

SHORT COMMUNICATION

Cryptosporidiosis among HIV-infected patients with diarrhea in Edo State, Midwestern Nigeria

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ABSTRACT

To determine the prevalence of cryptosporidiosis among HIV infected and HIV non-infected patients with diarrhea in Edo State, Nigeria, as well as the effect of CD4⁺ lymphocyte count on the prevalence of cryptosporidial infection among the HIV patients. Stool samples were collected from 300 patients consisting of 200 HIV-infected and 100 HIV non-infected patients with diarrhea. Blood samples were collected from the HIV-infected patients. The stool samples were processed to detect *Cryptosporidium* species using a modified Ziehl-Neelsen stain, as well as other intestinal parasites using saline and iodine preparations. The blood samples were used to determine CD4⁺ lymphocyte count. The prevalence of intestinal parasites was higher in HIV-infected patients compared with their HIV non-infected counterparts (39% vs 24% respectively, $p=0.0097$). Cryptosporidiosis was diagnosed only among HIV-infected patients and was the only parasite whose prevalence was significantly different between HIV-infected and HIV non-infected patients. CD4⁺ lymphocyte count of <200 cells/ μ L among HIV-infected was a risk factor for acquiring cryptosporidial infection (OR=18.776, 95% CI=6.299, 55.964). A cryptosporidial infection prevalence of 18% among HIV-infected patients was observed and CD4⁺ count of <200 cells/ μ L was a risk factor for acquiring the disease. Routine examination of diarrhogenic stools of HIV-infected patients for cryptosporidiosis is advocated.

Keywords: *Cryptosporidium* spp., HIV

INTRODUCTION

Cryptosporidium is a ubiquitous parasite that infects a wide range of vertebrates including humans (Leav *et al.*, 2003; Xiao *et al.*, 2004). *Cryptosporidium* spp. are major causes of diarrhoeal disease in both immunocompetent and immunodeficient individuals (Hunter and Nichols, 2002) though in immunocompetent individuals, the disease is usually self-limiting, while in immunocompromised persons, it results in chronic or cholera-like diarrhea that can be fatal if not properly managed (Zaidah *et al.*, 2008). The disease is mainly zoonotic. However, transmissions through contaminated water, food and sexual contact have been reported (Ghimire *et al.*, 2004). Human immunodeficiency virus (HIV)/Acquired immunodeficiency syndrome (AIDS) patients have been reported to have high prevalence of cryptosporidiosis (Ghimire *et al.*, 2004; Adesiji *et al.*, 2007; Zaidah *et al.*, 2008), though the prevalence varies depending on location, age of the study population, stage of disease and laboratory methods used (Zaidah *et al.*,

2008). In a similar vein, the few reports in Nigeria shows conflicting prevalence depending on the region of the country and age of patients (Adesiji *et al.*, 2007). In Edo State, the Midwestern part of the country, there are no data on cryptosporidiosis and the disease is not diagnosed routinely. Against the above background as well as generation of data that will improve the management of opportunistic infections in HIV/AIDS patients, this study is aimed at determining the prevalence of cryptosporidiosis among HIV-infected and HIV non-infected patients with diarrhea as well as the effect of CD4⁺ count on the prevalence of the disease among HIV-infected patients.

MATERIALS AND METHODS

Study population

The study was carried out within the period of January, 2008 to January, 2009 at the University of Benin Teaching Hospital, a tertiary health institution with referral status

and a center for HIV/AIDS management under the President's Emergency Plan for Aids relief (PEPFAR). A total of 300 patients with diarrhea consisting of 200 HIV-infected and HIV non-infected patients were included in this study. Verbal informed consent was obtained from all patients prior to specimen collection. Approval for the study was given by the Ethical Committee of the University of Benin Teaching Hospital, Benin City, Nigeria.

Collection and processing of specimens

Stool samples and 5 mL of venous blood were collected from each HIV patient while stool samples only were collected from non-HIV patients. The stool samples were collected into clean wide-mouthed container, while the blood samples were collected into ethylene diamine tetra acetic acid (EDTA) container.

The stool samples were examined microscopically for ova and cysts of parasites using saline and iodine mounts on grease-free slides. A sample of each stool was concentrated and used to detect *Cryptosporidium* oocysts using the modified cold Ziehl Neelsen stain as previously described (Adesiji *et al.*, 2007).

The blood samples were used for CD4⁺ T-lymphocyte count. The count was determined using flow cytometry (Partec, Germany).

The data were statistically analyzed using the Chi square (X²) test and Odd ratio analysis using the statistical software INSTAT[®].

