART and not on ART was correlated with $CD4^+$ count, it was found to be statistically significant (p-value=0.0000012,$p<0.05$) and the correlation was also significant, this finding equally agreed with the works of of Missage *et al.,* (2013), Akinbo *et al.*, (2011).

V. CONCLUSION

It is evident from this report that parasitic infections were highly prevalent among the HIV infected patients. This was particularly so in patients with very low CD4+ cells count and those that are not on ART. The majority of the infections were prevalent among the age range 30-44. The prevalence of opportunistic infection among those of HIV sero-positive was 25(14.7%). A significant difference ($p<0.05$) was observed in the prevalence of opportunistic parasitic infection, between $CD4^+$ count, ART use and age group.

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