THE PREVALENCE OF INTESTINAL OPPORTUNISTIC PARASITES AMONG HIV SEROPOSITIVE INDIVIDUALS IN THE CAPE COAST METROPOLIS

Abstract

Persistent diarrhoea and small bowel enteropathies are features of HIV infection. The coccidian parasites such as Microsporidium species, Cyclospora species, Cryptosporidium species, bacteria and cytomegaloviruses are implicated in HIV infections-associate diarrhoea. Determination of the prevalence of coccidian parasites and Microsporidium sp in HIV/AIDs seropositive patients visiting Cape Coast Teaching Hospital (CCTH) will contribute immensely to the management of diarrhoea.

Aims: The study estimates the percentage occurrence of Cryptosporidium, Cyclospora Microsporidium and Isospora among HIV seropositive patients within the Cape Coast.

Methods: The study was conducted from May 2012 to April 2013 at Cape Coast Teaching Hospital (CCTH). It was a cross-sectional study. The stool samples were examined microscopically using Wet mount, acid-fast staining methods. The protozoan was confirmed using species-specific PCR analysis. The data was analyzed using Pearson correlation.

Results: The overall prevalence of Cyclospora, Cryptosporidium, Microsporidium and Isospora spp as detected by microscopic examination were 32% (16), 46% (23), 16% (8) and 0% respectively. PCR test showed 40% (20), 48% (24), 0% and 0% for Cyclospora, Cryptosporidium, Microsporidium and Isospora spp. There was a negative correlation (R=-0.424) between

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the CD4+ T-cell counts and coccidian parasite counts among the subject.

Conclusions: There was a high prevalence of Cyclospora, Cryptosporidium, and Microsporidium species among HIV/AIDs patients experiencing diarrhoea in the Cape Coast.

Keywords: Coccidian parasites, Opportunistic; HIV seropositivity, diarrhoea, CD4+ T-cells;

I. INTRODUCTION

HIV (human immunodeficiency virus)/and AIDS (acquired immunodeficiency syndrome) are the most common causes of illness and death around the world [1, 2]. Opportunistic infections increase the morbidity of HIV/AIDS. It also has a direct effect on the diagnosis, treatment, and management of HIV/AIDS-related diseases [3]. Aids-associated protozoan infection has implications when it comes to improving Aids patients' health and well-being [4]. Diarrhoea, caused by opportunistic protozoa, is the most common complication seen in HIV/AIDs infection, and it is a significant cause of illness and death [5-7]. It affects roughly 90% of those living with AIDS across poor nations [8]. Investigations have highlighted the emergence of gastrointestinal opportunistic protozoa accounts for a significant number of diarrhoea episodes in this population. *Cryptosporidium*, *Isospora*, *Cyclospora*, *Microsporidium*, *Entamoeba histolytica* and *Giardia lamblia* are the major causes of diarrhoea in HIV/AIDs population [5, 9-11].

However, there is a paucity of data on the protozoan pathogens in the Cape Coast Metropolis and Ghana. Data on the correlations between the CD4+ levels and the opportunistic protozoan infections associated with diarrhoea in HIV/AIDs patients is also limited in the Central Region of Ghana. Most protozoan infections are treatable with early and accurate diagnosis [12]. This study determined the prevalence of coccidian parasites among HIV seropositive individuals in the Cape Coast Metropolis and the associations between the CD4 count and the opportunistic protozoan pathogens.

1. **Methods:** The study was conducted in the Cape Coast Teaching Hospital (CCTH) from May 2012 to April 2013 with ethical clearance from Ghana Health Service (GHS-ERC: 24/1/12). The study population comprised 50 HIV seropositive patients visiting CCTH. The patients' demographics such as gender, age, and CD4 counts were recorded from the patients' folders. The stool samples were also collected for examination. The study was cross-sectional.

