BRIEF COMMUNICATIONS

Isosporiasis in HIV/AIDS Patients in Edo State, Nigeria

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Abstract

***Background*: The role of opportunistic infections in accelerating disease progression in HIV-positive individuals, leading to quick death, is still receiving serious attention. The objective of this study was to determine the prevalence of *Isospora belli* infections in HIV-positive patients in Edo State, Nigeria between August 2007 and March 2008.**

***Methods*: A total of 268 samples from HIV-positive patients and 20 samples from HIV-negative patients were processed using the modified Ziehl-Neelsen staining technique to microscopically identify the presence of *I. belli* oocysts.**

***Results*: The overall prevalence of the coccidian was 3.1%. Gender and age had no correlation with the prevalence of the parasite (P > 0.05). There was a significant relationship between isosporiasis and CD4+ T cell counts in HIV-positive patients (OR=11.388, 95% CI= 2.797–46.371, P=0.0004).**

***Conclusions*: Routine investigation of *I. belli* in HIV-positive subjects is advocated in tertiary health institutions.**

***Keywords*:** *Isospora belli, HIV, CD4 + T lymphocytes, medical sciences*

Introduction

Human immunodeficiency virus (HIV) infection, a worldwide phenomenon, is a serious problem in the present day (1). *Isospora belli* is a coccidian protozoan parasite endemic to many regions of the world outside the United States, including the Caribbean, Central and South America, Africa and Southeast Asia. It is common in tropical and subtropical environments. The infection is common in immunosuppressed patients, particularly those with AIDS living in tropical areas. Sporadic outbreaks have occurred in mental institutions and in day-care centres in the United States (2). About a few hundred cases of human isosporiasis were described until it became an opportunistic infection in immunocompromised, predominantly human HIV-positive patients (3).

Transmission occurs via the faecal-oral route, mainly by ingestion of infectious oocysts in contaminated food and water. In immunocompetent individuals, isosporiasis is a self-limiting gastrointestinal disease characterised by watery diarrhoea (4). Some immunodeficient individuals are predisposed to severe and prolonged diarrhoea caused by opportunistic parasites, particularly *I. belli*, which is one of the most commonly identified causes of chronic diarrhoea in AIDS patients (5). Chronic diarrhoea in patients infected with HIV results in a significant morbidity and mortality, primarily caused by HIV wasting (6,7). The early detection of *I. belli* infection is very important in preventing complications and in prolonging a healthy life in HIV-positive patients. *I. belli* has recently been recognised as an opportunistic protozoan pathogen in patients with AIDS, though the coccidian rarely causes diarrhoea in patients with AIDS in the United States (8).

The objective of this study was to determine the prevalence of *I. belli* infections and the correlation between *I. belli* infection prevalence and CD4+ T cell counts among HIV-positive patients in Edo State, Nigeria.

Materials and Methods

The study was conducted at the University of Benin Teaching Hospital, Benin City, Edo State, Nigeria, between August 2007 and March 2008. The hospital is a tertiary care centre offering services to a large population from the mid-western part of Nigeria.

A total of 268 HIV-positive and 20 HIV-negative patients (131 males and 157 females) were analysed. The age of the study subjects ranged from 20 to 70 years (34.33±9.39 years). All enrolled subjects were outpatients that were on their first visit prior to HAART management. Verbal informed consent was obtained from each subject prior to specimen collection, and samples were taken only from those who indicated their willingness to participate in the study. The study was approved by the Ethical Committee of the University of Benin Teaching Hospital.

Faecal samples were collected during two different appointments separated by an interval of four months. Patients were given a universal container with 5 mL of 10% formol saline in which to put the faecal sample. The stool samples were processed using the modified Ziehl-Neelsen staining technique to identify oocysts of *I. belli* microscopically. A concentrated smear of the stool was made on a clean grease-free slide and was fixed in methanol for 3 minutes. The slide was immersed in cold carbol fuchsin and stained for 15 minutes. It was then thoroughly rinsed in tap water and decolourised in 1% acid alcohol for few minutes. After rinsing again in tap water, the slide was counterstained with 0.4% malachite green for 30 seconds (9). The slide was then air-dried and observed under a compound light microscope to look for the presence of oocysts of *I. belli*.

About 4 mL of whole blood was also collected from each patient for CD4+ T cell counts. The blood samples were analysed for CD4+ T cell counts using flow cytometry (Partec, Gmbh, Germany). Briefly, 20 µL of CD4 PE antibody and 20 µL of well mixed whole EDTA blood were put in a Partec test tube, mixed gently and incubated in the dark for 15 minutes at room temperature. The samples were mixed every 5 minutes during incubation. Eight hundred microlitres of CD4 buffer was added to each tube and the samples were mixed gently. The number of CD4+ cells in each tube was then measured with a flow cytometer.

