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Cryptosporidiosis in HIV infected patients with diarrhoea in Kano state, North-western Nigeria

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Cryptosporidium **is one of the agents associated with diarrhoea in human immune deficiency virus (HIV)/acquired immune deficiency syndrome (AIDS) patients. There is paucity of data in Northwestern Nigeria on the occurrence of this parasite among HIV patients. The study was to determine the prevalence of** ***Cryptosporidium*** **among HIV infected patients in Kano, Nigeria. In order to help in management of HIV related opportunistic infections. Stool samples were collected from 100 HIV positive and 50 HIV negative (control) patients presenting with diarrhoea at Murtala Muhammad Specialist Hospital, Kano (MMSH) in Northwestern Nigeria. Concentrated stool sample from each patient was screened for** ***Cryptosporidium*** **oocysts with the modified Ziehl Neelson method while direct sample was screened for other enteric parasites. The overall parasite prevalence rate in the diarrhoea patients is 22.7% (34/150) with** ***Cryptosporidium*** **spp. 4.0%,** ***Ascaris lumbricoides*** **7.3%, Hookworm 3.3%,** ***Schistosoma mansoni*** **0.7%,** ***Trichuris trichura*** **0.7% and** ***Entamoeba histolytica*** **6.7%. The parasite prevalence rate in HIV infected patients is 25% while in HIV-negative patients, the rate is 18%. The rate is significantly higher among HIV infected patients with diarrhea than among HIV negative with diarrhea (P < 0.0001). However,** ***Cryptosporidium*** **was found exclusively among HIV-infected patients. When** ***Cryptosporidium*** **prevalence was excluded from analysis, the parasite prevalence rates between the two groups was not significantly different (X^2 = 0.8002, df = 3, P = 0.8494).** ***Cryptosporidium*** **oocysts have been demonstrated in faeces of HIV infected patients attending Murtala Muhammad Specialist Hospital in Kano, Nigeria. It accounts for 6.0% of cases among this group. Hence should be considered in planning interventions aimed at optimizing management diarrhoeal diseases among HIV and other immune-suppressed patients.**

Key words: Cryptosporidiosis, human immune deficiency virus (HIV)/acquired immune deficiency syndrome (AIDS), prevalence, Northwestern Nigeria

INTRODUCTION

Cryptosporidium, an intracellular protozoan has changed from that of a rare largely asymptomatic disease to an important cause of diarrhoea in animals and humans worldwide (Flanigan et al., 1992). Reported Cryptosporidiosis prevalence is 3 to 4% in the USA (Mackenzie et al., 1994), 3.5 to 22.44% in Brazil (Rodrigues et al., 1991) and about 50% in Africa and Haiti (Colebunders et al., 1987). As in HIV infected patients, *Cryptosporidium* is the most frequent microbial cause of diarrhea, usually causing chronic bulky and intermittent diarrhoea with liquid

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non-bloody stools, accompanied by pain and abdominal colic, and a noticeable loss of weight (Kotler, 1995). In North-central Nigeria, Nwabuisi reported prevalence rate of 15.1% among children aged 0 to 14 years with diarrhoea (Nwabuisi, 2001) while a prevalence rate of 4.8% among malnourished children 0 to 5 years were reported (Bamwat et al., 2003).

In Southwestern Nigeria, 52.7% of *Cryptosporidium parvum* was reported as the cause of diarrhoea among HIV patients (Yemisi et al., 2007). Though no case of *Cryptosporidium* oocysts were detected in 2002 in Southeastern Nigeria as the cause of diarrhoea among HIV patients (Nwokediuko et al., 2002), its role as the causative diarrhoeal agent among the subjects is just emerging (Erhabor et al., 2011). In the Niger Delta of Nigeria, a prevalence of 3% was reported among HIV patients. Our study was therefore aimed at determining the prevalence and contribution of Cryptosporidiosis in HIV patients in Northwestern Nigeria in order to help in optimizing management of HIV related opportunistic infections in our environment.

