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Prevalence of intestinal parasites in HIV seropositive patients in infectious diseases Hospital, Kano (IDH)

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ABSTRACT: It has been increasingly recognized that enteric protozoan parasites are important in immuno-compromised individuals. These pathogens tend to aid disease progression or otherwise in such individuals. The association between intestinal parasites and HIV seropositive subjects in IDH was evaluated using fresh stool samples from 106 patients and 3op controls. Samples collected included both male and female in age ranges of 15-50. Stool samples were examined microscopically for consistency, presence of blood, mucus and adult worms. Microscopic examination was done using fresh mounts, ether-formalin sedimentation technique and modified Ziehl – Beelsen stain. *Blastocystis hominis*, *Isospora belli* were detected in 1 (2.6%) each while *Cyclospora cayentanensis* and *Cryptosporidium parvum* were detected in 2 (5.1%) and 4 (10.3) respectively in HIV infected patients with chronic diarrhoea. *Entamoeba histolytica* and *Giada lambia* were also associated with HIV patients with diarrhoea but were not exclusive with diarrhoea or HIV status. Diarrhoea is associated with intestinal parasites in HIV infected persons ($P < 0.01$). HIV, diarrhoea and presence of parasites are related.

Key Words: Human Immunodeficiency Virus (HIV); Intestinal parasites; Enteric parasites; Diarrhoea.

Introduction

Parasitic infections are important in immune compromised individuals including HIV/AIDS and enteric parasites have been implicated. Framm and Soave (1997) indicate that diarrhoea occurs in 30-60% of AIDS patients in developing countries and in about 90% of AIDS patients in Africa and Haiti. The etiology for such diarrhoea coule be either parasitic, bacterial, fungal, enteric virus or HIV itself may contribute to the diarrhoea. In addition to microbes, other factors such as medication, immune deregulation, autonomic dysfunction and nutritional supplementation play substantial role in diarrhoea of HIV/AIDS patients (Harris and Beeching, 1991; Essex *et al* (1994) and Framm and Soave, 1997). Pathogens include opportunistic agents that consistently cause severe, chronic or frequent gastro-intestinal disease and non-opportunistic agents that usually cause acute, treatable diarrhoea illness (Smith *et al*., 1988; Fleming, 1990; Gilks and Ojoo, 1991; Gurerrant and Boback, 1990; Harries and Beeching, 1991; Mohandas *et al*., 2002).

Several species of protozoa have been associated with acute and chronic diarrhoea in HIV diseases. These include *Cryptosporidium parvum*, *Isospora belli*, *Microsporidia* species, *Giardia intestinalis*,

Entamoeba histolytica, Cyclospora species, *Blastocystis hominis* and *Dientamoeba fragilis*, but convincing evidence isd lacking as to the causality of the last two protozoans. besides these, the *Strongyloides stercoralis* can cause diarrhoea and overwhelming infestation (hyper infection syndrome) in patients with a variety of immuno suppressive disorders including HIV/AIDS, Enton *et al* (1991); Essex *et al* (1994); Pollock and Farthing (1997) and Thomas (2001).

Materials and Methods

The study was carried out between January to April, 2004 at Infectious Diseases Hospital (IDH). IDH is located at Weather head Street in Sabongari Area, Fagge Local Government Area "L.G.A." in Kano, Kano State. The choice of IDH was made because it is a referral hospital for HIV/AIDS patients in Kano.

Study Design: A cross-sectional study was conducted on HIV infected and HIV non-infected patients in order to determine the prevalence of intestinal parasites and their association with diarrhoea of HIV seropositive patients. Serological test for HIV antibodies was done using ELISA tests kits by medical laboratory Scientists at the Hospital Laboratory. Clinical information was obtained from the study subjects by verbal interview and from patient cards and recorded in record schedule which included serial number, sex, age, nature of stool, HIV status parasites detected and date. All stool samples were processed for investigation at the IDH Laboratory.

Patients Selection: For this study, both out-patients and in-patients stool samples were investigated. Seropositive HIV persons stool specimens were obtained during ward rounds in the case of in-patients. Out-patients who came for HIV test were included in the study and were considered controls if such subjects later tested negative. For all out-patients, all stool samples for stool parasitology investigations were processed in the laboratory without prior knowledge of the study subjects HIV status, so that each stool sample had equal emphasis during laboratory investigations. From each patient, multiple sufficient stool samples (a sample each, for three consecutive days because oocyst excretion is usually low and variable) were requested for parasitology investigation. Chronic diarrhoea was defined as three or more loose stools passed daily for more than two weeks (Harries and Beeching, 1991).