Statistical analysis was done using the chi-square ($\chi 2$) test and odds ratio analysis. A *P* value <0.05 was considered significant.

Results

During the eight months of this study, 288 patients were examined for isosporiasis. Among these patients, 268 were HIV-positive and 20 were HIV-negative patients. An overall prevalence rate of 9 (3.1%) was observed among HIV-positive patients in this study, with 4 (3.1%) males and 5 (3.2%) females infected with *I. belli* (Table 1). There were no *Isospora* infections observed in the HIV-negative patients.

Table 1: Prevalence of Isospora belli infection according to sex

Sex	No. Examined	*I. belli* infection (%)
Male	122	4(3.3%)
Female	146	5 (3.4%)
Total	268	9 (3.4%)

Statistical analysis of the information displayed in Table 1 showed no significant association between infection with the parasite and gender (P > 0.05). Isosporiasis was only observed in HIV-positive patients, and there was a significant difference in the prevalence of *I. belli* infection between HIV-positive and HIV-negative patients (*P* < 0.001). There was a significant association between the CD4+ T cell counts and isosporiasis (OR = 11.388, 95% CI = 2.797–46.371, *P* = 0.0004) (Table 2).

Table 2: Isosporiasis in HIV-positive patients and its association with CD4+ T cell counts

CD4+ (cells/µL)	Number tested	Number positive (%)
<200	21	4(19.05)
200 – 500	247	5 (2.02)

The results showed that the highest prevalence for males (5.0%) was found in the 41–50 year age group and the highest prevalence for females (4.5%) was found both in the 20–30 and in the 51–60 year age groups. There was no significant association between isosporiasis and age group (*P* > 0.05) (Table 3).

Table 3: Prevalence rate of Isosporiasis according to age and sex

Age (years)	Males		Females	
	No. examined	*I. belli* (%) infection	No. examined	*I. belli* (%) infection
20-30	30	0	22	1 (4.5%)
31-40	45	2 (4.4%)	51	2 (3.9%)
41-50	40	2 (5.0%)	49	1 (2.0%)
51-60	11	0	22	1 (4.5%)
61+	5	0	13	0

Discussion

To our knowledge, this is the first study to be carried out on the prevalence of *I. belli* infection in HIV-positive patients in Edo State, Nigeria. The results obtained in this study can provide important information for future understanding of isosporiasis in HIV-positive patients.

The overall prevalence rate of 3.1% is higher than that found in several other studies. Wiest (9) observed a prevalence rate of 0.9% in Egypt, Fisseha recorded a prevalence rate of 1.4% in Addis Ababa (10). On the other hand, the prevalence rate we found is lower compared to some other studies. Cardoso reported a rate of 4.04% in the northern region of Sao Paulo state, Brazil (11), Mohammed observed a prevalence rate of 7.4% in Jimma, Ethiopia (12), Bialek recorded a prevalence rate of 23.9% in Tabingen, Germany (13), Escobedo noted a prevalence rate of 27% in India (14) and Ambrioise-Thomas observed an 85% prevalence rate in Haiti (15). The observed prevalence rate (3.1%) in this study is similar to that found in Brazil (4.4%) (12).

The low prevalence rate that we found may be to the result of the low abundance of this parasite in the study area. It is possible that the observed prevalence rate would be higher with the use of a diagnostic real time PCR assay for *I. belli*, as this is regarded as a gold standard in detecting *Isospora*. It has been noted that with the commencement of HAART treatment in these patients that a low prevalence may also be observed, but with our study, the subjects were those on their first visit who are yet to commence the HAART treatment. There was no significant association between isosporiasis and gender or age group ($P > 0.05$).

There appeared to be a significant difference in I. belli infection rates between HIV-positive and HIV-positive subjects ($p<0.001$). This is in agreement with work of Cardoso (11) and Mohammed (12). This could be due to a shift in the immune status of the subjects. HIV patients with CD4+ T cells counts of <200cells/μL were at increased risk of *I. belli* infection (OR= 11.388, 95% CI= 2.797–46.371, P = 0.0004). This higher *I. belli* infection rate may be the result of the low immune status of these patients, which exposes them to opportunistic infections. The effects of isosporiasis may lead to increases in morbidity and mortality in these patients.

This study has demonstrated that *I. belli* infection is prevalent in Edo State, Nigeria and, as a result, may increase the burden on HIV-positive patients.

In conclusion, routine investigation of *I. belli* infection is advocated in various tertiary hospitals as this will enhance better management of HIV-positive patients.

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