RESULTS

HIV-infected patients with diarrhea have significantly ($p=0.0097$) higher prevalence of intestinal parasites than their HIV non-infected counterparts with diarrhea (39% and 24% respectively) (Table 1). However, when considering the parasites individually, only *Cryptosporidium* species found among HIV patients had a significantly higher prevalence ($p<0.0001$) as it was not found among HIV non-infected patients. Other parasites causing diarrhea among the study population includes *Entamoeba histolytica*, *Ascaris lumbricoide*, Hookworm, *Trichuris trichuria* and *Strongyloides stercoralis*.

HIV-infected patients with diarrhea and CD4⁺ count of <200 cells/μL have approximately 6 to 56 fold increase risk of acquiring cryptosporidial infection compared with those with CD4⁺ count ≥200 cells/μL (OR=18.776 95% CI=6.299,55.964, $p<0.0001$) (Table 2).

DISCUSSION

Diarrhea is a common complication of HIV infections while *Cryptosporidium* is a well established cause of diarrhea among HIV-infected patients (Adesiji *et al.*, 2007). Cryptosporidiosis is not diagnosed routinely in our setting – Edo State, Midwestern Nigeria, perhaps, due to lack of data. This study focused on determining the prevalence of cryptosporidial infection among HIV-infected and HIV non-infected patients with diarrhea as well as the effect of

CD4⁺ count on the prevalence of cryptosporidial infection among the HIV-infected patients.

The data in this study reveals that 102 (34%) out of the 300 patients with diarrhea were infected with various intestinal parasites. HIV-infected patients have a

Table 1: Frequency of intestinal parasites among patients with diarrhea

Parasites	HIV-infected n=200 (%)	HIV non-infected n=100 (%)	p-value
<i>Cryptosporidium</i> sp.	36 (18.0)	0 (0.0)	$p<0.0001$
<i>Entamoeba histolytica</i>	8 (4.0)	2 (2.0)	$p>0.05$
<i>Ascaris lumbricoides</i>	14 (7.0)	10 (10.0)	$p>0.05$
Hookworm	5 (2.5)	7 (7.0)	$p>0.05$
<i>Trichuris trichuria</i>	3 (1.5)	1 (1.0)	$p>0.05$
<i>Strongyloides stercoralis</i>	12 (6.0)	4 (4.0)	$p>0.05$
Total	78 (39.0)	24 (24.0)	$p= 0.0097$

Table 2: Effect of CD4⁺ count on the prevalence of cryptosporidiosis among HIV-infected patients

CD4 ⁺ count (cells/μL)	Number tested	Number Positive (%)
≥200	119	4 (3.4)
< 200	81	32 (39.5)
<200 vs ≥ 200		
OR = 18.776		
95% CI = 6.299, 55.964		
$p<0.0001$		

Significant higher prevalence of intestinal parasite infection (HIV-infected vs HIV non-infected: 39% vs 24%, $p=0.0097$). Though, diarrhea is a common complication of HIV/AIDS infection (Adesiji *et al.*, 2007), the immunosuppression seen in these patients predisposes them to opportunistic infections (Ghimire *et al.*, 2004). It is important to note that the higher prevalence of intestinal parasites observed among HIV-infected patients in comparison to their HIV non-infected counterparts is due to *Cryptosporidium* sp. as none was recovered from HIV non-infected subjects. This finding is similar to that of Adesiji *et al.* (2007), with the exception of *S. stercoralis*, which was detected in this study. However, the prevalence of cryptosporidial infection in this study (18%) is lower than that of Adesiji *et al.* (2007) (52.7%) while another study in the Southeastern Nigeria did not detect any *Cryptosporidium* spp. in the stool of diarrhoeagenic HIV-infected and HIV non-infected patients (Nwokediuko *et al.*, 2002). This difference could be due to location - a known factor for observed differences in the prevalence of cryptosporidial infection (Zaidah *et al.*, 2008)

The finding of *Cryptosporidium* species among HIV-infected patients only, indicates that it is an opportunistic

parasite that readily causes infection among immunodeficient persons. Indeed, an immunodeficient state has been reported as a risk factor for cryptosporidial infection (Ghimire *et al.*, 2004). In this study HIV-infected patients with CD4⁺ count <200 cells/ μ L are at increased risk for acquiring cryptosporidial infection.

It has been reported that cryptosporidiosis occur principally at CD4⁺ count of <200 cells/ μ L (Timothy, 1994). The diagnosis of cryptosporidiosis of greater than one month duration in HIV-infected patient is taken as an AIDS defining condition (Zaidah *et al.*, 2008). Therefore, there is need to diagnose cryptosporidiosis in HIV-infected patients, in order to commence management on time so as to increase the longevity and quality of life of the affected patients.

In conclusion, this study reveals a cryptosporidiosis prevalence of 18% among HIV-infected patients with diarrhea. CD4⁺ count <200 cells/ μ L is a risk factor for acquiring cryptosporidial infection among HIV-infected patients. The routine examination of stool of HIV-infected patients for presence of *Cryptosporidium* spp. is advocated.

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