

Opportunistic Intestinal Protozoan Infections and Immunological Status of HIV/AIDS Patients in Jalingo Local Government, Taraba State

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Abstract: The Human Immunodeficiency Virus (HIV) is a virus that attacks the immune system. Opportunistic intestinal protozoans are among the major contributors to the global intestinal parasite load. This study investigated the danger and complication due to intestinal protozoans' infection among HIV/AIDS patients in Jalingo metropolis. The study also compared the prevalence of opportunistic intestinal protozoa infection and CD⁴⁺ T-Cell count between HIV/AIDS and non HIV patients. The procedure of wet mount technique and faecal concentration methods were employed. The CD⁴⁺ count of patients were determined using the Partee Cyflow Counter, which determines the percentage of CD⁴⁺ cells of individuals. The prevalence of opportunistic intestinal protozoan parasites was higher among female positives, 68 (19.60%) than the male, 50 (16.50%) with no significant difference ($p > 0.05$). The infection based on age groups revealed that the age group 10-15 years had the highest prevalence of 27 (48.21%) while the age group 41-45 years had the least prevalence of 2 (2.63%). The prevalence of intestinal parasites was highly significant among the study participants with CD⁴⁺ count < 200 cells/ μ l. *Cryptosporidium spp* was significantly associated with HIV positive subjects who had CD⁴⁺ count of less than 200 cell/ μ l. The prevalence of intestinal parasites was highly significant among the study participants with CD⁴⁺ count < 200 cells/ μ l. The implications of these findings are discussed and solution proposed to curb opportunistic infections among HIV/AIDS patients.

Keywords: Immunological, Opportunistic, Protozoans, HIV/AIDS, Taraba

1. Introduction

The Human Immunodeficiency Virus (HIV) is a virus that attacks the immune system which is the body's natural defense against illnesses. A person infected with HIV finds it more difficult to fight infection diseases. The viruses destroy white cell called T-helper cells, replicating inside them. T-helper cells are also known as CD⁴⁺ cells. There are two types of HIV: type-1 is the most common type and it is found worldwide. HIV-2 is found mostly in West Africa and in some cases in India and Europe [1].

The status of HIV/AIDS scourge in sub-sahara Africa. According to UNAIDS report on global HIV/AIDS, sub-Saharan Africa is the world's epicenter of HIV/AIDS despite the fact that this region contains only about 11% of the earth's population. Prevalence of HIV in adult is 1.2% worldwide. (0.6% in North America but 9.0%) in sub-Saharan Africa). Five million adults and children became infected with HIV in 2001, 3.5 million people from sub-sahara Africa alone [2]. Of all people living with HIV/AIDS global, 9% of them live in Nigeria [2], as of 2014 in Nigeria, HIV among adults aged between 15-49 years was 3.17% [2]. According to a recent report of News Agency of Nigeria, Rivers State has the

highest prevalence (15.2%) of HIV/AIDS in Nigeria. HIV infection causes a progressive destruction of the cell mediated (CMI) system by eliminating CD⁴⁺ or T-helper lymphocytes. Decreased immunity leads to opportunistic infections and certain cancers [1].

Opportunistic intestinal protozoans are among the major contributors to the global intestinal parasite load. In different parts of the world a wide variety of intestinal parasites like *Ascaris lumbricoides*, *Entamoeba* spp, *Toxoplasma*, *Cyclospora*, *Giardia* and *Cryptosporidium*.

HIV/AIDS is a scourge in sub-Sahara Africa despite the ameliorating effects of highly active antiretroviral Therapy (HAART). The prevalence of HIV/AIDS in Taraba State is 10.5% [2]. Opportunistic intestinal protozoan infections pose a great danger to the HIV/AIDS patients with complications. There is therefore the need to investigate the activities of intestinal protozoa parasites in AIDS related complexes in Jalingo Local Government. This study investigated the danger and complication due to intestinal protozoans' infection among HIV/AIDS patients in Jalingo metropolis. The study also compared the prevalence of opportunistic intestinal protozoa infection and CD⁴⁺, T-Cell count between HIV/AIDS and non HIV patients.

