THE PREVALENCE OF INTESTESTINAL PARASITES AND IMPACT OF CRYPTOSPORIDIUM INFECTIONAMONG DIARRHOEAL - INFECTED HIV PATIENTS VISITING THE SPECIALIST HOSPITAL, BENIN-CITY, EDO STATE, NIGERIA

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Abstract

Background of the study: Diarrhea is one of the world's worst health problems, especially in immunocompromised persons.

Aim: The study's main goal was to determine the prevalence of intestinal parasites and the clinical impact of *Cryptosporidium parvum* in HIV-positive diarrhea patients at the Specialist Hospital in Benin City, Edo State, south-central Nigeria.

Materials and methods: stool concentration and modified Zehl-Neilson staining was used to evaluate 196 stool samples for intestinal parasites.

Results: A total of 196 HIV/AIDS-related diarrhea patients (116 women and 80 men) were examined for intestinal parasites. The overall parasite prevalence, density, and age prevalence among diarrhea-infected HIV/AIDS patients were 21.35% and 5.0%, respectively, with *Ascaris lumbricodes (5.6%*), *Cryptosporidium parvum (5.1%), and Entamoeba histolytica (5.1%)* being the second most frequent intestinal parasites surveyed.

HIV-infected people of all ages had 5.0% *Cryptosporidium*-related diarrhea. Patients aged 46–55 years had 2.5% of all cases, whereas those aged 6–15 years, 16–25 years, and 56–60 years had 0%. *Cryptosporidium* was more common in women (4.0% vs. 1.0%) than in men.

Both groups showed a statistical difference (P 0.05). Regimen 1A had the most *cryptosporidium* (3.1%). HIV-related diarrhea was more common among patients with CD4 levels below 350 (4.1% vs. 1.0%, respectively) compared with CD4+ > 350 (136 individuals), who made up the bulk of those tested with a statistical difference between groups. HIV patients with only bloody diarrhea had a 3.1% prevalence of cryptosporidium, compared to 0% in those with bloody diarrhea and mucus or only mucus; the odd ratio (OR) was 1.06 (95% CI 0.20–85.27) with P> 0.05. *Cryptosporidium* oocysts have been found in HIV-infected patients' feces at the Specialist Hospital in Benin City, Edo State, suggesting a relation to HIV diarrhea, particularly in the late stages of illness. It causes 5.0% of diarrhea in this population.

Conclusion: Cryptosporidiosis is frequent in HIV-infected diarrhea patients. Thus, HIV and immunosuppressed patients' diarrhea treatments should incorporate this study's findings.

Keywords: *diarrhea, intestinal parasites, HIV/AIDS*

Introduction

Diarrhea is one of the most serious health problems that people with weakened immune systems can face. Over 50 million people have died worldwide from it[1,2]. Diarrhea, also known as "loose stools," occurs when regular bowel movements are disrupted, most commonly by an increase in bowel transit time, stool consistency, or stool volume [1, 2]. Two common causes of acute diarrhea are excessive alcohol consumption and ingesting contaminated food or water, and regardless of treatment, attacks typically resolve within a day or two [1] and [2]. If diarrhea lasts for a long time, it could be a sign of something more serious, such as an intestinal disorder caused by bacteria, viruses, and parasites [1,2]. Diarrhea caused by protozoan parasites has been a concern for humans since at least the nineteenth century, when it became a threat to humans, apes, rodents, and insects [1, 2]. The most common parasites that can cause diarrhea are Giardia lamblia, Entamoeba histolytica, Balantidium coli, Isospora belli, Cyclospora cayetensis, Microsporidia, and Cryptosporidium parvum[3-8]. Chronic diarrhea is experienced by 70% of HIV-positive Africans[9]. Diarrhea is a common HIV symptom and has affected 0.9% to 14.0% of people in outpatient studies with low CD4 cell counts and homosexual males as risk factors. The apicomplexan protozoan parasite Cryptosporidium causes the disease cryptosporidiosis, whose commonest routes of transmission are the feculo-oral route and contaminated water[10]. People with weakened immune systems, such as those suffering from HIV/AIDS, succumb to the disease's symptoms and eventually die. Symptomatic treatments include rehydration, electrolyte correction, and pain relief. Symptoms typically appear after a week of incubation and include red, watery diarrhea, abdominal pain, vomiting, and fever[11,12]. Because the parasite Cryptosporidium is so widely distributed throughout the environment and is disseminated by the ingestion of these organisms (and others), the prevention and control of this disease have become a major public health issue. Techniques for prevention include things like improved filtration systems, consistent water quality testing, and public education about the disease's symptoms and preventative measures[13]. Cryptosporidiosis remains a major opportunistic infection and an important source of morbidity and mortality in people living with HIV/AIDS in underdeveloped countries due to the lack of a universal treatment program and inadequate access to care.

