

Prevalence of *Cryptosporidium* Species and Other Intestinal Parasites Among HIV Infected Patients at LAUTECH Teaching Hospital, Osogbo, Nigeria

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Abstract: *Background:* Cryptosporidiosis though an opportunistic infection in HIV infected patients, increases the mortality and morbidity to the diseases by its effect suppression of the immune system, our quest is to highlights the clinical correlation of diarrhea in immunocompromised individuals and the economic losses associated with managing infected individuals. *Methods:* Stool samples were collected from 96 subjects. Their personal information regarding their names, sex, age was also obtained. The stool samples were processed for non-opportunistic parasite using the direct and formol-ether concentration method. For *Cryptosporidium*, Modified Ziehl-Neelsen Technique was used. A total of ninety-six confirmed HIV individuals were enrolled for this study. *Results:* The mean age of the patients was 24.7 years with the youngest been 3 years old and the eldest 58 years old. 77 (74%) were females while 22 (23%) were males. Table 1 shows the general characteristics of the HIV subjects recruited into the study. The distribution of intestinal parasites detected in patient's stool is shown in Table 2. Of the 96 stool samples examined, intestinal parasites were seen in 87 samples, while 9 samples showed absence of parasites. The diagnosed parasitic infections were: *Cryptosporidium* spp (54.2%), *A. lumbricoides* (9.4%), Hookworm (5.2%), *E. histolytica* (3.1%), *S. stercoralis* (1%) and *Teania* spp (1%). Double parasitic infections with *Cryptosporidium* were observed in 13 (13.5%) HIV/AIDS patients stool samples. Regarding age and sex distribution of the participants, there were 74 (77%) females and 22 (23%) males. Males (68.2%) were more infected than females (55.4%) but the difference was not statistically significant. Table 3 shows relationship between intestinal parasitic infection and diarrhea in the HIV/AIDS patients. 45 (86.5%) of patients with *Cryptosporidium* infection presented with diarrhea. *Conclusion:* The association between diarrhea and *Cryptosporidium* infection was statistically significant. It may be concluded that in HIV infected patients, both opportunistic intestinal parasitic infections are prevalent.

Keywords: *Cryptosporidium* Spp, HIV/AIDS, Intestinal Parasites

1. Introduction

The connection between certain enteric parasites and human immunodeficiency virus (HIV) infection is well

documented. Gastrointestinal involvement in HIV/AIDS is almost universal, and significant diseases occur in 50-90% of immunocompromised and malnourished patients [1] while diarrhea can be presenting manifestation or a life threatening complication in HIV patients sometimes during the course of

the disease [2]. The etiology for such diarrhea could either be parasitic, bacterial, fungal, enteric virus or HIV itself [3]. Several species of protozoa have been associated with acute and chronic diarrhea in HIV diseases. These include: *Crptosporidium parvum*, *Isospora belli*, *Microsporidia species*, *Giardia intestinalis*, *Entamoeba histolytica*, *Cyclospora species*, *Blastocystis hominis* and *Dientamoeba fragilis* [2]. Nematodes like *strongloides stercoralis* can also cause diarrhea and overwhelming infestation in patients with variety of immunosuppressive disorders including HIV/AIDS [4, 5]. Helminths, including hookworm, *Ascaris lumbricoides*, *Teania saginata* and *Trichuris trichura*, are considered non opportunistic, except on rare occasions [6, 7].

Opportunistic infections are caused by either organisms of low or no virulence which are nonpathogenic for a person with intact immune system. The infection caused by known pathogen in such immunocompromised patients may present in different form like increased virulence, recurrence, multidrug resistant, or atypical presentation [8]. Intracellular intestinal protozoans *Cryptosporidium parvum*, *Clyclospora cayetanensis*, *Isospora belli*, and the microsporidia are most frequent opportunistic in patients with acquired immunodeficiency syndrome (AIDS) and are often the major cause of uncontrollable, debilitating diarrhea [9]. *Cryptosporidium spp* is one of the enteric microorganisms most commonly associated with persistent diarrhea (PD) and is associated with wasting in immunologically compromised individuals involving mostly people with HIV/AIDS [10]. This parasite causes morbidity and mortality in AIDS patient worldwide, and these outcomes would be expected to be appreciably higher in developing countries due to higher prevalence of infection in the general population. About 42 million people are infected globally with HIV while sub-Saharan Africa account for more than half (29.4 million) of this number (10). In Nigeria, infection rate of HIV range between 4.9% and 5.8% [11].