2. Materials and Methods

2.1. Study Area

Jalingo is the capital of Taraba State and the headquarters of Jalingo Local Government. The Local Government borders Ardo-Kola in the south, Lau Local Government in the North, Yorro Local Government to the East. Majority of the inhabitants of the L. G. A live in rural agricultural communities with civil service and farming as the major occupation. Jalingo Local Government is located between latitude 8°89'N, longitude 11°38'E.

2.2. Ethical Permission

Ethical permission was sought and obtained from Postgraduate review committee of Department of Biological Sciences, Taraba State University, Jalingo. Furthermore, additional permission was sought and obtained from the various hospital management before the commencement of study.

2.3. Research Design and Sample Collection

The design of the study is hospital base, cross sectional study. A total of 650 stool samples were collected from both male and female HIV/AIDS patients and 200 stool samples were also collected from non-HIV/AIDS patients to serve as control. Permission of the parents, hospital and patients was sought for, before the commencement of the study. Each patient was provided with a pre-labelled 60ml universal sample bottle for stool collection. The patients were instructed on how to collect the sample. The samples were preserved and transported to the laboratory Department of Taraba State Specialist hospital Jalingo, for analysis.

2.4. Questionnaires' Administration

A questionnaire relevant to the study was issued to each patient to obtain information on: age, sex, level of education, occupation of parents, and history of previous health status. Qualitative data on environmental/predisposing factors such as type of toilet facility, source of drinking water were obtained from the participants.

2.5. Microscopic Examination of the Stool

Two methods to examine the stool were employed in this study: the wet mount and the concentration techniques as described [3].

For the wet mount technique, a microscope slide was obtained and one drop of normal saline was placed on one end of the slide. A drop of iodine was placed on the other end to incorporate into the faeces-saline mixture after an initial examination for motile trophozoites. A small amount of the stool was introduced to the drop or saline and another similar specimen into dilute iodine before mounted on the microscope to be examined under low-power magnification.

Faecal concentration was processed by the sedimentation method. Half tea spoonful of faeces was thoroughly mixed in 10ml of water and strained through two layers of gauze in a funnel. The filtrate was centrifuged at 2000rpm for 2 minutes, the supernatant was discarded and the sediment was re-suspended in 10 ml of physiological saline, centrifuged again and the supernatant discarded. The sediment was re-suspended in 7ml of formalin saline and allowed to stand for 10 minutes for fixation. About 3 ml of ether was then added. The tube was stoppered and shaken vigorously to mix, then the stopper was removed and the tube was centrifuged at 2000rpm for 2 minutes. The tube was allowed to rest in a stand and four layers became visible. The top layers consisted of ether, second a plug of debris, third a clear layer of formalin saline and the fourth was the sediment. Then the plug of debris was detached from the side of the tube with a glass rod and the liquid was poured off leaving a small amount of formalin saline for suspension of the sediment. It was poured on a clean glass slide covered with cover slip and examined under a microscope using x40 objective. The sediment was also mixed with a drop of iodine and examined in the same manner.

2.6. Method for CD⁴⁺ Count Analysis

The CD⁴⁺ count of patients were determined using the Partec Cyflow Counter, which determines the percentage of CD⁴⁺ cells of individuals.

Procedure for Adults: Twenty (20) µl of whole blood (K-EOTA as anticoagulant) was added to sample tube. Then, 20µl of CD⁴⁺ mAb PE was added; mixed gently and incubated for 15 minutes at room temperature and protected from light. About 800µl of no-lyse buffer was added and mixed gently; and analyzed using the Partec Cyflow Counter Machine.

Procedure for children: About 20µl of whole blood (K-EDTA) as anticoagulant was added to a sample tube. Then, 10µl of CD⁴⁺ mAb PE and 10µl of CD45 mAb were

added and was protected from light. Four hundred (400) µl of buffer 1 was added and mixed gently. Directly prior to the measurement 400µl of buffer 2 was added and analysed using the Partec Cyflow Counter machine.