There is no one who is immune to the ripple effects of cryptosporidium. It causes half of all parasitic illnesses spread by water (50.8%). Diarrhea caused by Cryptosporidium accounts for 8–19% of cases in low-income regions[15]. A recent study discovered oocysts in the urine of 10% of low-income individuals, and less than 5% live in developed nations, where the youngest victims are those under the age of 9. Those that are susceptible to cryptosporidium infection are travelers, swimmers, cattle handlers, homosexual partners, and those caring for children[17]. There is a possibility of considerable weight loss, along with nausea, vomiting, watery diarrhea, stomach cramps, an upset stomach, a low-grade fever, and fatigue[17]. Some of the issues are dehydration, inadequate absorption, pancreatitis, and anorexia[18, 19, 20, 21]. Cryptosporidium is very fatal in infants, the elderly, and those with impaired immune systems, with symptoms like cholecystitis and cholangitis[20]. Diarrhea of a milder, self-limiting kind can be caused by parasite infections in otherwise healthy patients and in immunocompromised persons, such as those with HIV/AIDS, Cryptosporidium spp. can cause severe, persistent, and sometimes deadly diarrhea and wasting[22]. In the industrialized world, the AIDS epidemic has been greatly diminished by highly active antiretroviral therapy (HAART) over the past 20 years. However, drug-resistant HIV variations and the failure or discontinuation of HAART in these patients have been associated with the re-emergence of Cryptosporidium spp. Even though HAART is effective, those with advanced AIDS may still experience symptoms[23]. Although ART is accessible in certain developing nations, treatment remains out of reach in the vast majority of countries where HIV/AIDS is prevalent[23]. Seventy-six percent of AIDS patients and one hundred percent of HIV-positive people in underdeveloped countries suffer from chronic diarrhea in the absence of ART[24,8]. Diagnosis can be made by checking stool samples for parasite ova. Use of metronidazole, sewage treatment, frequent handwashing, and the use of bottled water are all effective preventative strategies[25.]

Materials and methods

Study Area

This is a cross-sectional study on the occurrence and impact of *Cryptosporidium* infection and other intestinal parasites among diarrhoeal-infected HIV-positive patients.

It was conducted at the Specialist Hospital, Benin City, Edo State, one of the centers owned by the Edo State Ministry of Health. HIV/AIDS patients visit the Comprehensive HIV/AIDS Services Center established by the Global HIV/AIDS Initiative Nigeria (GHAIN), Edo Zone, as part of an ongoing prospective cohort study. The hospital serves a large number of HIV/AIDS patients from within and outside the metropolis, including referred patients from other health facilities, NGOs, other related agencies, and organizations within the state, as well as voluntary visitors from the neighboring states of Delta and Ondo. According to the 2006 Nigeria census, there were 11,47188 people living in the urban area of Benin City, which serves as the capital of Edo State. Its coordinates are 6.340 degrees north, 5.60 degrees east, and 87.88 meters in elevation. Participants are largely of African descent and come from a range of educational backgrounds.