MATERIALS AND METHODS

Study area

The study was conducted at Murtala Muhammad Specialist Hospital Kano, a secondary health care facility of the Kano State government between July and September, 2010. HIV/AIDS patients visited GHAIN Laboratory established by the Global HIV/AIDS initiatives Nigeria (GHAIN), Kano Zone – as part of an ongoing prospective cohort study. The hospital serves a large number of HIV/AIDS patients from within and outside the metropolis.

Subjects

One hundred HIV-infected individuals presenting with diarrhoea were recruited among the HIV – positive patients who attended GHAIN Lab/clinic routinely at 3-monthly intervals as well as at interim visits when acutely unwell, and 50 HIV-negative individuals with diarrhoea who attended outpatients' clinics of the hospital were included as controls. Patients were counseled and recruited into the study after providing written informed consent. HIV was diagnosed using a combination of an HIV-1 enzyme-linked immunosorbent assay (ELISA) and a Western blot confirmatory technique. Diarrhoea was defined as passage of more than 3 loose or watery stools in 24 h and was acute if it lasts for less than 14 days and persistent/chronic if it lasts for 14 or more days (World Health Organization (WHO), 1991).

Specimen collection

Stool samples were collected from each subject into a clean Universal bottle and transported to the medical microbiology laboratory of Murtala Muhammad specialist Hospital, Kano for analysis. Subjects were interviewed to collect relevant demographic data.

Parasitological techniques

A direct sample of the stool was made with both saline and iodine mounts on clean grease free slides and examined under the microscope first with 10× and then 40× objective lens for ova and cysts of parasites. A sample of the stool was concentrated using formol ether method concentration technique (Cheesbrough, 1999). Detection of *Cryptosporidium* oocysts in the concentrated stool was done using the modified cold Ziehl Neelsen staining technique (Smith, 1995). Briefly, a concentrated smear of the stool was made on a clean grease-free slide and fixed in methanol for 3 min. The slide was immersed in cold Carbol fuchsin and stained for 15 min. It was then thoroughly rinsed in tap water and decolorized in 1% HCl (v/v) in methanol for 10 to 15 min. After rinsing again in tap water, the slide was counterstained with 0.4% malachite green for 30 s. The slide was then air-dried and observed under the compound light microscope using 40× objective lens for the presence of *Cryptosporidium* oocysts, which was confirmed under the oil-immersion objectives as small pink to red spherules on pale green background

Data analysis

Demographic and other data obtained were analysed with a PC containing GraphPad software (GraphPad Software Inc, San Diego, USA). Significant differences between categorical variables were determined using Chi square or Fisher exact tests, and $P < 0.05$ was taken as significant value.

RESULTS

One hundred and fifty patients with diarrhoea were investigated for cryptosporidiosis and other parasites in Murtala Muhammad specialist Hospital Kano, North-western Nigeria. Total of 100 (43 males, 57 females, age range 9 to 54 years, mean age 32.04 ± 9.62) were HIV infected and 50 (22 males, 28 females, age range 9 to 56 years, mean age 28.8 ± 11.9) were HIV negative and served as controls (Table 1). Figure 1 shows the oocysts of *C. parvum* stained by the acid-fast method.

The overall parasite prevalence rate in the diarrhoea patients is 22.7% (34/150) with *Cryptosporidium parvum* 4.0%, *Ascaris lumbricoides* 7.3%, Hookworm 3.3%, *Schistosoma mansoni* 0.7%, *Trichuris trichura* 0.7% and *Entamoeba histolytica* 6.7% (Table 2). The parasite prevalence rate in HIV infected patients is 25% while in HIV-negative patients, the rate is 18%. The rate is significantly higher among HIV infected patients with diarrhea than among HIV negative with diarrhoea ($P < 0.0001$). *Cryptosporidium* spp. was exclusively detected among HIV- patients in the study. Thus there was no significant difference in term of parasitic infestation among the study groups if *Cryptosporidium* spp. was excluded from the data ($X^2 = 0.8002$, df = 3, $P = 0.8494$). The incidence of *Cryptosporidium* among HIV infected patients who had diarrhoea was 6.0%. With the highest prevalence rate in patients aged 46 to 55 years age

Table 1. Age and sex distribution of patients presenting to the MMSH with diarrhea.