2. **Wet mount** : An iodine-stained wet mount stool preparation [13] and examined using a trinocular clinical microscope (Olympus CX3, Japan) with magnification (X40) followed by (X100) to detect the parasites under study.

3. **Formalin-ethyl acetate sedimentation method**: About 4g of each remaining stool sample was transferred into 10% formalin (10 ml) in a test tube. The stool and the formalin were mixed thoroughly and allowed to stand for 30 minutes. The sample was strained through a wet gauze into a 15 ml centrifuge tube, and 0.85% NaCl was added and centrifuged at 500g for 10 minutes [14]. The supernatant was decanted, and the sediment

was suspended in saline solution and centrifuged for 10 minutes at 500g followed by 10% formalin-ethyl acetate centrifuged for 10 minutes at 500 X g. The sediments were observed at X40 followed by X100 magnification using a trinocular clinical microscope (Olympus CX3, Japan).

4. **Modified Ziehl-Neelsen acid fast staining** : The stool samples were stained using a modified Ziehl-Neelsen staining method to enhance the identification of the parasites [15].

II. EXTRACTION OF GENOMIC DNA AND SPECIES-SPECIFIC PCR ANALYSIS

The genomic DNA was extracted using a QIAamp DNA stool mini-Kit (Cat. No. 51504, UK). A 636-bp, 844-bp, 607-bp and 89-bp fragment of the 18S rRNA genes of *Cyclospora, Cryptosporidium, Microsporidium and Isospora species* were amplified using PCR [16-19]. The PCR reaction mix of 25 µl contained 2.5 µl of 10x PCR buffer (100 mM Tris-Hcl, pH 8.3, 500 mM KCl, 15 mM MgCl2 and 0.01% gelatin), 200 µM of each deoxynucleotide triphosphates (dNTPs) mix, 0.2µM of each primer pairs (Table 1), 2.5 U of the Taq DNA polymerase enzyme (5 U/ µl in 20 mM Tris-HCl, pH 8.0, 100 mM KCl, 0.1 mM EDTA, 1mM DTT, stabilizers, 50% glycerol), 5 µl of DNA template and 11.625 µl nuclease-free water (PS Park Scientific Ltd, UK) was added to make up the volume. The amplification reaction was carried out using a PCR thermal cycler (TECHNE TC-512, UK). The cycling conditions were as follows: 95 ˚C for 15 minutes as initial activation, followed by 94˚C for 30 seconds for denaturation, annealing at 53˚C for 30 seconds, extension at 72 ˚C for 90 seconds and final extension at 72˚C for 10 minutes. The number of cycles was 35, and for each reaction, a negative control that contained no DNA but dH2O was added to the amplification. The amplified products were analyzed by agarose gel electrophoresis and were visualized using a transilluminator (Clear View, UK).

Table 1: Primer pairs sequences targeting the 18S RNA of protozoan parasites

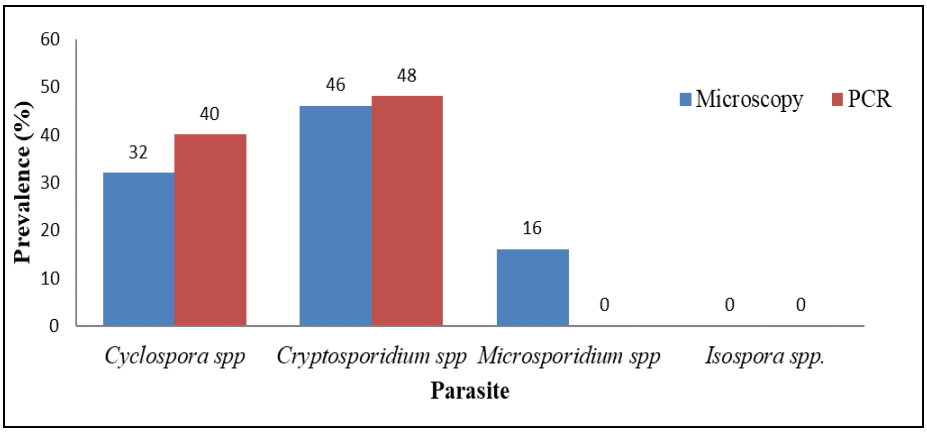
ORGANISM	**PRIMER**	**SEQUENCE**	**EXPECTED AMPLICON SIZE (bp)**
Cyclospora sp	F1E R2B	Forward 5′-TACCCAATGAAAACAGTTT-3′ Reverse 5′-CAGGAGAAGCCAAGGTAGG-3′	636 [16]
Microsporidium sp	EBIEF1,EBIER1	Forward 5′-GAAACTTGTCCACTCCTTACG-3′ Reverse	607 [17]

