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Full Length Research Article

PREVALENCE OF INTESTINAL PARASITOSIS AND IMMUNOLOGICAL STATUS OF HIV/AIDS PATIENTS ON ANTIRETROVIRAL THERAPY IN NYANYA GENERAL HOSPITAL ABUJA, NIGERIA

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ABSTRACT
Intestinal parasites, especially in HIV/AIDS patients, are a leading cause of morbidity and mortality worldwide. The aim of this study was to determine the prevalence of intestinal parasitosis and the immune status of HIV/AIDS patients on ART. Two hundred (200) HIV/AIDS patients were recruited from the ART clinic at Nyanya General Hospital in Abuja, Nigeria, for this cross-sectional institution-based research. To collect detailed demographic data, questionnaires were sent out. The direct wet mount, formol-ether concentration, and adjusted Ziehl-Neelsen staining were used to analyze stool samples. Patients' records were analyzed during this study period to assess their $CD4^+$ T-cell count and viral load status. The findings were compared using a contingency table analysis and the chi-square test. The Mann-Whitney test was used to compare quantitative data sets. P value of 0.05 was described as statistically significant ($P \leq 0.05$).The findings of our study were 80 (40 percent) of the patients had intestinal parasitosis, with 11 (13.8 percent) of them having multiple parasitosis. *Cryptosporidium parvum* had the highest prevalence (16%), *Entamoeba histolytica /dispar* (13%), *Giardia lamblia* (7%), and *Entamoeba coli* (3.5%). *Strongyloides stercoralis*, *Ascaris lumbricoides*, and *Cystoisospora belli* each had a prevalence of 2.5 percent. The findings revealed that diarrhoea was linked to cryptosporidiosis, giardiasis, and cystoisosporiasis. In this study, there was no correlation between intestinal parasitosis and immune system function. In HIV/AIDS patients, prolonged and untreated diarrhoea can be crippling and fatal.

Keywords: HIV/AIDS, Parasitosis, $CD4^+$ T-cell count, Cryptosporidiosis, Giardiasis, Cystoisosporiasis.

INTRODUCTION

In the United States of America (USA), the first case of acquired immunodeficiency syndrome (AIDS) was reported in 1981. More people are becoming infected with the human immunodeficiency virus (HIV), particularly in Asia, South America, and Sub-Saharan Africa (SSA).

Since the epidemic began, an estimated 76 million people have contracted HIV. About 38 million individuals have acquired HIV since the AIDS epidemic began, and tens of millions more people have died from AIDS-related illnesses (UNAIDS, 2020).
Over the past 20 years, considerable global efforts have been conducted to battle the disease with notable progress. While the number of persons seeking care for HIV has climbed to 25.4 million in 2019, the number of new HIV infections and AIDS-related deaths have decreased over time (WHO, 2016).

Despite this, underlying issues make managing HIV more challenging. There is now no cure for HIV, and many individuals are either infected with it or are at risk of contracting it. The virus primarily affects people who are in the prime of their careers, and it has an effect on the development of society, the economy, and the nation in addition to the health of the individual. Numerous countries with high HIV prevalence rates also experience other infectious diseases, food shortages, and other issues related to public health and development (UNAIDS, 2020).

Furthermore, COVID-19's global proliferation has already had a detrimental effect on the HIV/AIDS response in low- and middle-income countries, upsetting antiretroviral therapy and prevention programs. Although the full effects of COVID-19 on HIV care and development are unknown, such interruptions are anticipated to cause a sharp increase in the number of HIV-related deaths (UNAIDS, 2020).

Despite significant drops in new infections since the mid-1990s, 1.7 million infections were reported as new in 2019, with an average of nearly 5,000 cases each day. Recent data demonstrates that while progress has been made, it has not been uniform within and between nations. Additionally, the pace of decline varies by location, gender, and age (UNAIDS, 2020).
HIV/AIDS is still the world's number one killer, particularly among women who are sexually active (Gbadamosi *et al*., 2019). On the other hand, AIDS-related mortality have decreased as antiretroviral treatment (ART) has been ramped up. According to UNAIDS (2022), 650,000 people died from AIDS in 2021, down from 1.7 million in 2004 and 1.1 million in 2010, respectively. An estimated 4.9 million people in Western and Central Africa are HIV-positive.
Nigeria is home to one of the world's largest populations of HIV-positive people. The current national HIV prevalence is estimated to be 1.4 percent (15-49 years), with 1.9 million people living with HIV in Nigeria (Federal Ministry of Health (FMOH), (2019).

Human Immunodeficiency Virus, a member of the Retroviridae family and genus Lentivirus, an RNA virus causes acquired immune deficiency syndrome (AIDS). The disease is as a result of HIV replication in CD4 lymphocytes, which kills CD4 T cells and

reduces their population. Patients with impaired immune systems are more vulnerable to opportunistic and evolving infections caused by bacteria, fungi, viruses, and parasites, as well as malignancies including Kaposi's sarcoma and brain lymphoma (Garcia & Estrada, 2016).