Age group	HIV infected patients (test patients)			HIV Negative patients (control patients)		
	Male	Female	Total	Male	Female	Total
6-15	1	3	4	2	3	5
16-25	10	12	22	9	8	17
26-35	19	23	42	6	10	16
36-45	12	14	26	3	6	9
46-55	1	5	6	2	1	3
Total	43	57	100	22	28	50

Mean age = 28.4 ± 11.19.

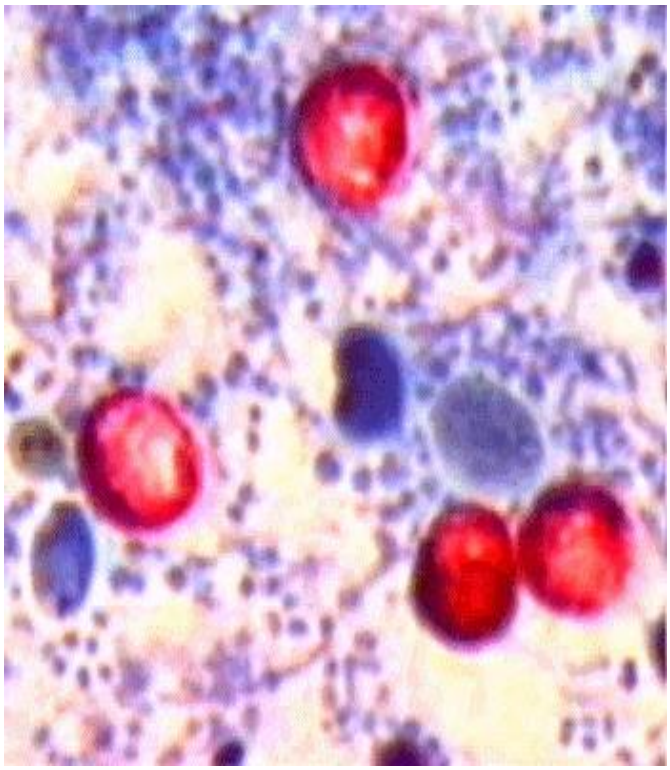

Figure 1. Oocysts of *Cryptosporidium parvum* stained by the acid-fast method.

group, it was also common among female patients (Table 3).

DISCUSSION

Diarrhoea is a significant problem in HIV infected patients in Africa (Mann et al., 1986; Mukhopadhya et al., 1999). *Cryptosporidium* is a well established cause of diarrhoea among HIV infected patients worldwide with prevalence of infection ranging from 3% in developed countries to 50% in developing countries (Goldstein et al., 1996). However, the role of this parasite in the occurrence of diarrhoea among adult patients with HIV infection in Nigeria is just emerging (Erhabor et al., 2011). Though *Cryptosporidium* was not detected in stool samples of 189 HIV infected and non-infected patients with diarrhea in Southeastern Nigeria (Nwokediuko et al., 2002). In Southwestern Nigeria, 52.7% of *C. parvum* was reported as the cause of diarrhoea among HIV patients (Yemisi et al., 2007). A 3% prevalence of *Cryptosporidium* spp. prevalence in HIV patients with diarrhoea was reported in Niger Delta, Nigeria (Erhabor et al., 2011). The reason for these differences can be related to the known fact that HIV opportunistic infections, cryptosporidiosis inclusive, tend to vary from one locality to another and from one country to the other depending on the level of contamination of water, foodstuff and contacts with animals, which are important factors in dissemination of the parasite (Widmer et al., 1996).