2.7. Calculation of Results in CD⁴⁺ Sample

During the staining procedure with the CD⁴⁺ easy count kit, the blood sample was diluted by a factor of 42. The machine already takes this dilution factor into account and displays directly the number of CD⁴⁺ µT-cells per µl of undiluted blood sample.

$$\text{Dilution} = \frac{20\mu\text{l} + 10\mu\text{l} + 10\mu\text{l} + 80\mu\text{l}}{20\mu\text{l}}$$

2.8. Statistical Analysis

Microsoft Excel and SPSS were used to analyze data. Frequency distribution table, percentages, prevalence of infection attributed to intestinal protozoans in HIV/AIDS patients was estimated. Furthermore, Chi-square test was used to compare prevalence of infection between sexes, ages

and location. The level of significance was set at $P \leq 0.05$.

3. Results

Table 1 shows the age and gender specific prevalence of opportunistic intestinal protozoan among HIV positive patients which reveals that the prevalence was higher among female positives (19.60%) than the male (16.50%). Chi-square analysis showed no statistically significant difference ($p > 0.05$) in infection among sexes. On the other hand, the occurrence of intestinal protozoan infection based on age group revealed that the age group between 10-15 years had the highest prevalence of 27 (48.21%), followed by 26-30 years age group (28.13%). The least prevalence of 2 (2.63%) was recorded among 41-45 years in a decreasing order respectively. The Chi square analysis revealed that there is association between age and gender and the occurrence of Opportunistic Intestinal protozoan infection among HIV sero-positive $p \leq 0.05$.

Table 1. Age and Gender Specific Infections of Intestinal protozoan in HIV Subject in study area.

Age (Years)	No. Exam	No. Inf (%)	No. Exam	No. Inf (%)	No. Exam	No. inf
10-15	33	12 (36.36)	23	15 (65.21)	56	27 (48.21)
16-20	33	7 (21.21)	42	11 (26.19)	75	18 (24.0)
21-25	29	7 (24.13)	42	8 (19.04)	71	15 (21.13)
26-30	34	11 (32.35)	30	7 (23.33)	64	18 (28.13)
31-35	47	4 (8.51)	54	11 (20.37)	101	15 (14.85)
36-40	41	3 (7.32)	54	6 (11.11)	95	9 (9.47)
41-45	33	1 (3.03)	43	1 (2.33)	76	2 (2.63)
46-50	32	2 (6.25)	40	4 (10.0)	72	6 (8.33)
51 above	21	3 (14.28)	19	5 (26.32)	40	8 (20.00)
TOTAL	303	50 (16.50)	347	68 (19.60)	650	118 (18.15)

Table 2 shows the prevalence of opportunistic intestinal protozoan parasites in relation to CD⁴⁺ T-cell count of HIV and non-HIV infected patients in Jalingo. The result showed that 34.75% of the subjects had a CD⁴⁺ cell count of less than 200 cells/mm³ while 55.93% of the HIV sero-positive subject had a CD⁴⁺ cell count between 200-500 cells/mm³ and 9.32% had a CD⁴⁺ count greater than 500 cells/mm³. For the

sero-negative subjects, 60.00% had a CD⁴⁺ count of greater than 500 cells/mm³ while 13.33% had a CD⁴⁺ count less than 200 cells/mm³. The Chi square analysis revealed that there is association ($p \leq 0.05$) between the occurrence of opportunistic intestinal protozoan infection and CD⁴⁺ T-cell count among HIV and Non HIV infected patients.

Table 2. Prevalence of Opportunistic Intestinal Protozoan Infection in relation to CD⁴⁺ T-cell count among HIV and HIV infected subjects in Jalingo Metropolis.