Stool Examination: All stool specimens were examined for possible parasites. Parasitic infections were diagnosed by examination of stool specimens as fresh wet mounts, formol-ether and modified acid fast stain (AFS). fresh stool specimens were examined as saline wet mount to detect mobile trophozoites. Direct examination of stool may not always be able to reveal presence of a parasite; if they are present in small numbers. The concentration technique increases the ability to detect protozoan cyst and helminth eggs and larvae by decreasing the amount of background materials in the preparation and by an actual concentration of organisms (Reynolds, 1998). Air dried smears from concentrated stool samples were stained by modified AFS to detect *Cryptosporidium Isospora* and *Cyclospora* species.

(i) Fresh Mount

A drop of sodium chloride is put on a slide, one on the middle left half of the slide and the other on the middle right half. This provides a double mount for the specimen, one is unstained (saline) and the other stained (iodine).

Both are covered with cover slips. They are then observed in turns under low dry power objective (x10) for screening and then high dry power objective lens (x 40) to examine trophozoites.

(ii) Formalin – ether centrifugal method

This method is more efficient than the simple sedimentation method. Formalin treatment preserves the morphology of parasite components and ether treatment dissolves the fatty substances. As a result, the parasitic components are set free and they settle at the bottom of the llighter density organic solvent. The procedure followed is as described by Reynolds (1998).

(iii) Modified AFS

- A thick smear from concentrated stool sample is made and air dried.
- The slide is fixed in methanol for 3 minutes
- It is then stained with carbol fushin and left standing for 10 minutes
- It is decolourised in acid alcohol (1% HCL in ethanol) for 3 minutes.
- After these, it is washed in runningwater and air dried
- *Cryptosporidium, Isospora* and *Cyclospora* speciescysts are then observed under oil immersion.

Results

During the 4-month study period, 136 subjects were examined for intestinal parasites, of which 106 tested positive for HIV/AIDS and 30 controls were included. Tables of result obtained are presented below.

Table 1: Age and sex distribution of HIV seropositive and non-seropositive subjects in IDH, 2004.

Age Group	**HIV seropositive = 106**		**HIV non-seropositive = 30**	
	Number (%)		**Number (%)**	
	Male	**Female**	**Male**	**Female**
15 – 19	3 (2.8)	1 (0.9)	4 (13.3)	1 (3.3)
20 – 24	8 (7.5)	3 (2.8)	3 (10.0)	2 (6.7)
25 – 29	24 (22.6)	12 (11.3)	8 (26.7)	3 (10.0)
30 – 34	32 (30.2)	9 (8.5)	4 (13.3)	1 (3.3)
35 – 39	2 (1.9)	1 (0.9)	2 (6.7)	1 (3.3)
40+	11 (10.4)	0 (0.0)	1 (3.3)	0 (0.0)
Total	**80 (75.4)**	**26 (24.6)**	**22 (73.3)**	**8 (26.6)**

The age and sex distribution of the study presented in Table 1 shows that the age range 30 – 34, is the most predominant in overall size. The number of females is much lower in comparison to the males, markedly less than the proportionate values given for Nigeria (FMOH, 1999). This is partly due to the fact that there are more male wards than female in the Hospital (3 to 1) and perhaps fewer women turning up for HIV tests.

Diarrhoea is more common in HIV positive patient than in HIV non-infected subjects, 36.8% and 20% respectively. Irrespective of diarrhoea, the general prevalence of parasites in HIV infected and non-infected are 16.7% and 13.3% respectively. Among the 106 infected patients, 25(23.6%) and 14(13.2%) had chronic and acute diarrhoea respectively. Intestinal parasites were detected in patients with or without diarrhoea and include both protozoans and helminths. *Blastoystis hominis* and opportunistic coccidian parasites i.e. *C. pavum, I. belli* and *C. cayetanensis* oocysts were detected only HIV patients with chronic diarrhoea. Multiple parasites were detected in 5 cases of Hiv patients in combination of 2 and 3, while in controls this was observed once (three). Chi-tests show that there is relationship between diarrhoea and intestinal parasites in HIV patients (P 0.01) and a relationship exists between HIV status, diarrhoea status and presence of intestinal parasites (P 0.001).

Table 2: Prevalence of intestinal parasites among HIV seropositive subjects in relation to their diarrhoea in IDH, 2004.