Study population

For this study, we enrolled 196 HIV/AIDS patients with diarrhea, of whom 116 were female and 80 were male. The sample size was determined using a formula in line with a previous prevalence of 15.0% of Cryptosporidium in humans in Nigeria[26,27].

Inclusion and exclusion criteria

The study population included HIV patients presenting with diarrhoea in a specialist hospital in Benin City, Edo State, categorized as HIV positive patients presenting with diarrhoea only, HIV and tuberculosis co-infected patients presenting with diarrhoea, both HIV positive and co-infected with CD4 below or above 350, and both HIV positive and co-infected on different drug combination codes referred to as regimens as follows:

1.1a (AZT, 3TC, and EFV) for TB/HIV co-infected
2.1b (AZT, 3TC, and NVP) for HIV infection only.
3.1c (TDF-FTC-EFV) for those with Hb 8 g/dl.
4.1d (TDF, FTC, NVP)
5. 1e, (TDF, 3TC, EFV) alternate first line due to side effects.
6 1f (TDF, 3TC, NVP) for pregnant clients and others

Patients of all ages who tested positive for HIV and who presented with diarrhea during follow-up visits were included in the study. However, HIV-positive patients who did not present with diarrhea, who did not have their consent sought, or who did not show a willingness to participate in this study, were not. The study was designed to determine the occurrence and impact of cryptosporidium infection and other intestinal parasites among diarrheal HIV-positive patients. The study instruments used for this study were hospital general request and report forms, ART, PMTCT, HCT Laboratory, Clinic, and Pharmacy Registers. Other instruments were clinical evaluations and pharmacy order forms.

Sample Collection

Stool samples were collected in UV-safe, wide-mouth plastic containers along with information on the subjects' ages, sexes, and the types of stool they passed. Samples were examined as they were collected.

Parasitological Technique (direct wet preparation)

Stool samples were examined by direct techniques as described. The appearance of the specimens was noted, including the color, consistency (formed, semi-formed, or watery), the presence of blood and mucus, and the presence of worms or their segments. A drop of fresh physiological saline was placed on a glass slide. A piece of wood was used to pick a small amount of the specimen, which was mixed with the saline to make a smooth preparation. This was then covered with a cover slip and examined microscopically under x10 and x40 objectives for the presence of parasite cysts, trophozoites, eggs, or larvae[25].

In a centrifuge tube containing 4 mL of formalin-saline solution, about 2 g of feces were emulsified thoroughly. The fecal suspension was sieved, and 2 ml of ether was added and shaken. After that, the mixture was centrifuged for 2 minutes at 1000 rpm. Using an applicator stick, the debris layer was loosened and, together with the supernatant, poured away. The sediment was re-suspended by tapping the bottom of the tube, and smears were then made with the sediment[28,25].

The smears made with sediment were stained by the modified Ziehl-Nelson staining method[28]. The smear was fixed by heat, flooded with carbol fuchsin solution, and heated gently until steam rose.

After 5 minutes, this was rinsed with tap water. In order to remove the color, 1% acidic alcohol was used until the film turned yellow.

It was washed with water and counterstained with malachite green for 3 minutes.

It was washed with water, bottled, dried, and examined under an oil immersion lens for the presence of oocysts of Cryptosporidium[28,25].

Ethical Clearance

Ethical clearance was obtained from the Edo State Ministry of Health, Benin City. Permission and consent were sought from patients, the heads of a specialist hospital in Benin City, the Edo State governor, and relevant stakeholder groups supporting the facility where the study was conducted. All information collected from patients who participated in the study was kept strictly confidential. Patients' records were kept confidential within the research team and those who needed access for the research to proceed.

Statistical Analysis

Descriptive statistics of frequency and percentages were used to present the results. The levels of association between the variables used in this study were determined using the Chi-square test. The statistical significance was set at $p<0.05$.