Except for a few published cases, there is insufficient information on the prevalence and magnitude of the clinical importance of *Cryptosporidium* infection in HIV/AIDS patient in tropical and developing countries. Some studies in Africa suggested that the incidence of infection might be less as might be expected given the higher prevalence of immunosuppression due to HIV/AIDS in the continent [12]. However, this could be attributed to a lack of adequate diagnostic methods specific to these parasites in this part of the world of which research are currently ongoing [13, 14].

This study seeks to highlights the opportunistic significance of *Cryptosporidiosis* infection and clinical correlation of diarrhea in immunocompromised individuals, the economic losses associated with management of infection and persistent debilitating diarrhea experience by infected individuals as major concerns.

2. Materials and Methods

2.1. Study Area

The study was carried out at the HIV clinic of Ladoke

Akintola University of Technology (LAUTECH) Osogbo. Osogbo is the capital city of Osun State, Nigeria located in the rain forest zone. It is located between latitude 7° 641N and 4° 341E with a human population of about 845,000 (National Census, 2006).

2.2. Sample Collection and Analysis

Stool samples were collected from consenting HIV patients attending the HIV clinic for routine checkup, drug collection and other clinical complaints. Clean sterile universal bottle was given to after being educated on how to properly collect the stool into it.

2.3. Analysis of Stool Sample

The stool was examined microscopically using direct saline and iodine preparation for the identification of trophozoites and cysts of protozoan parasites while the formal-ether concentration technique was used to identify cysts and ova of intestinal parasites. Ziehl-Neelsen staining was carried out for the identification of oocyst of *Cyptosporidium spp* as described by Cheesbrough, 2005.

2.4. Direct Saline Preparation

A piece of the fecal sample was mixed on the slide with physiological saline and iodine. The resulting suspension was sufficiently thin in order to transmit light easily. The sample was spread out and covered with a coverslip (N0.1) and examined microscopically at a magnification of x10 and x40.

2.5. Modified Formal-ether Sedimentation Technique

All stool samples, about 1g of faeces was emulsified in 7ml of 10% formalin in a centrifuge tube. The suspension was strained through a brass wire sieve into a beaker to collect the filtrate. The filtrate was poured into a centrifuge tube and 3ml of ether as added. The tube was shaken vigorously by hand for one minute and then centrifuge immediately at 3000rpm for one minute. The layer of fecal debris was loosen with the aids of an applicator stick from the side of the centrifuge tube and inverted quickly to discard the supernatant. The sediment was resuspended by tapping the bottom of the tube. A drop of resuspended mixture was transferred onto a clean microscope slide and covered with a cover slip. This was examined microscopically using x10 objective lens, then x40 for confirmation and identification of parasites [12].

2.6. Modified Ziehl-Neelsen Technique

Ziehl-Neelsen staining was carried out as described by Cheesbrough, [15]. Smears were prepared from the sediment obtained by formol-ether concentration technique. The smears were air dried and fixed with methanol for 2 minutes. The fixed smears were stained with carbol fuschin for 15 minutes, and were washed with water. This was followed by decolourization with 3% acid alcohol for 10 seconds and washed immediately with water. The smears were counter

stained with 0.5% methylene blue, and the slides were stood in a draining rack for the smears to dry. The stained smears were examined microscopically for oocysts, using x100 oil immersion objective lens to identify them.

2.7. Statistical Analysis

Test for statistical significance and association was done using T-test and Chi-square.

3. Results

A total of ninety-six confirmed HIV individuals were enrolled in this study. The mean age of the patients was 24.7 years with the youngest been 3 years old and the eldest was 53 years old. 74 (77%) were females while 22 (23%) were males. Table 1 shows the general characteristics of the HIV subjects recruited into the study.

The distribution of intestinal parasites detected in patient's stools is shown in Table 2. Of the 96 stool sample examined, intestinal parasites were seen in 87 samples, while 9 samples showed absence of parasites. The diagnosed parasitic infections were: *Cryptosporidium spp* (54.4%), *A. lumbricoides* (9.4%), Hookworm (5.2%), *E. histolytica* (3.1%), *S. stercoralis* (1%) and *Teania spp* (1%). Double parasitic infections with *Cryptosporidium* were observed in 13 (13.5%) HIV infected patients stool samples examined.