Human Immunodeficiency Virus infections are categorized clinically based on the viral load (VL) and the quantity of lymphocytes present. Helper T-cell counts in a patient's blood are used to measure the severity of the infection and to assess the immune system's damage. Helper T-cell counts (cells per mm^3) can range between 500 and 1,500 in healthy adults who are not HIV-positive, and HIV infection lowers T-cell numbers, making patients more susceptible to opportunistic infections or malignancies (Garcia & Estrada, 2016).

A viral load test determines the amount of HIV in a blood plasma sample. It does not show the extent of the immune system's damage, but it does shows how quickly the infection can spread. Even if a person has a large number of helper T-cells, a high viral load means that the person is at risk of rapidly deteriorating and developing AIDS if medical treatment is not begun immediately (Nettles *et al*., 2005)

Increased plasma HIV viral load (VL) was seen in HIV-positive patients during parasitic opportunistic infections (POIs), suggesting active HIV replication in response to POIs. POIs can raise the risk of HIV transmission as well as the risk of death due to their effects on HIV RNA VL (Ekwaru *et al*., 2013; Urgessa *et al*., 2018).

The viral load rises as HIV reproduces inside the body thereby destroying the immune cells ($CD4^+$ T- cells). This weakens the immune system and reduces the body's ability to combat infections. Laboratory tests are needed on a regular basis to assess the patient's reaction to ART and any possible side effects. Viral resistance assays can be used in the event of ART failure to detect antiretroviral drug resistance mutations.

In clinical practice, regular CD4 count monitoring in patients who are chronically ill and have CD4 T- cell counts <200 cells/mm^3 is no longer useful. The most precise and practical test of ART effectiveness is monitoring HIV RNA levels to confirm effective treatment response and long-term viral suppression (Gale *et al*., 2013; Shoko & Chikobvu, 2019).

The CD4 T- cell count is used to determine immunologic status, forecast clinical progress, and make treatment decisions for opportunistic infections (Shoko & Chikobvu,2019).
In patients with HIV infection, opportunistic infections continue to cause significant morbidity and mortality. The risk of parasitic opportunistic diseases can clearly be reduced with the introduction of more potent ART. In HIV patients with advanced disease, improved immune function along with particular prophylaxis will lower the risk of parasitic opportunistic infections and enhance survival (Urgessa *et al*., 2018).
It is critical to realize that there is a reciprocal relationship between POIs and HIV infection (Khalil *et al*., 2015; Laksemi *et al*., 2020; HIV.gov., 2022). Immune suppression caused by HIV makes it easier for opportunistic infections to infect HIV-positive individuals. The typical course of HIV infection can be altered by POIs, like other co-infections, by inducing reversible elevations in circulating VL, which can speed up HIV development and enhance HIV transmission.
The most prevalent symptom of HIV is diarrhoea, which is defined as having watery stools at least twice per day. Depending on the disease's stage of development, it can be caused by drugs or parasitic opportunistic infections (Garcia & Estrada, 2016).
In AIDS' patients, POIs are a common cause of diarrhoea, which is often more severe and prolonged than in healthy people (Garcia & Estrada, 2016). When diarrhoea is combined with other symptoms including fatigue, nausea, intestinal cramps, and flatulence, it may make HIV infection worse.

Owing to a variety of demographic factors such as climate change, population, poverty, poor environmental sanitation, shortage of potable water, and medical service inadequacies, parasitic opportunistic pathogens have been isolated frequently from immunocompromised individuals (Akinbo *et al*., 2010; Okafor-Elenwo *et al*., 2020). *Cryptosporidium parvum, Cystoisospora belli, Entamoeba histolytica, Giardia lamblia, Cyclospora spp, Strongyloides stercoralis,* and *Microsporidium spp* are the most common intestinal parasites isolated from HIV patients (Akinbo *et al*., 2010; Devulapally *et al*., 2019).

Patients with HIV/AIDS who are already experiencing difficulties are at high risk for intestinal parasitic infections. Due to a dearth of information about the impact of intestinal parasitic infections on HIV/AIDS patients' immunologic status in the research area, this study assessed the immunologic status of HIV/AIDS patients receiving ART in Abuja, Nigeria, as well as the prevalence of intestinal parasitosis.

MATERIALS AND METHODS

Study Design and Subjects
The research was a cross-sectional institution-based study. The research included 200 HIV-positive patients who were registered at the Nyanya ART clinic in Abuja. Only adults (male and female) who gave their informed consent were enrolled in this study

Demographic Information
Related demographic information was collected from the patients using a standardized questionnaire. The questionnaire was used to determine socioeconomic status, age, sex, educational level, toilet facilities, housing structure, source of drinking water, heterosexuality, homosexuality, IDUs, and animal interaction.