Results

A total of 196 HIV/AIDS patients with diarrhea visited the Specialist Hospital in Benin City for treatment and follow-up investigations for intestinal parasites. Out of this number, 116 (59.4%) were females, and the remaining 80 (41.0%) were males. Female patients (4.0% vs. 1.0% prevalence) more frequently tested positive for Cryptosporidium. Both groups were statistically different from one another (P 0.05). Diarrheic HIV/AIDS patients of varying ages show the cryptosporidium age distribution in Table 1.0. It was observed that overall age prevalence was 5.0%, which was highest among those aged 45–55 years (2.5%), followed by the age group 36–45 (1.5tinal parasites. Out of this number, 116 (59.4%) were females, and the remaining 80 (41.0%) were males. Female patients (4.0% vs. 1.0% prevalence) more frequently tested positive for Cryptosporidium. Both groups were statistically different from one another (P<0.05). Diarrheic HIV/AIDS patients of varying ages show the cryptosporidium age distribution in Table 1.0. It was observed that overall age prevalence was 5.0%, which was highest among those aged 45–55 years (2.5%), followed by the age group 36–45 (1.5%), and then those aged 26–35 (1.0), 116 (59.4%) were females, and the remaining 80 (41.0%) were males. Female patients (4.0% vs. 1.0% prevalence) more frequently tested positive for Cryptosporidium. Both groups were statistically different from one another (P<0.05). Diarrheic HIV/AIDS patients of varying ages show the cryptosporidium age distribution in Table 1.0. It was observed that overall age prevalence was 5.0%, which was highest among those aged 45–55 years (2.5%), followed by the age group 36–45 (1.5%), and then those aged 26–35 (1.0%), when compared to a 0.0 % prevalence recorded in those aged 6–15, 16–25, and 56–60,respectively.

Table 2.0 showed the overall parasite prevalence density among diarrheal HIV/AIDS patients. It was observed that the prevalence rate was 21.35% (42/196) with Cryptosporidium parvum (5.1%), Ascaris lumbricoides (5.6%), Hookworm (2.55%), Schistosoma mansoni (1.5%), Trichuris trichura (1.5%), and Entamoeba histolytica (5.1%). Table 3.0 showed the prevalence of cryptosporidium among diarrhoeal infected HIV/AIDS patients on an ART regimen. Patients with HIV and tuberculosis co-infection on regimen 1A had a higher prevalence of cryptosporidium (3.1%), followed by those on regimens 1D and 1F (1%) and 1B, C, and E (0%), respectively. 1A and 1F differ significantly at the 95% confidence limit, whereas 1B and 1C do not.

Table 4.0 showed the percentage of HIV/AIDS patients with diarrhea who also had cryptosporidium infection. It was observed that patients with CD4 350 have the highest prevalence of (4.1%) compared with patients with CD4 >350 with a prevalence of (1.0%). ($p<0.05$). Table 5.0 showed the prevalence of cryptosporidium in the stool consistency of diarrhea-infected HIV/AIDS patients. It was observed that the prevalence of cryptosporidium was highest with diarrheal stools mixed with blood (3.1%), OR 1.06, 95% CI 0.20-85.27, $P > 0.05$ compared with diarrheal stools mixed with mucus (0.0%).

TABLE1: PREVALENCE OF CRYPTOSPORIDIUM BY AGE AMONG DIARRHOEAL- INFECTED HIV/AIDS PATIENTS

		Male(A)		Female(B)		
Age groups (yrs) Positive	No Examined % Prev.	No % Prev	%	No	No	
		Positive	Prev.	Examined		
6-15	10	0	0.0	3	0	0.0
16-25	2	0	0.0	15	0	0. 0
26-35	20	0	0.0	30	2	1.0
36-45	25	1	0.5	50	2	1.0
46-55	20	1	0.5	15	4	2.0
56 -60	3	0	0.0	1	0	0.0
Total	**80**	**2**	**1.0**	**116**	**8**	**4.0**