Cryptosporidiosis in Nigeria has been reported from tertiary institutions mainly among children in North-central (Nwabuisi et al., 1998; Nwabuisi, 2001; Banwat et al., 2003) and South-south Nigeria (Nkanginieme et al., 1996). It is similar to rates reported from other African countries (Colebunders et al., 1987) indicating that cryptosporidiosis is an important opportunistic parasitic disease causing diarrhoea among HIV infected patients in Nigeria. This is especially so as this parasite was detected in stool samples of only HIV infected patients and this further highlights the association between immunodepression and cryptosporidiosis. Although the overall parasite prevalence rate was higher among HIV infected patients when compared to HIV negative patients with diarrhoea in this study, this was mainly due to the *Cryptosporidium* prevalence of 6.0% among the HIV infected patients. It is pertinent to note that *Cryptosporidium* was not detected among non-HIV subjects. The mechanism by which *Cryptosporidium* cause diarrhoea is not well known, inflammatory response to the

Table 2. Distribution of parasites isolated from stool of patients with diarrhoea in MMSH, Kano, Nigeria.

Parasitic type	HIV – infected (n=100)	HIV – negative (n=50)	Total (%) (n=150)
Cryptosporidium spp	6	0	6 (4.0)
Ascaris lumbricoides	8	3	11 (7.3)
Hookworm	3	2	5 (3.3)
Schitosoma mansoni	1	0	1 (0.7)
Trichuris trichura	1	0	1 (0.7)
Entamoeba	6	4	10 (6.7)
Total	25	9	34 (22.7)

Fisher exact P < 0.0001. X^2 = 0.8002, df = 3, P = 0.8494 (Minus *Cryptosporidium* prevalence).

Table 3. Distribution of *Cryptosporidium* by age and sex.

Age group	Male			Female		
	No. examined	No. +ve	Prevalence (%)	No. examined	No. +ve	Prevalence (%)
6-15	1	0	0	3	0	0
16-25	10	0	0	12	0	0
26-35	19	1	5.3	23	1	4.3
36-45	12	1	8.3	14	2	14.3
46-55	1	0	0	5	1	20.0
Total	43	2	4.7	57	4	7.0

Mean age = 28.4 ± 11.19.

infection is variable and may be modified by co-pathogens such as *Cytomegalovirus*. However, histological evidence of gastrointestinal mucosa injury has been reported with clinical manifestations influenced in part by the anatomic distribution of the infection with extensive infections involving both small and large intestines producing the most severe illness (Lumadue et al., 1998).

Higher prevalence of *Cryptosporidium* was observed in females than in males. This may be attributed to higher prevalence of HIV-1 in females than males as reported elsewhere (Atzori et al., 1993) which reported females acquiring HIV-1 infection at a younger age than males. The relatively high prevalence of HIV/AIDS in women of child-bearing age is of particular concern, given the possibility of vertical transmission from mother to child and the strong association between HIV/AIDS and cryptosporidiosis. However, in other studies, the prevalence of *Cryptosporidium* was reported to be higher in males than females (Onah et al., 1998; Akujobi and Ogunsola, 2005). The study has several limitations because only a single stool specimen was examined; the prevalence of the infection may have been under-estimated (Blanshard et al., 1996). Furthermore, the role of bacterial and viral pathogens was not addressed. Finally, CD_4 cell count and HIV viral load was not determined and therefore the true level of immunosuppression is unknown.

CONCLUSION

Cryptosporidium oocysts have been demonstrated in faeces of HIV infected patients attending Murtala Muhammad Specialist Hospital in Kano, Nigeria. It accounts for 6.0% of cases among this group. It is therefore suggested that this should be considered in planning interventions aimed at managing diarrhoeal diseases among HIV and other immune-suppressed patients.

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