Ethical Approval
Ethical approval was obtained from the Federal Capital Territory Authority (FCTA) ethical committee and Federal Capital Development Authority (FCDA) hospital management boards, Abuja.

Sample Collection
A total of 200 stool samples from HIV-positive patients were obtained. Each patient was given a stool sample container with a big mouth and instructions on how to collect the sample. The samples were taken to the Karu General Hospital to be processed and analyzed. During the study period, the patients' records at the ART clinic were reviewed to determine their CD4 cell count and viral load.

Microscopic Examination of the stool samples
All of the samples were subjected to a direct wet mount with normal saline and iodine, formol-ether concentration, and the modified Ziehl-Neelsen techniques outlined by (Mohammed *et al*., 2014). The stool samples were immediately processed and examined. Wet mounts were made with saline and iodine on clean grease-free slides with cover-slips and analyzed under the microscope with X10 and X40 objectives.
The formol-ether process was used to concentrate the faeces. When parasites and their ova were present in limited numbers, this improved the chances of finding them. In a test tube, 1g of stool was emulsified with 4ml of 10% formalin, then the mixture was centrifuged after being thoroughly mixed by vigorous shaking of the stool. After the supernatant was decanted, the sediment was examined under a microscope. The sediment from the formol-ether concentration technique was used to make a smear, which was then used in the updated Ziehl-Neelsen method. It was air dried and methanol fixed for 3 minutes, then painted with carbol-fuchsin for 15 minutes before being decolorised for 15 seconds with 1 percent acid alcohol. Methylene blue was used as a counterstain for 30 seconds. The slide was washed with water after each stage before being examined under low power objective to for oocysts and an oil-immersion objective (X100) for identification.

Statistical Analysis
To evaluate the data using contingency table tests, the Chi-square test was used. Quantitative data were compared using the Mann-Whitney test, using P ≤0.05, for statistical significance.

RESULTS
The patients were between ages 20 and 69, with a median age of 34. 139 (69.5%) patients were females and (61) (30.5%) patients were males. Promiscuous heterosexuality, accounted for 103 (51.5%), homosexuality, 40 (20%), and bisexuality, 13 (6.5%), injection drug users (IDUs), 37 (18.5%), and others, 7 (3.5%), were the key risk factors for HIV infection.

Intestinal parasites were discovered in 80 (40%) of the patients' stool samples, with 11 (13.8%) of them having multiple parasitosis. *Cryptosporidium parvum* was the parasite with the highest prevalence of both single and multiple parasitosis. *Giardia lamblia* and *Entamoeba histolytica* / *dispar* were also common parasites (Table 1).

Table 1: Prevalence of Intestinal Parasites among HIV/AIDS Patients

Intestinal Parasites	No. of Patients (N=200) (%)
C. parvum	32 (16.0)
E. histolytica / dispar	26 (13.0)
G. lamblia	14 (7.0)
E. coli	7 (3.5)
A. lumbricoides	5 (2.5)
S. stercoralis	5 (2.5)
Cystoisospora belli	5 (2.5)

These patients' CD4 T-lymphocyte counts ranged from 1 to 199 cells/mm^3, with an average of 88.1162.18 cells/mm^3. There was no statistical correlation in terms of amount of CD4+ T cells and the presence of parasitic intestinal infections (Table 2).

Table 2: Total CD4$^+$ T- cell counts among HIV/AIDS Patients

Intestinal Parasites	CD4+ T-cells (cells/mm^3)	Median	Min.	Max.	No. of Patients
Parasites +	76.65± 65.50	61.50	1.00	199.0	80
Parasites -	97.75± 58.90	97.50	3.00	199.0	120

Patients with intestinal parasites had 76.65± 65.50 CD4+ cell counts, while those without parasites had 97.75 ±58.90 cells/mm^3 (Mann-Whitney test= 3885.5; P-value =0.23).
Forty-five (45) (22.5%) of the 200 samples studied were diarrhoeic stools, while 155 (77.5%) were not; of the 120 stools without intestinal parasites, 108 (90%) were non-diarrhoeic and 12 (10%) were diarrhoeic. In comparison, diarrhoea was recorded in 47 (58.8%) of patients with intestinal parasites, suggesting that there is a correlation between intestinal parasites and diarrhoea (X^2=25.12; P<0.0001). The prevalence of *C. parvum, G. lamblia*, and *Cystoisospora belli* was significantly associated with the incidence of diarrhoea with P-values of 0.037, 0.001, and 0.036, respectively), with a P-value of 0.076 for *S. stercoralis*.