Mean age 33.7, SD 9.5, Median 30

TABLE 2: DISTRIBUTION OF OTHER INTESTINAL PARASITES ISOLATED FROM STOOL OF DIARRHOEL-INFECTED HIV/AID PATIENTS

Parasite types	Number tested	Frequency	% infected
Cryptosporidium Parvum	196	10	10 (5.1)
Ascaris lumbricides	*?*	11	11(5.6)
Hook worm	*?*	5	5 (2.55)
Schistosoma mansoni	*?*	3	3 (1.5)
Trichuris trichura	*?*	3	3(1.5*)*
Entamoeba histolytica	?	10	10(5.1)
Total	**196**	**42**	**42 (21.35)**

TABLE: 3 PREVALENCE OF CRYPTOSPORIDIOSIS AMONG DRUGS COMBINATION REGIMEN

REGIMEN	No Tested	No. positive	Percent positive
1A	140	6	3.1 %
1B	3	0	0.0 %
1C	2	0	0.0 %
1D	9	2	1.0%
1E	3	0	0.0 %
1F	39	2	1.0 %
Total	**196**	**10**	**5.1 %**

Confidence limits: 0.41 - 5.7 %, 0.16 - 4.73 %, and 15.07 - 28.76 %

TABLE 4: PREVALENCE OF CRYPTOSPORIDIOSIS IN RELATION TO HIV PATIENTS CD4 GROUP

CD4GRP	No tested	No positive	Percent positive
<0 - 350	136 (4.1%)	8	
>350 - 700	60 (1.0%)	2	
Total	**196 (5.1 %)**	**10**	

C. I = 2.38% - 10.44%, P<0.05

TABLE 5: PREVALENCE OF CRYPTOSPORIDIUM IN RELATION TO STOOL CONSISTENCY

	Diarrhoea with Blood		Total	
	Yes	No	NO.	%
Cryptosporidium	10 (3.1 %) 3.1 %	0	6	
No Cryptosporidium	0 (0.0%) 96.9 %	190	190	
Total	**6 100 %**	**190**	**196**	

OR 1.06, 95% CI 0.20 -85.27, P >0.05.

Discussion

Death and illness are still caused by parasitic illnesses in developing nations, especially among HIV/AIDS patients[29]. Biological illnesses are among the many that HIV/AIDS patients may experience. Morbidity and mortality from AIDS are exacerbated by opportunistic bacterial, viral, and parasitic infections[3]. HIV and helminths are typically found together because they both have an effect on the immune system. People in Africa who are HIV positive often suffer from debilitating diarrhea. Infected persons with HIV all around the world suffer from diarrhea because to *Cryptospcridium*[4,30,31]. This research was conducted in Benin-City, Edo State, South-South, Nigeria to determine the prevalence of intestinal parasites and clinical impact of *Cryptosporidium* infection among diarrhoeal- infected HIV patients. A total of 189 patients with HIV, who were suffering from diarrhoea had negative results for Cryptosporidium in their stool samples when examined using a modified Ziehl Nelsen staining method[21,44,45,32]. Parasite prevalence was found to be 21.35 percent overall and 5 percent for cryptosporidium among HIV-positive individuals using the same diagnostic method. It was observed that the parasite(*Cryptosporidium*) is responsible for diarrhoea in HIV-positive people of all ages[5,32-34], In contrast to previous studies[35], this study found that 5 percent of people with diarrhea also had cryptosporidium. Patients with HIV/AIDS and diarrhea had a prevalence of 4.1% for those with CD4+<350 and 1.0% for those with CD4+ >350 (P< 0.05). HIV patients with low CD4+ cell counts are more likely to get opportunistic infections[36,37].