	No examined	CD ⁴ T-cell count (%)		
		< 200 cells/mm ³	200-500 cells/mm ³	≥500 cells/mm ³
HIV sero-positive	118	41 (34.75)	66 (55.93)	11 (9.32)
HIV sero-negative	15	2 (13.33)	4 (26.67)	9 (60.00)
Total	133	43	70	20

The sex specific prevalence of intestinal opportunistic protozoan parasites in relation to CD⁴⁺ T-cell count among HIV sero-positive subjects as depicted in Table 3 revealed that large proportion of the female subjects (58.54%) had CD⁴⁺ count less than 200 cells/mm³ compared to their male counterpart (41.46%). The Chi-square analysis showed no significant association ($p \leq 0.05$) between the occurrence of opportunistic intestinal protozoan infections with gender and CD⁴⁺ T-cell count among HIV Sero-positive subjects.

Table 4 showed the occurrence of opportunistic protozoan infection and CD⁴⁺ T-cell count among HIV infected patients, majority of sero-positive HIV subjects with intestinal protozoan parasites had a CD⁴⁺ count of less than 200 cells/mm³ at 68 (57.63%) while 11 (9.32%) had CD⁴⁺ count of above 500 cells/mm³. Based on species, *Cryptosporidium* spp 29 (24.58%) had the highest association with CD⁴⁺ count less than 200 cells/mm³. *Giardia lamblia* had no significant association with CD⁴⁺ count level <200 cells/mm³ to >500

cells/mm³. However, *Entamoeba histolytica* had varying CD⁴⁺ count level of 19 (16.10%) to 5 (4.24) out of the 30 tested positive subjects. Of the 40 tested positive subjects *Entamoeba coli* had varying CD⁴⁺ count levels of 20 (16.95%) to 4 (3.39) with <200 cells/mm³ to <500 cells/mm³ respectively in decreasing order. There was a statistically significant association between CD⁴⁺ count in relation to *Entamoeba histolytica*, *Cryptosporidium parvum* and *Entamoeba coli*. Chi-square analysis showed that there is association (p<0.05) between the occurrence of opportunistic

protozoan infection and CD⁴⁺ T-cell count among HIV and non-HIV infected patients.

Table 3. Distribution of Opportunistic Intestinal Protozoan Infection in relation to CD⁴⁺ T-cell count among HIV sero-positive subjects.

CD ⁴⁺ (Cells/mm ³)	Male (%)	Female (%)	Total (%)
<200	17 (41.46)	24 (58.54)	41 (6.31)
200-500	29 (43.94)	37 (56.06)	66 (10.15)
>500	5 (45.45)	6 (54.54)	11 (1.06)
Total	51 (43.22)	67 (56.78)	118 (18.15)

Table 4. Association between parasites species and CD⁴⁺ T-cell count of HIV sero-positive subjects in the study area.

CD ⁴⁺ T-Cell count (%)	No. pos	CD ⁴⁺ T-Cell count (%)		
		<200 Cells/mm ³	200-500 ells/mm ³	>500 Cells/mm ³
<i>Giardia lamblia</i>	2	0(0.00)	2 (1.69)	0 (0.00)
<i>Entamoeba histolytica</i>	30	19 (16.10)	6 (5.08)	5 (4.24)
<i>Cryptosporidium parvum</i>	46	29 (24.58)	15 (12.77)	2 (1.6900)
<i>Entamoeba coli</i>	40	20 (16.95)	16 (13.56)	4 (3.39)
Total	118	68 (57.63)	39 (33.05)	11 (9.32)

4. Discussion

It was observed that the prevalence of opportunistic Intestinal protozoan infection was lower among patients whose CD⁴⁺ count was higher than 200 cells/μl while prevalence of infection increased when CD⁴⁺ count was low. This is because deficiency of immune system due to HIV/AIDS infection reduces the immune response to intestinal parasite infection. This supports the findings of Rossignol *et al.* [4] who observed similar pattern in the State place of his research while working among HIV/AIDS patients. The use of CD⁴⁺ t-lymphocytes to monitor HIV patients on ART has been shown to be highly indicative of their morbidity status. It has been established that a weakened immune system depicted by low CD⁴⁺ count result in high susceptibility of the individual to co-morbidities. This is why the World Health Organization recommended the use of CD⁴⁺ count to monitor opportunistic infections [5].