Table 3: Prevalence of Intestinal Parasites among Diarrhoeic and Non-Diarrhoeic HIV/AIDS Patients

Organisms	Diarrhoeic N=45 (%)	Non-Diarrhoeic N=155 (%)	P
C. parvum	12 (26.7)	20 (12.9)	0.0368
E. histolytica / dispar	8 (17.8)	18 (11.6)	0.3147
G. lamblia	11 (24.4)	3 (1.9)	<0.001
Cystoisospora belli	3 (6.7)	2 (1.3)	0.0363
E. coli	2 (4.4)	5 (3.2)	0.6559
S. stercoralis	3 (6.7)	2 (1.6)	0.0764
A. lumbricoides	2 (4.4)	3 (1.9)	0.3140

P≤ 0.05, statistically significant

DISCUSSION
Intestinal parasites were found to be present in 40% of the patients. The majority of patients had a single parasitosis, but 11 (13.8%) had multiple parasitosis.

The most prevalent parasite was *C. parvum* (16%), *E. histolytica /dispar*, *G. lamblia* accounted for 7% of the total. *E. coli* was found in 3.5 percent of the population, while *A. lumbricoides, S. stercoralis*, and *Cystoisospora belli* were found in 2.5 percent of the population.

Since diarrhoea is a common gastrointestinal symptom in HIV patients, this study compared the intestinal parasites found in

diarrhoeic and non-diarrhoeic patients with and without parasites. The non-diarrhoeic patients were higher 108 (90.0%) than the diarrhoeic group 12 (10.0%) among the faecal samples without intestinal parasites, indicating that the diarrhoeic states are strongly linked to intestinal parasitosis and that diarrhoea is an important manifestation of it (Obateru *et al*., 2017).

The presence of *C.parvum, G. lamblia*, and *Cystoisospora belli*, as well as *S. stercoralis* infection, were found to have a significant link between positive parasitologic examinations and diarrhoea.

The prevalence of intestinal parasites varies by geographical location among HIV patients. The rate found in this study corresponds to 59.5 percent in West Cameroon and 50.9 percent in Kenya, respectively (Awofala & Ogundele, 2018; Nkenfou *et al*., 2013; Olopade & Idowu, 2017). However, this contrasts with the findings of Akinbo *et al*. (2010) in Benin, Nigeria, who found a prevalence of 15.3 percent and Jegede *et al*. (2017) found a prevalence of 11.4 percent in Kano, Nigeria.

C. parvum was found to be the most prevalent intestinal protozoan parasite in this study. Cryptosporidiosis is a disease caused by the coccidian parasite, a microscopic protozoan organism, and it has gotten a lot of attention recently because of its potential for opportunistic infection in HIV/AIDS patients (Nissapatorn & Sawangjaroen, 2011).

Cryptosporidiosis causes a number of health problems that are becoming more commonly recognized around the world, and it has been included in the World Health Organization's neglected diseases initiative since 2004 (Otegbayo *et al.*, 2008). The prevalence observed in this study matched that found by Nkenfou *et al*. (2013) in Cameroon. In Abeokuta, Nigeria, Okodua *et al*.(2003) discovered a lower prevalence of 3.9 percent.

Intestinal parasite transmission, especially of opportunistic protozoal species, may be influenced by a variety of factors, including zoonotic transmission. According to Acácio *et al*. (2018) and UNAIDS (2016), dogs have been found to ingest *C. parvum* oocysts and *E. histolytica* / *dispar* cysts in the field, and these can be transmitted to humans by close contact with these animals and the contaminated environment (Dalhatu et al., 2016).

This study considered the relationship between intestinal parasites and immune function (CD4 count). Also, the length of diarrhoea is inversely correlated with CD4 T-cell levels. The duration of the diarrhoea was greater in patients with lower CD4 levels. Non-statistically significant inverse relationship between CD4 levels and the frequency of intestinal parasites in India was discovered by Janagond *et al*. (2013). However, the effectiveness of the immune system is measured using the CD4 count.

A viral load of >30,000 copies per mL is linked to a reduced risk of AIDS and death. Most of the patients in these study had low viral load, therefore, there is viral load suppression as a result of antiretroviral therapy. But, the higher the viral load, the greater the immunosuppression and the chance of Parasitic Opportunistic Infections (POIs). Nearly 80% of AIDS patients die from AIDS-related infections like POIs rather than HIV when $CD4^{+}$T-cell counts are drastically reduced, especially below 200 cells/mm^3 (Kindie & Bekele, 2016).

Conclusion

This study discovered the presence of parasitic intestinal pathogens in HIV/AIDS patients. The most common intestinal parasites present in both diarrhoeic and non-diarrhoeic patients were *C. parvum*, *E. histolytica* / *dispar*, *G. lamblia*, and *E. coli*. In determining patient immune status, ART efficacy, and AIDS prognosis, the CD4 count and viral load values are extremely useful.

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