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Cryptosporidiosis among People Living with HIV/AIDS on Highly Active Anti-Retroviral Therapy (HAART) at Mukono Church of Uganda Hospital, Uganda

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Abstract

This study was carried out to estimate the prevalence of cryptosporidiosis among People living with HIV (PLWH) on highly active antiretroviral therapy (HAART) and relate the prevalence to possession of pets and the immune status of the individual attending ART at Mukono church of Uganda hospital. A cross sectional study was carried out among 232 people living with HIV between June and July, 2014. Interview with questionnaires and document reviews were used to collect data.

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Stool samples were obtained from each patient and processed using formal-ether concentration method, stained by modified Ziehl-Neelsen staining method and parasites were examined by direct microscopy. Univariate and multivariate analysis were carried out. Level of significance was set at p-value of 0.05. A total of 232 patients participated in the study. The mean age was 36.0 (±1 SD of 13.3) years. Females constituted 73.7% (171) of the study participants. All the study participants were those initiated on HAART. The most common HAART regimen being used by the study participants was AZT/3TC/EFV accounting for 61% (141/232) of the patients. More than half of the study participants were on HAART for more than one year (54%) whereas 46% were within the first year of treatment. The prevalence of Cryptosporidium spp was 4.31% (10/232). A significant difference in the risk of developing infection with Cryptosporidium spp was observed between groups based CD4+ T-lyphocyte counts, duration on HAART and possession of pets ($p < 0.05$ for all variables). The low prevalence of Cryptosporidiosis in this study may imply that immune restoration through ART is an important factor in prevention and management of Cryptosporidiosis. Though the prevalence reported in this study was low, it is still important for clinicians to maintain a high index of suspicion for cryptosporidiosis in gastro intestinal infections among PLWH with keen attention to those with CD4+T-lymphocyte count <100cells/mm^3.

Keywords: Cryptosporidiosis; Highly Active Antiretroviral Therapy (HAART); People Leaving with HIV(PLWH).

1. Introduction

Cryptosporidiosis, also known as crypto, [1] is a parasitic disease caused by Cryptosporidium, a protozoan parasite in the phylum Apicomplexa. It is spread through the fecal-oral route, often through contaminated water [1]. The main symptom is self-limiting diarrhea in people with intact immune systems. Cryptosporidium infection is cosmopolitan. Low economy status, poor sanitation and water treatment will result in high prevalence of infection and diarrhea epidemic. Infection mostly happens in children less than two years old and immunocompromised individuals [2]. In immunocompromised individuals, such as people living with HIV/AIDS, the symptoms are particularly severe and often fatal. Treatment is symptomatic, with fluid rehydration, electrolyte correction and management of any pain. Despite not being identified until 1976, it is one of the most common waterborne diseases and is found worldwide. The parasite is transmitted by environmentally hardy microbial cysts (oocysts) that, once ingested, exist in the small intestine and result in an infection of intestinal epithelial tissue. Cryptosporidium prevalence varies in different groups/population. It causes 50.8% of water-borne diseases that are attributed to parasites [3]. In developing countries, 8-19% of diarrheal diseases can be attributed to Cryptosporidium [4]. In [5] reported that 11.9% of HIV patients (n= 318) with chronic diarrhea in Jakarta were positive for Cryptosporidium species oocysts, while in Medan General Hospital only 2.9% of children with diarrhea (n= 172) were positive. In West Africa the prevalence was 7.7% in children of less than 3 years old [6] whereas in Iran the prevalence was 25.6 and 3.7% in adults with and without diarrhea respectively [7]. In Uganda, Cryptosporidium species has emerged as an enteric pathogen that causes persistent diarrhea in immunologically compromised individuals particularly in association with HIV/AIDS [8]. Though it was once thought to be rare and host specific, Cryptosporidium is now known to be ubiquitous and to have many hosts. Once thought to be non-pathogenic, some isolates are now known to cause severe illness. Although recognized and named in 1907, most information on its identification, clinical significance,

epidemiology, and treatment has been obtained only within the past few years. This research was aimed at determining the prevalence of cryptosporidiosis among people living with HIV/AIDS on HAART at Mukono Church of Uganda Hospital.