Regarding age and sex distribution of the participants, there were 74 (77%) females and 22 (23%) males. Males (68.2%) were more infected than females (55.4%), it equally shows the relationship between intestinal parasitic infection

and diarrhea in HIV patients. 45 (86.5%) of patients with *Cryptosporidium* infection presented with diarrhea.

Table 1. General Characteristic of Subjects.

Patients Characteristics	Number observed
Number of subject studied	96
Sex: Male: Female	22:74
Mean age	24.7 yrs
Subjects positive for <i>Cryptosporidium spp</i> (%)	52 (54.2%)
Subjects positive for other intestinal parasites (%)	19 (19.8%)
Co-infection of <i>Cryptosporidium</i> and other intestinal parasites (%)	13 (13.5%)
Number with diarrhea	60 (62.5%)

Table 2. Prevalence of intestinal parasites in HIV infected patients.

Parasite seen	AIDS Patients N=96 (%)
SINGLE	
<i>Cryptosporidium spp</i>	55 (54.2)
<i>Ascaris lumbricoides</i>	9 (9.4)
Hookworm	5 (5.2)
<i>E. histolytica</i>	3 (3.1)
<i>S. stercoralis</i>	1 (1.0)
<i>Teania spp</i>	1 (1.0)
DOUBLE INFECTION	
<i>Cryptosporidium</i> + <i>A. lumbricoides</i>	4 (4.2)
<i>Cryptosporidium</i> + Hookworm	5 (5.2)
<i>Cryptosporidium</i> + <i>E. histolytica</i>	3 (3.1)
<i>Cryptosporidium</i> + <i>S. stercoralis</i>	1 (1.0)

Table 3. Intestinal parasitic infection among HIV positive patients according to age.

Age Range	Male		Female		P-value
	No. Examined	No.(%) +ve	No. Examined	No. (%) +ve	
<20	3	2 (67)	5	3 (60)	0.0001
21-30	4	2 (50)	35	22 (63)	
31-40	5	4 (80)	26	9 (53)	
41-50	6	5 (83)	7	6 (86)	
>50	4	2 (50)	1	1 (100)	
Total	22	15 (68.2)	74	41 (55.4)	

4. Discussion

HIV infection and its health and economic consequence is a threat to people in sub-Saharan Africa [16]. It has also been associated with chronic diarrhea which is usually as a result of opportunistic parasitic infection. Intestinal parasitic infections are among the leading causes of morbidity and mortality in patients infected with HIV. *Cryptosporidium spp* is a well-established cause of diarrhea among HIV infected patients worldwide with prevalence of infection ranging from 3% in developed countries to 50% in developing countries [17]. An overall prevalence of 54.2% of *Cryptosporidium* infection was noted in HIV/AIDS patients in this study. The prevalence rate reported in this study is similar to what was previously reported in this area and other African countries [18].

The prevalence of 73.9% was recorded for overall intestinal parasitic infection among the HIV subjects. This is in line with the reports from other part of Nigeria. [19], observed a prevalence rate of 70.8% in Ilorin, Northern Nigeria, while [20], recorded prevalence rate of 89.5% in Lagos, Nigeria. Results of this study also reveal the trend in the occurrence of specific parasites in HIV positive persons in Osogbo, Nigeria. *Ascaris Lumbricoides*, (9.4%), Hookworm (5.2%), *Entamoeba Histolytica* (3.1%), *Teania spp* (1%) and *Strongyloides stercoralis* (1%). These findings are in agreement with those of Okodua *et al.*, [21], who reported Hookworm (5.7%), *Entamoeba histolytica* (5.7%) and *Strongyloides stercoralis* (2.9%). Although, *Stongyloides stercoralis* can cause overwhelming infection in HIV/AIDS patients, its detection among HIV patients in this study is also in agreement with study from Addis Abba (3.4%) [22, 23].

While non opportunistic intestinal parasites infections are

still an important problem in HIV-infected patients at any immunity level with or without the symptoms of diarrhea. With adequate medication, diarrhea as a result of immunosuppression will be tackled.

5. Conclusion

In conclusion, this study confirmed the widespread existence of *Cryptosporidium spp* in HIV/AIDS patients in Nigeria. Therefore, considering the severe and untreatable nature of Cryptosporidiosis and other opportunistic parasites should be done routinely for this category of patients, and control measures should be taken to achieve improved management among HIV/AIDS population.

Further study need to be done to characterize the species of *Cryptosporidium* as this will help to identify the pathogenic forms and increase our understanding of their genomic sequence. The use of Polymerase Chain Reaction technique in diagnosis may proof more efficient.

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