		5′-CCATGCACCACTCCTGCCATT-3′	
Cryptosporidium sp.	ExCry1 (forward) ExCry2 (reverse)	Forward 5′-GCCAGTAGTCATATGCTTGTCTC-3′ Reverse 5′-ACTGTTAAATAGAAATGCCCCC-3′	844 [18]
Isospora sp	Ib-40F Ib-129R	Forward 5′-ATATTC CCT GCA GCATGT CTG TTT-3′ Reverse 5′-CCA CAC GCG TAT TCC AGA GA-3′	89 [19]

1. **Data analysis:** The information was entered into Excel and analyzed using SPSS version 16. The infection frequency was represented by simple counts and proportions. The association between CD4 and the frequencies of pathogens were tested using Chi-square analysis and Pearson correlation.

III. RESULTS

In all, 50 participants with an age range of 1-70 years with a mean age of 38.66 ± 15.8006 years (Males: 28% (14); Females: 72% (36). Their CDT-cell counts/µL of blood were in the range of 200<CD4+ T-cells>500. Microscopic examination showed a prevalence of 32% (16), 46% (23) and 16% (8) for Cyclospora, Cryptosporidium and Microsporidium spp. with *Isospora* spp recording no prevalence (Figure 1). On the other hand, *Cyclospora* spp. and *Cryptosporidium* spp recorded a prevalence of 40% and 48% and *Microsporidium* and *Isospora* spp had no prevalence. There was a significant difference between the prevalence by microscopic examination and that of PCR ($\chi 2$=16.752, P=0.000).



The CD4+ count was significantly associated with of *Cyclospora* ($\chi 2$=13.90, P=0.001) and *Cryptosporidium* spp ($\chi 2$=13.75, P=0.001) infections. However, no significant association ($\chi 2$=5.626, P=0.06) between CD4+ T-cells count and Microsporidium spp prevalence (Table 2).

Table 2: Association between opportunistic pathogens and CD4 count

CD4$^+$ T-cell count (µ/L)	No. of subjects tested	No. of positive cases (%)		
		***Cyclospora* spp**	***Cryptosporidium* spp**	***Microsporidium* spp**
>500	15	0 (0)	1 (6.7)	0 (0)
200-499	19	6 (31.6)	11 (57.9)	3 (15.8)
<200	16	6 (37.5)	11 (68.8)	5 (31.3)
χ^2		13.9	13.75	5.626
P-value		0.001	0.001	0.06

The CD4+ count was negative correlation with the number of opportunistic parasites in diarrhoea-HIV/AIDs cases (Pearson Correlation R= -0.424, p<0.0001) (Table 3).

Table 3: Correlation of CD4$^+$ counts and Number of Opportunistic protozoan in diarrhoea-HIV/AIDS

No. of pathogens	CD4$^+$ T- cells count /µL			Pearson's correlation (R)
	>500	200-499	<200	
1	2	12	5	-0.424
2	0	2	6	
3	0	0	4	p< 0.0001
None	13	5	13	
Total	15	19	28	

The pathogens were Cyclospora, Cryptosporidium and Microsporidium spp. 1= a single pathogen, 2= two of the pathogens, 3= all three pathogens were present and none= no pathogen present.