2. Materials and Methods

2.1 Study site and population

The study was carried out at Mukono church of Uganda hospital, in Mukono district. The hospital includes HIV clinic where counseling and testing is routinely done before enrollment on ART if found positive. Baseline investigations such as $CD4^+$ T lymphocyte count, Liver function tests (LFTs), Renal function tests (RFTs) and complete blood count (CBC) were carried out. The study included those patients on HAART and willing to consent and excluded clients on HAART but declining consent and/ or HAART naïve clients. Stool and blood samples were taken from all HIV positive patients on ART. The study was conducted only on HIV/AIDS positive subjects and total of 232 stool and blood samples were collected from these patients at Mukono church of Uganda Hospital. The fresh stool samples were collected in sterile wide mouth containers. The consistency of the stool specimens was graded by categories (soft, watery, etc) while waiting to be processed for cryptosporidium oocysts. The blood samples were used to determine the CD_4 T lymphocyte count.

2.2 Study design and sample collection

A cross sectional study was conducted at Mukono church of Uganda hospital focusing on HIV positive patients that came to the hospital for follow-ups. A total of 232 HIV positive patients on ART participated in the study. The fresh stool samples were collected in sterile wide mouth containers and blood was collected aseptically through venipuncture using EDTA blood collection tubes to determine the CD_{4+} cell count. Each specimen was labeled, containing information about patient's name, age of patient then the anticoagulated blood stored at room temperature (20^oC to 25^oC) was stained with in 24hours of collection and analyzed for CD_4 T lymphocytes with in 48hours of staining.

2.3 Laboratory Methods

2.3.1 Formal ether concentration method

A portion of each fresh stool sample was taken and processed. Briefly, 1 g of stool was placed in a clear 15 ml falcon tube containing 7 ml formalin saline by using applicator stick. The resulting suspension was filtered through a sieve into another conical tube. After adding 3 ml of diethyl ether to the formalin solution, the content was centrifuged at 3200 rpm form 3 minutes. The supernatant was poured away and the smear prepared from the sediment and left to air dry before staining using Modified Ziehl-Neelsen staining method [9].

2.3.2 Modified Ziehl-Neelsen staining method

Air dried thin smears were fixed with methanol for 5 minutes and stained with strong carbol fuchsine for 30 minutes. After washing the slides in tap water, they were decolorized with acid alcohol for 1–3 minutes and

stained in methylene blue for 1 minute. The slides were then washed in tap water, left to air dry and observed under an oil immersion objective[10].

2.3.3 Determination CD_{4+}T lymphocyteCount

After collecting the blood aseptically in a BD vacutainer EDTA collection tubes, a quality control was performed to check the BD facs count's system accuracy and linearly. Reagents tubes were then prepared with the patient accession number that identifies the tube of the blood where each tube was vortexed while upside down and opened with coring station. Each Patient's blood was then added in a different tube by first mixing it and then pipetting 50ul of whole blood in the tubes. There after the tubes were capped and vortxed while upright for 6minutes and then incubated for 30minutes at room temperature in the work station. The tubes were a gained uncapped and a fixative added in each tube and run in the system by entering the patient accession number which is done for each patient.

2.4 Sample size and sampling procedure

Based on the current prevalence of cryptosporidiumin HIV/AIDS patients attending ART in Uganda that was 17.6%, [8] the sample size was calculated using the formula:

$$N=4P\ (100-P)/d^2$$

Where, P was the previous prevalence of cryptosporidium in Uganda

N was the sample size

d was the marginal error on P

P =17.6 and d =5

$N =4*17.6(100-17.6)5^2$

N =232

Therefore, the minimum size sample was 232 people living with HIV/AIDS.