Intestinal protozoan infection in relation to CD⁴⁺ count between males and females showed that, female had higher CD⁴⁺ count than their male counterparts. This could be due to maternal age/gestational age and PCV at booking that did not influence the CD⁴⁺ cell count in HIV-infected pregnant women. Akinsegun *et al.* [6] reported such cases in his study.

Intestinal protozoan infections in HIV/AIDS subjects require a careful study in a developing world where poor nutrition is associated with poor hygiene and several tropical diseases. The parasite detected in this study for HIV/AIDS subjects include *Cryptosporidium species* (7.10%), *Entamoeba coli* (6.15%), *Entamoeba histolytica* (5%) and *Giardia lamblia* (2%). *Cryptosporidium species* was found to be significantly higher in HIV/AIDS subject than in the control samples. This could be transmitted to relatively low immune status of subject with HIV/AIDS. This is in agreement with the research carried out by Babatunde *et al.* [7] who reported intestinal parasitic infections are the major causes of morbidity and mortality in HIV positive patients

according to the study of Assefa *et al.* [8] and Adamu and Petros [9]. A significant number of parasites-positive participants in this study had CD⁴⁺ count levels of 200-500 cells/μl i.e. 200-500 cells/mm³ and below 200 cells/mm³. It was found that, participants with *Cryptosporidium spp* were significantly associated with CD⁴⁺ count below 200 cells/mm³ this is because HIV/AIDS infection has been shown to predispose patients highly to intracellular opportunistic intestinal parasites such as *Cryptosporidium*. The association between opportunistic intestinal parasites have been explained by various researchers; this is in agreement, with the findings of Weber *et al.* [10]. The association of opportunistic parasites with CD⁴⁺ counts below 200 cells/μl in this study was significant because of the depletion of the immune system due to HIV/AIDS. This was in line with studies done elsewhere which include, that done in Ethiopia, India and Brazil [8, 9, 11, 12]. Another study done in Benin City, Nigeria by Akinbo *et al.* [13] also reported that *Cryptosporidium* infections were significantly associated (p≤0.05) with CD⁴⁺ count below 200 cells/μl.

The other pertinent finding was that in immunocompromised patients whose CD⁴⁺ T cell count fell below 500 cells/mm³; they were more likely be infected with intestinal parasites. This is in agreement with previous finding in Nigeria by Akinbo *et al.* [13], in Nepal by Tiwari *et al.* [14] and in Ethiopia by Teklay *et al.* [15].

5. Conclusion

This study report the prevalence of intestinal parasites among HIV/AIDS patients with special emphasis on the sero-positive status and CD⁴⁺ count levels of the infected subjects. The prevalence of intestinal parasites was highly significant among the study participants with CD⁴⁺ count <200 cells/μl. *Cryptosporidium spp* was significantly associated with HIV positive subjects who had CD⁴⁺ count of less than 200 cell/μl. Routine stool examination should be enforced and performed regularly during the follow-up of

HIV/AIDS patients who attend HIV clinics for early diagnosis and treatment of the specific parasites in these patients. Since HIV/AIDS disease coexist with intestinal parasite infections as indicated in this study, it is imperative that the National AIDS Control Programme provides the necessary logistics required to diagnose specific opportunistic intestinal parasites. This should include PCR, Isoenzyme Analysis and Antigen detection which has proven to be a very effective means of diagnosing intestinal parasites. All diagnostic techniques for parasites detection especially opportunistic parasites should be made widely available at HIV clinics. It is recommended that clinicians caring for HIV/AIDS patients should request for routine stool examination for the specific diagnosis of opportunistic intestinal parasites, especially in patients at the symptomatic stage CD⁴⁺ T-cell count: 50-200 cells/ μ l) of HIV disease.

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