According to the results of this study, the three most prevalent parasites are *E.histolytica* (5.1%), *A.lumbricodes* (5.6%), and *cryptosporidium* (5.6%). Intestinal parasites such *Ascaris lumbricoides, hookworm, Giardia intestinalis, Entamoeba histolytica,* and *Taenia trichiura* should be ruled out in HIV-positive persons. *Cryptosporidium* infections have been reported by people living with HIV type [16]. The results of this research indicate opportunistic infections caused by *Cryptosporidium species* are common.

Females (4%) were more likely to be infected with *Cryptosporidium* than males (1%). One possible explanation is that, as shown in other regions, females are disproportionately affected by HIV-1[30,40,35].Because of their increased propensity for sexual activity, women of reproductive age have a higher HIV/AIDS prevalence than typical. However, men are more likely to contract *Cryptosporidium* [41,25,42]. There was a 2.5% increase in prevalence between the ages of 46 and 55 compared to the rest of the age groups (P<0.05). Variation in prevalence by age may have causes in the community's exposure, practices, and behaviors. However, studies conducted in tropical areas have shown that the most susceptible age range is between 15 and 26.

It is not known how *Cryptosporidium* causes diarrhea, Inflammation can be altered by co-pathogens like *Cryptosporidium*. Previous work done by Scholars from Southeastern ,Nigerian university on *Cryptosporidium* observed histologic change of gastrointestinal mucosa and the anatomic location of the infection has been shown to influence the severity of symptoms[1,41,43.] Cryptosporidiosis is an HIV opportunistic disease that can be spread by contaminated water, food, and animals, and its prevalence varies greatly from place to country. Children in postsecondary institutions in north central Nigeria have an unusually high prevalence of cryptosporidiosis [23, 30].

Researchers in both developing and developed countries observed a range of 11.4%-18.4% when studying the prevalence of Cryptosporidium in diarrhoea patients, therefore the 5% prevalence found here is in line with those findings. Thiry-eight(38%) of HIV-positive Haitians have *Cryptosporidium* infection. The small sample size of this study may explain the low prevalence, or the excellent immune responses of individuals on anti-retroviral medication may be to blame. The most common combination was the regimen1A , which consists of Zidovudine, Lamivudine, and Efavirenz, and accounted for 3.0% of all cases. This population is at a higher risk for opportunistic infections[16,47,48,36] due to the high prevalence of tuberculosis among them. The majority of the clinic's patients with diarrhea were part of this group, too.

Although the vast majority of reviews of the literature link cryptosporidiosis and mucus in the stool, this study only observed diarrhea with blood. One limitation is that just one stool sample was analyzed from each subject. This suggests that the true scope of the infection may have been underreported [31]. The cyst may have been removed by centrifugation with only formal ether concentration, rather than flotation with zinc sulfate. Most people living with HIV who are on ART rarely experience diarrhea because of their enhanced immune response.

Oocysts of the parasite *Cryptosporidium* have been found in the feces of HIV-positive patients at the Specialist Hospital in Benin's HIV-positive City. It's the root cause of diarrhea for 5% of the locals. The highest risk of *Cryptporidium* (3.0%) was seen in TB/HIV co-infected patients, who had CD4 levels that were often below 350. Stool samples revealed cryptosporidium, and they were bloody and watery, indicating that the diarrhea was bloody. Researchers in this study analyzed the correlation between CD4 count and antiretroviral medication regimen in order to ascertain the incidence of Cryptosporidiosis in people with HIV and diarrhea.

Conclusion

Diarrhea is one of the most serious health problems that people with weakened immune systems can face and Over 50 million people have died worldwide. From this study, it is clear that HIV/AID- diarrheal- induced patients are prompt to Cryptosporidium infection as a result of weak immune system, which reported 5% diarrheal cases due to *Cryptosporidium* infection. TB/HIV co-infected patients exhibited the highest rate of Cryptporidium, likely because to inadequate immunity, and all cryptosporidium was from CD4 <350 patients. Hence, all *Cryptosporidium* was from bloody diarrhoea.

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