2.5 Quality control

New slides were used make smears and after the first reader reading the slide, a second reader also read the slide and gave his independent opinion. An agreement of the two readers was taken as the final result. A quality control for the BD facs count was done daily to check system accuracy and linearly before running patients' samples.

2.6 Data analysis and presentation

The data was entered and organized in Microsoft excel and then imported to STATA ver11 statistical analytical tool for analysis. It was then interpreted with Odds ratios, Qi- square test and their p-values at 95% CI and presented as percentages, graphs, and tables.

3. Results

3.1 Characteristics of study participants

A total of 232 people living with HIV/AIDS and on HAART participated in the study. The overall mean age of the study participants was 36 (±1 SD of 13.3). Females constituted 73.7% (171) of the study participants.

Tabe1: Socio-demographic, treatment and immunity characteristics of study participants

Characteristic	**Proportion (%)**					
	Male			**Female**		
	No.	**Positive**	**Negative**	**No.**	**Positive**	**Negative**
Age						
1-20	7 (11)	0 (0)	7 (100)	12 (7)	2 (17)	10 (83)
21-40	26(43)	3(12)	23 (88)	116 (68)	5(4)	111(96)
41-60	25(41)	0 (0)	25(100)	37 (22)	0(0)	37(100)
61-80	3(5)	0 (0)	3(100)	6 (3)	0 (0)	6(100)
Living with pets						
Yes	8(13)	4(50)	4(50)	7(4)	3(43)	4(57)
No	53(87)	0 (0)	53(100)	164 (96)	4(2)	160(98)
Duration on HAART						
1-6 Months	12(20)	4 (25)	9 (75)	25 (15)	6(24)	19(76)
7-12 months	19(31)	0 (0)	19(100)	51 (30)	0(0)	51(100)
> 12 months	30(49)	0 (0)	30(100)	95 (55)	0(0)	95(100)
HAART regimen						
D4T/3TC/NVP	13(48)	1(8)	12(92)	14 (8)	1(7)	13(93)
D4T/3TC/EFV	10(32)	1 (10)	9 (90)	21 (13)	1(5)	20(95)
AZT/3TC/NVP	13(39)	0 (0)	13 (100)	20 (11)	1(5)	20(95)
AZT/3TC/EFV	25(18)	1(4)	24(96)	116 (68)	4(3)	112(97)
CD4 count						
<100	7(11)	3(43)	4(57)	12 (7)	7(58)	5(42)
101-200	6(10)	0(0)	6(100)	14(8)	0(0)	14(100)
201-300	11(18)	0(0)	11 (100)	17(10)	0(0)	17(100)
>300	37(61)	0(0)	37(100)	128 (75)	0(0)	128(100)

All the study participants were initiated on HAART. The commonest HAART regimen being used by the study participants was AZT/3TC/EFV accounting for 61% (141/232) of the patients. Of the 232 patients, 30% (70/232) had taken the therapy for at least 7 -12 months and 54% (125/232) of patients had taken the therapy for more than one year (Table 1).

Table 2: Association between sex, age, living with pets, CD4 counts and treatment status with Cryptosporidiosis

Characteristic	***P*- value**	***95% CI***	
		lower	***upper***
Age group	0.009	0.0761	0.0112
Sex	0.287	0.0760	0 .0226
Living with pets	0.000	0.1461	0.3267
Duration on HAART	0.001	0.0028	0.0003
HAART regimen	0.858	0.0253	0.0211
CD4 count	0.000	0.1194	0.0725

3.2 Prevalence of cryptosporidiosis.

A summary of characteristics of cryptosporidiosis among PLWH is shown in Table 1. Overall, 10 (4.31%) of the PLWH had cryptosporidiosis. The age group (21-40 years) had the highest prevalence of cryptosporidiosis with a total of 80% (8/10), 37.5% (3/8) being male and 62.5% (5/8) being female. The age group (1-20year) had the second highest prevalence of cryptosporidiosis that constituted 20% (2/10) who were all female.