	Status of diarrhoea HIV seropositive			**Status of diarrhoea HIV negative**		
	Number (%)	**Number (%)**		**Number (%)**	**Number (%)**	
		Parasite+	**Parasite-**		**Parasite+**	**Parasite-**
Acute	12 (10.4)	4 (33.3)	8 (66.7)	4 (13.3)	1 (25)	3 (75)
Chronic	27 (26.4)	8 (29.6)	19 (70.4)	2 (6.7)	1 (00)	2 (50)
No diarrhoea	67 (63.2)	5 (7.5)	62 (92.5)	24 (80.0)	3 (12.5)	21 (87.5)
Total	**106 (100)**	**13 (16.3)**	**67 (83.7)**	**30 (100)**	**4 (13.3)**	**26 (86.7)**

Key:
Parasite+ = Parasite present
Parasite- = Parasite absent

Table 3: Prevalence of specific intestinal parasites among HIV seropositive and HIV negative subjects in their diarrhoea status in IDH, 2004.

Parasites detected	**Diarrhoea status**						**Total**
	Acute		**Chronic**		**No diarrhoea**		
	+	-	+	-	+	-	
Ascaris lumbricoides	2	1	1		3	1	8
Hook worm	1				2	1	4
Hymenolepsis nana	1				2	2	5
Entamoeba histolytica	2		1		1	1	5
Giardia lamblia	1		1				2
Blastocytis hominis			1				1
Cryptospium parvum			4				4
Isospora belli			1				1
Cyclospora cayetanensis			2				2

Table 4: Prevalence of intestinal parasite in HIV positive individuals and their correlation with diarrhoea.

Parasite detected	Number	Diarrhoea (%)
Ascaris lumbricoides	6	3 (50)
Hook worm	3	1 (33)
Hymenolepsis nana	3	1 (33)
Entamoeba histolytica	4	3 (70)
Giardia lamblia	1	1 (100)
Blastocytis hominis	1	1 (100
Crystosporidium parvum	4	4 (100)
Isospora belli	1	1 (100)
Cyclospora cayetanensis	2	2 (100)

Discussion

In this study, HIV prevalence is higher in the 30 – 34 age group range. This agrees with the 1999 sentinel survey in which the 30-34 age bracket has the highest prevalence range in the North West and the North East (FMOH, 1999(but is different from the nationwide prevalence on age group which indicate that HIV is most wide spread among adult age 20 to 24 with recorded prevalence rate of 8.1% (i.e. prevalence in each age group) in 1999 (FMOH, 1999). The association of HIV with pulmonary tuberculosis is synergistic majority of in-patients (over 90%) had tuberculosis.

The findings of this study shows that pathogenic and opportunistic intestinal parasites can be detected in HIV/AIDS patients with diarrhoea. This study is consistent, although to varying degrees with studies conducted by other workers such as Mengesha (1994) and Fisseha *et al* (1998) in Addis Ababa, Swankamb *et al* (1987) in Uganda and Mohands *et al* (2002) in Northern India among others. Among the parasites *Blastocystis hominis* and *I. belli* were detected in 1(2.6%) each, *C. parvum* 4(5.1%), *G. lamblia* 1(2.6%)*; E. histolitica* (7.7%) in HIV infected parents with diarrhoea respectively. There is paucity of data for comparison in case of *B. hominis*(2.6%). This is lower than 7.5% recorded by Mohandas *et al* (2002). *B. hominis* is still controversial and some workers still doubt its pathogenicity while others suggest it may be a commercial that may become opportunistic (Editorial, 1991). prevalence of *C. parvum* is similar tyo studies conducted by El-Naga *et al* (1998) (10%), Escobedo and Nunez (11.9%) (1999). Other studies report a magnitude of (15 – 48%), Swankamb *et al* (1987), Colebounders *et al* (1998), Fleming (1990), Mengesha (1994), Tarimo *et al* (1996) and Mohandas *et al* (2002). It is also known that many patients develop *Cryptosporidium* associated disease at the advanced stage of HIV infection or AIDS.

Some of these studies which had high figures were conducted on AIDS patients only while in this study was conducted on HIV seropositive patients irrespective of whether it has progressed to full blown AIDS or not. *I. belli* (23.5%) closely agrees with 3% recorded by El-Naga *et al* (1998) but is higher than 1.4% in Addis Ababa (Fisseha *et al*., 1998) 1.5% in Cuba (Escobedo and Nunez, 1999) and (Moahandas *et al*., 2002) in Northern India. Other researchers recorded higher figures such as (7.4%) by Colebounders *et al* (1998) in Zaire and Awole *et al* (2003) in Jimma, Ethiopia respectively. Yet higher figures were recorded (10-15%) by Swankamb (1987), pape (1989) and harries and beeching (1991). Prasand (2000) reports about 27% in India while Ambriose (2001) recorded in 85% of AIDS patients in Haiti. *Cyclospora cayetanensis* with 2(5%) is higher than 3% (Mohandas *et al*., 2002) and 2.2% (Tarimo *et al*., 1996). *C. parvum, I. belli* and *C. cayetanensis* are coccidians, which are causes of opportunistic infections that are consistently found in HIV/AIDS patients with chronic diarrhoea as also confirmed in this study. On the other hand, in immunocompetent adults, *C. parvum* and *C. cayetanensis* are often associated with water borne outbreaks of acute diarrhoea/illness (Thomas, 2001)_. Mohandas *et al* (2002) recorded *C. parvum* in