IV. DISCUSSION

Opportunistic infections constitute a health problem in patients infected with HIV/AIDs [20, 21]. Parasitic diseases are the common cause of illness and death in HIV/AIDs infected individuals worldwide [22]. The coccidian parasites Cyclospora, Cryptosporidium, Isospora and Microspora spp. are foremost among the enteric parasites in these patients [23]. These organisms usually cause a self-limiting illness in immunocompetent individuals but cause life-threatening profuse watery diarrhoea [24, 25]. The high prevalence of Cryptosporidium spp, Cyclospora spp, and Microspora spp in HIV patients should be a concern for the HIV control program in Ghana as these infections contribute to the wasting disease among HIV patients. The Cryptosporidium infection is most challenging due to auto-infectivity and causes severe diarrhoea [5, 26]. Stool microscopic examination remains the diagnostic method for HIV/AIDs patients experiencing chronic diarrhoea, but microscopy diagnosis has a limited diagnostic value with low sensitivity for oocysts in stool samples [27, 28]. There was a significant difference between the prevalence by microscopic test examination and that of PCR detection. The PCR diagnosis has higher sensitivity and specificity for pathogen detection than microscopy [29, 30]. However, the deployment of PCR as a diagnostic method for protozoan among HIV patients is challenged by limited resources, lack of stable electricity and technically skilled personnel and remains a surveillance tool in the research institution. It recommended that PCR diagnosis be reserved for HIV/AIDs patients with unknown chronic diarrhoea.

Interestingly, the study did not record Isospora spp infection among the participants. The widespread usage of Trimethoprim-sulfamethoxazole (TMP-SMZ) as prophylaxis against Pneumocystis carinii in HIV patients has led to a very low prevalence of Isospora *spp* among HIV patients [31, 32]. A similar study from the Komfo Anokye Teaching Hospital in Ghana also could not find Isospora infections among HIV/AIDs patients [33, 34]. Several studies have revealed that antibiotics, antimalarial agents, mineral oil, bismuth and non-absorbable anti-diarrhoeal preparations potentially interfere with the detection of Isospora spp [34-36]. In Ghana, antimalarials and antibiotics drugs are highly abused and probably account for the very low prevalence of Isospora *belli* among the study participants [37, 38]. In addition, the incidence of intestinal parasites in HIV/AIDS patients may vary depending on pathogen endemicity in a population or environment [39, 40].

The study discovered a link between CD4 count and the incidence of Cyclospora spp. Patients with high CD4+ cells had lower levels of intestinal protozoans. There was yet

another negative association between CD4+ cells and the amount of opportunistic parasites among research participants. The study mirrors a similar report from India that shows a 0%, 12.5% and 4.9% prevalence for CD4+ counts > 500 cells/ µl, 200 – 499 cells/ µl, and < 200 cells/ µl respectively [41]. The cell-mediated immune response is well known to play a role in modulating cyclosporiasis and Cryptosporidium spp infections [42, 43]. Thus, an increase in CD4+ T-cell count decreases the number of opportunistic parasites by spontaneous clearance of parasites [44, 45]. However, there was no significant association between CD4+ T-cell count and the prevalence of Microsporidium species.

V. CONCLUSION

Cyclospora and *Cryptosporidium* were more prevalent among HIV/AIDs patients in Cape Coast. The Species-specific PCR had a high detection rate than microscopy. Also, the a high CD4+ cells count negatively correlated to the number of opportunistic parasites among study subjects.

1. Declarations

- **Ethics approval:** The Ghana Health Service approved the study (GHS-ERC: 24/1/12). All methods were carried out in compliance with the Helsinki Declaration 2013 (64th World Medical Association General Assembly, Fortaleza, Brazil, October 2013).

2. **Consent :** All recruited study participants provided written informed consent including assent.
   - **Authors' contributions**
   - All authors contributed equally,
   - **Acknowledgement**
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