3.3 Possession of pets and distribution of cryptosporidium

A total of 15 patients out 232 HIV positive patients lived with pets in their homes which accounted for 6.5%. People living with pets at their homes and had no cryptosporidiosis constituted 53% (8/15) and those with pets and had cryptosporidiosis constituted 47% (7/15). PLWH that were positive for cryptosporidiosis and did not possess pets in their homes were 4/164 accounting for 2%.

3.4 Association of cryptosporidiosis in PLWH with the duration on HAART

Cryptosporidiosis was found in all patients under the group of HAART duration 1-6months with a prevalence of 10 (100%), 4(40%) male and 6(60%) female. A total of 37(15.9%) patients had attended therapy for 1-6months, 70(30.2%) had been on the therapy for 7-12months and 125(53.9%) had attended the therapy >12months.

3.5 Prevalence of cryptosporidiosis in PLWH in association with HAART regimen

A total of 232 patients that participated in this study had HAART. 27(11.3%) of the patients were on D4T/3TC/NVP with 20% (2/10) having cryptosporidiosis, 31(13.4%) of the patients were on D4T/3TC/EFV with 20% (2/10) being affected with cryptosporidiosis, 33(14.2%) constituted those on AZT/3TC/NVP accounting for 10% (1/10) cryptosporidiosis and 141(60.7%) for those on AZT/3TC/EFV with 50% (5/10) with cryptosporidiosis.

3.6 Prevalence of cryptosporidiosis with patient's immune status as measured by CD4 T lymphocyte count

The study participants who were on HAART consisted of 8.2% (19) with CD4+ T lymphocyte count of <100cells/mm^3, 20 (8.6%) patients with CD4+ T lymphocyte count 101-200cell/mm^3, 28(12.1%) with CD4+ T lymphocyte count 201-300cells/mm^3, 165(71.1%) patients with CD4 T lymphocyte >300cell/mm^3.all patients that were positive for cryptosporidiosis were those with <100cell/mm^3 CD4+ T lymphocyte counts.

4. Discussions, conclusions and recommendations

This study was carried out on a total of 232 HIV infected individuals that had been initiated on HAART and the prevalence of cryptosporidiosis among these PLWH on HAART in Mukono church of Uganda Hospital HAART clinic was 4.31%.

The prevalence of cryptosporidiosis among HAART patients in this study was lower than that reported in Brazil[11], South Ethiopia (34.3%)[12], Congo (24.6%), Nigeria (30%)[13]with that of Cameroon study (40.5%) [14], Gondar (43.5%), in selected ART centers of Adama, Afar and Dire-Dawa (52%)[15], Arbaminch Chencha and Gideo (45%), and in different parts of Ethiopia (57.2%,)[15].This low prevalence in this study might be due to geographic difference, difference in sample size (more than one study area for most reports), considering those patients with, time gap where those studies were done averagely four years ago but nowadays there is a better awareness of the patients about intestinal parasite infection and their cause. The results could also be explained by the fact that all our study participants were on HAART.

This study showed statistical significant difference between the number of males and female recruited for the study. Out of 232patients, 61 (26%) were male and 171 (73.71%) were female, $P< 0.05$ indicating a significant difference in gender representation of the patients.

In this study, modified Ziehl-Neelsen staining method was used for detection of Cryptosporidia. Water-ether sedimentation method for Microsporidia and other methods like Molecular techniques and immunoflouscent techniques sensitive for parasites were not used. In addition to this, patients may be diagnosed for parasites and treated as well before. Antihelminthics may be given for deworming purpose. Because of this fact prevalence of cryptosporidium species may have been under estimated in this study.