an HIV negative control without diarrhoea. I do not know of any reported case of water borne *C. parvum* and/or *C. cayetanensis* in kano or Nigeria. The other protozoan infections i.e. *G. lablia* and *E. histolytica* have not been found to be opportunistic in HIV infected patients because there is no evidence for increased prevalence in other studies conducted. In this study, *E. histolytica* was detected in both diarrheic and non-diarrheic stools of HIV patients. Although it was detected in a higher number of cases with diarrhoea of varying severity, its presence in a control non-diarrheic indicate that it is not exclusive to HIV and/or diarrhoea alone. There is also no evidence of altered natural history of these parasites infections in HIV patients in spite of the fact important immune deficiencies against them may be expected to be deranged by HIV infection. Hence, exposure to *G. lamblia* and *E. histolytica* are likely to occur independently of Hiv infection. Heavier parasite loads may accumulate as well as delayed clearances of these parasites in individual with concurrent HIV induced immune suppression. They can cause diarrhoea and intestinal absorption regardless of patients' HIV status, Gurerrant and Boback (1990); Pollock and farthing, 1997 and Cox (2001).

This study was made with a light microscope which can not distinguish between *E. histolytica* and *E. dispar* which are morphologically indistinguishable. New and sophisticated techniques such as polymerase chain reaction (PCR) techniques, isoenzyme analysis and antigen detection are necessary for specific indentification. Although the appearance of characteristic *Giardia* cysts in stool still remaines the principal method of diagnosis, more recently the detection of salivary 1gA antibodies to *Giardia* has been shown to have a good correlation with the presence of cysts in stools of children and appear much more sensitive than faecal examination (Hashkes, *et al*, 2003). *Strongyloides stercoralis* and *Schistosoma species* were not detected in the present study. New evidences suggest there is a close association. In Jamaica, a strong association was shown to exist between HIV infection and parasitologically proven *S. stercoralis* (Monica, 2000 and Robinson *et al*., 1990). However, in HIV infected, hyperinfection syndrome has been noted to be very rare, Fleming (1990). Evidences indicate that there is an association between schistosomiasis and HIV/AIDS (Texeira *et al*., 1998).

Previous studies have not associated presence of parasites in diarrhoea with specific CD4+ counts although Mohandas *et al* (2002) report an exception in *G. lamblia* infections, in which infections appear to associated CD4+ counts less than 30 cell/mm^3.

The number of parasites detected in this study is likely to have been higher because in-patients are placed on anti-viral medication which obviously improves immune capacity and this can clear opportunistic infections or reduce parasite loads. Nurses report that most patients who had a history of diarrhoea which stopped when they were placed on this medication. In addition, flagyl is also administered on some of the patients which is effective against *Giardia, E. histolytica* and *B. hominis* (Martinezx-Palomo, 1993).

Conclusion

This study highlights the importance of testing for intestinal parasites in patients that are HIV positive and the need to increase awareness of clinicians regarding the occurrence of these parasites in this population. It also confirms that opportunistic protozoan infections occur in cases of chronic diarrhoea in HIV/AIDS patients. Studies like these can often guide therapy when resource limitations hamper the exact diagnosis of etiological agent in HIV associated diarrhoea. Since most opportunistic infections are treatable except cryptosporidiosis, information on prevalence can be very valuable to clinicians as prevalence differ from place so that this can be put into consideration during prescription of treatment regimen.

Recommendations

Because the etiologic agent could not be identified in a large number of patients, it is suggested that further comprehensive etiological studies should be conducted in future including viral, fungal, bacterial and parasitic causes of diarrhoea not included in this study.

Limitations of Study: Presence of microsporidia spores such *Enterocytozoan bieneusi*could not be determined because of lack of reagents i.e. modified trichrome stain. Also CD4 counts were not available for most patients as result correlation could not be made with diarrhoea and parasitosis.

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