This study indicated that all of cryptosporidiosis infections among PLWH on HAART were found significantly associated with lower <100 cells/mm^3 CD4 Tlymphocyte count when compared to the HAART experienced

patients without this parasite infection in any of CD4 category. This may be due to the fact that opportunistic parasites are known to resolve spontaneously with immune restoration among HIV/AIDS patients on HAART. The association of these parasite with <100 cells/mm^3 CD4 count was in line with that of Varasani (97.8%), India (83%), [16]in selected ART centers of Adama, Afar and Dire-Dawa (62.5%)[15]in different part of Ethiopia (76.9%)[10].

4.1 Conclusion

The low prevalence of Cryptosporidiosis in this study may imply that immune restoration through ART is an important step in prevention and management of Cryptosporidiosis. Though the prevalence reported in this study was low, it is still important for clinicians to maintain a high index of suspicion for cryptosporidiosis in gastro intestinal infections among PLWH with keen attention to those with CD4+T-lymphocyte count <100cells/mm^3. The higher prevalence of cryptosporidiosis was associated with a lower CD4 count implying that it could even be higher among ART naïve patients. Living with pets was also associated with the high prevalence of cryptosporidiosis.

4.2 Limitations of the study

The study was limited to patients on ART. It is likely that we missed cases of cryptosporidiosis in ART naive patients.

4.3 Recommendation

Public health measures should continue to emphasize the importance of environmental and personal hygiene as well as providing and monitoring the quality of drinking water aiming to obtain a better quality of life for those patients.

Stool examination should be routinely performed in the follow-up of patients with HIV/AIDS attending ART clinic in order to optimize treatment of cryptosporidiosis and other preventive measures such as an anthelmentics should be given for deworming purposes or designing a strategy in which every HIV infected person should be routinely dewormed whether on HAART or not. The ministry of health should sensitize the public about opportunistic parasites more so in immunocompromised individuals causing chronic diarrhea especially in HIV/AIDS patients to increase the awareness about cryptosporidiosis and its effect on people living with HIV/AIDS. The public health measures should also continue to offer HAART to people living with HIV/AIDS in order to improve on their immune system and to reduce the chances of opportunistic parasites not forgetting cryptosporidiosis as being one of them in the HIV/AIDS patients.

5. Ethical Approval

Ethical clearance was received from the Faculty Ethical Review Committee of Kyambogo University. Permission to conduct the study was also obtained from Mukono Church of Uganda hospital. Informed consent was obtained from the study participants after explaining the importance, purpose and procedure of the study.

Confidentiality of the study participants was highly prioritized. Even though the names were included on the sample specimens, there were eliminated at analysis. The Case Report Forms and all the other source documents were secured under locker and key.

Results were included in the patients' reports for appropriate management by the attending clinicians through the routine laboratory reporting system.

6. Competing interests

The authors declare that they do not have competing interests.

References

[1] Centers for Disease Control and Prevention (CDC). "Outbreak of cryptosporidiosis associated with a splash park - Idaho," MMWR Morb Mortal Wkly Rep, vol. 58(22), 2009

[2] Smith HV, Cacciò SM, Cook N, Nichols RA, Tait A. "Cryptosporidium and Giardia as foodborne zoonoses.," Vet Parasitol. , vol. 21;149(1-2), pp. 29-40, 2007

[3] Gideon AA Njiné T, Nola M, Menbohan SF, Ndayo MW. "Measuring resistant forms of two pathogenic protozoa (Giardia spp and Cryptosporidium spp) in two aquatic biotopes in Yaoundé (Cameroon)]." Année de parution, vol. 17(3), pp. 167-72. , 2007.

[4] Wangeci Gatei Claire N. Wamae, Cecilia Mbae, Anthony Waruru, Erastus Mulinge, Tabitha Waithera, Simon M. Gatika, Stanely K. Kamwati, Gunturu Revathi, And Charles A.. Hart "Cryptosporidiosis: Prevalence, Genotype Analysis, And Symptoms Associated With Infections In Children In Kenya," Am. J. Trop. Med. Hyg.,, vol. 75(1), pp. 78–82, 2006.

[5] Kurniawan A, Karyadi T, Dwintasari SW, Sari IP, Yunihastuti E, Djauzi S, Smith HV. "Intestinal parasitic infections in HIV/AIDS patients presenting with diarrhoea in Jakarta, Indonesia," Trans R Soc Trop Med Hyg vol. 103: , pp. 892-898., 2009

[6] Perch MS, Morten, Jakobsen, Marianne S, Valentiner-Branth, Palle, Steinsland, Hans, Fischer, Thea K, Lopes, Dina Duarte, Aaby, Peter; Mølbak, Kåre. "Seven years' experience with Cryptosporidium parvum in Guinea-Bissau, West Africa," Annals of Tropical Paediatrics: International Child Health,, vol. 21(4) pp. 313-318 2001.

[7] Mirzaei M. "Prevalence of Cryptosporidium sp. infection in diarrheic and non-diarrheic humans in Iran. ," The Korean Journal of Parasitology, , vol. 45(2), , pp. 133–137. , 2007.

[8] Tumwine JK, Kekitiinwa A, Bakeera-Kitaka S. "Cryptosporidiosis and microsporidiosis in Ugandan children with persistent diarrhea with and without concurrent infection with the human immunodeficiency virus.," Am J Trop Med Hyg. , vol. 73(5):921-925. , pp. 921-925, 2005. .

[9] Assefa Missaye, Mulat Dagnew, Abebe Alemu and Agersew Alemu, "Prevalence of intestinal parasites and associated risk factors among HIV/AIDS patients with pre-ART and on-ART attending dessie hospital ART clinic, Northeast Ethiopia," AIDS Research and Therapy vol. 10, p. 7, 2013.

[10] Telele NF, Damte DG, Selassie S. "Intestinal parasitic infections among HIV seropositives and seronegatives adult patients presented with diarrhea in Gondar, North west Ethiopia.," Revi Ant Infec Dis, vol. 3, pp. 7–8, 2010.

[11] Rodrigues Bachur TP, Vale JM, Branco Coêlho IC, de Sales Queiroz TR, Souza Chaves BC , "Enteric parasitic infections in HIV/AIDS patients before and after the highly active antiretroviral therapy in Brazil. ," Bra J Infe Dis., vol. 12, pp. 115–122. , 2008.

[12] Girma M, Teshome W, Petros B, & Endeshaw T, "Cryptosporidiosis and Isosporiasis among HIV-positive individuals in south Ethiopia: a cross sectional study. ," BMC Infectious Diseases, , vol. 14, p. 100. , 2014. .

[13] Wokem GN, Chukwu C, Nwachukwu BC , "Prevalence of intestinal parasites seen in HIV sero-positive subjects in Port Harcourt, Nigeria. ," Niger J Parasitol., vol. 29 pp. 115–120, 2008.

[14] Sarfati C, Bourgeois A, Menotti J, Liegeois F, Moyou-Somo R, Delaporte E, Derouin F, Ngole EM, Molina JM, "Prevalence of intestinal parasites including microsporidia in human immunodeficiency virus–infected adults in Cameroon: a cross-sectional study.]," Am J Trop Med Hyg., vol. 74, pp. 162–164, 2006.

[15] Adamu H, Petros B, "Intestinal protozoan infections among HIV positive persons with and without antiretroviral treatment (ART) in selected ART centers in adama, afar and dire-dawa, Ethiopia. ," Ethiop J Health Dev. , vol. 23 pp. 133–140, 2009.

[16] Tuli L, Gulati A. K, Sundar S., & Mohapatra T. M., "Correlation between CD4 counts of HIV patients and enteric protozoan in different seasons – An experience of a tertiary care hospital in Varanasi (India). ," BMC Gastroenterology,, vol. 8,, p. 36. , 2008