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***CRYPTOSPORIDIUM PARVUM* AND *ISOSPORA BELLII* INFECTIONS AMONG PATIENTS WITH AND WITHOUT DIARRHOEA**

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ABSTRACT

Objective: To assess the importance of *Cryptosporidium parvum* and *Isospora belli* infections as a cause of diarrhoea among patients admitted to the Medical Wards in Queen Elizabeth Central Hospital (QECH) in Blantyre, Malawi.

Design: Prospective case control study.

Subjects: One hundred and twenty one patients with diarrhoea and 122 patients without diarrhoea.

Main outcome measures: Demonstration of *C. parvum* and *I. belli* oocysts by examination of at least one stool sample per patient using phenol auramine-O-fluorescence staining and an immuno-fluorescent assay with monoclonal antibodies against *Cryptosporidium*, seropositivity for HIV and AIDS.

Results: In 22% of the patients with diarrhoea an infection with *C. parvum* or *I. belli* was found. Thirteen (11%) of them had a *C. parvum* and 14 (12%) an *I. belli* infection; a mixed infection was found in one patient. In the control group, three (3%) *C. parvum* and three (3%) *I. belli* infections were seen. The prevalence of both infections was very significantly higher in the cohort of diarrhoea patients than in the controls, 13/108 versus 3/119 ($p=0.0099$) for *C. parvum*, and 14/107 versus 3/119 ($p=0.0056$) for *I. belli*. Infections were only seen in HIV positive patients. Two hundred and four (84%) patients were HIV positive and 145 (60%) of them had AIDS.

Conclusions: *C. parvum* and *I. belli* infections are a significant cause of diarrhoea among medical in-patients at QECH. Examinations of stool specimen for parasites among hospitalised patients with diarrhoea provide data for a more appropriate management.

INTRODUCTION

Diarrhoea is a common clinical problem in tropical areas and may be caused by many pathogens. The acquired immunodeficiency syndrome (AIDS) is commonly associated with diarrhoea and weight loss. In developing countries 95% of the HIV infected patients develop episodes of diarrhoea during the course of their disease. A wide range of pathogens has been found, but in 50% of the patients with chronic diarrhoea no gut pathogens were detected (1-3). *Cryptosporidium parvum* and *Isospora belli* have been recognised as opportunistic enteric pathogens in patients with AIDS(4). Previous studies of African patients with AIDS showed the prevalence of *C. parvum* to be between 7 and 31 % and of *I. belli* between 5 and 19 %(5).

In immunologically competent individuals these infections cause an acute self-limiting diarrhoeal illness, but in immunocompromised patients these infections may become chronic and may lead to severe and even life-threatening conditions. The typical presentation is profuse watery diarrhoea without blood. Other less frequent symptoms include abdominal pains, nausea, and vomiting

and low-grade fever. Dehydration can easily occur, especially in children.

For *Cryptosporidium* enteritis, no effective therapy is currently available. At present paromomycin is widely used but trials showed only modest improvements and no cure(6, 7). Paromomycin plus azithromycin showed good response in terms of diminishing clinical symptoms and a decrease in oocyst excretion(8). Symptomatic treatment with rehydration and anti-diarrhoeal agents as loperamide, bismuth sub-salicylate and nutritional supplements are presently the cornerstone of management. Isosporiasis can be effectively treated with co-trimoxazole (9). Prophylaxis with co-trimoxazole in AIDS patients is now recommended by UNAIDS to prevent diarrhoea by *Isospora* infections as well as other infections such as toxoplasmosis cerebri, pneumocystis carinii pneumonia and bacterial infections(10).

No studies have been carried out in Malawi on the aetiology of diarrhoea, although it is a frequent reason for hospitalisation. In absence of laboratory services, current guidelines for treatment of chronic diarrhoea in the Department of Medicine include symptomatic treatment with co-trimoxazole, albendazole and metronidazole in

addition to supportive treatment. The objective of this study was to estimate the prevalence of *C. parvum* and *I. belli* infections among patients admitted to the medical wards.

MATERIALS AND METHODS

Between May and August 2001, patients of the non-paying medical wards of the Queen Elizabeth Central Hospital (QECH), Blantyre, were enrolled in the study immediately after admission. All patients who presented with diarrhoea (defined as "loose to watery stools, more than three times a day, for more than three days") were included. All patients not showing diarrhoea on admission were included as controls. This was continued until a similar number as for the diarrhoea group was reached. Patients presenting with 'acute diarrhoea' (diarrhoea shorter than three days) and patients unable to communicate or those that were severely constipated were excluded.

Patients were fully informed about the aim of the study and written informed consent was obtained. The patients were pre-counselled for HIV testing according to current guidelines in the hospital. Patients were informed about the results of the test unless they indicated otherwise. A patient record was completed and included name, age, sex, urban or rural place of residence, stool description (loose to watery, bloody, duration of having loose stools), and reason for admission. The patients were instructed to collect two samples from different stools. The first stool sample was obtained before any chemo therapy started. After collection the stool samples were stored at +4°C and processed the next morning.

The "Expanded WHO case definition for AIDS surveillance" was used to decide whether the patient met the criteria for the diagnosis of AIDS(11). The other patients were designated as "HIV-positive but not AIDS" or "HIV-negative".

Blood samples were centrifuged at 3000 r.p.m. (1600g) for five minutes. Serum was tested with a rapid test (HIV-SPOT kit, Genelabs Diagnostics, Singapore Science Park, Singapore or Capillus™ HIV Type 1/Type 2, Cambridge Diagnostics Ireland, Galway, Ireland) and confirmed with ELISA (Vironostika® HIV Uni-form II plus O, Organon teknika BV, Boxtel, The Netherlands).

All stool samples were investigated for *Cryptosporidium* and *Isospora* using phenol auramine-O-fluorescence staining and fluorescence microscopy(12). Two investigators examined each slide. In case of disagreement a new slide of the stool was prepared and examined. In every staining procedure a positive control slide was processed. Observations were verified by examination of one in every five samples in an immuno-fluorescent assay with the use of monoclonal antibodies against *Cryptosporidium* (Crypto-cel IF test, Cellabs, Brookvale, Australia). All phenol auramine-O-positive readings were also assayed in this test for confirmation. Fisher's exact test was performed with the Instat® package V2.05a (Graphpad Software, San Diego, CA, USA) in order to substantiate differences between data. P-values<0.05 were considered significant.

Ethical approval for the study was obtained from the College of Medicine Research Committee.

RESULTS

From 354 patients that were initially included in the study, 249 submitted at least one stool sample. Six patients with acute diarrhoea were excluded from the study. Two stool samples were obtained from 187 patients. There were 121 diarrhoea cases and 122 controls. Of these 243 patients, 203 (84%) were HIV positive and 145 (60%) of them had AIDS.

Table 1

Demographic and clinical characteristics of 243 patients enrolled in the study

Characteristic	Patient group				P-value ^a
	Diarrhoea		Control		
Number of patients providing one stool sample	121	(100%)	122	(100%)	
Number of patients with additional 2nd stool sample	96	(79%)	91	(75%)	n.s.
Number of male/female patients	48/73		58/64		n.s.
Number with urban/rural residency	73/48		70/52		n.s.
Overall age of patients: median (range)	32 (13-65)		35 (18-70)		
Age of male patients: median (range)	35 (21-60)		35 (18-70)		
Age of female patients: median (range)	32 (19-65)		35 (20-64)		
Number of HIV positive patients	107	(88%)	96	(79%)	n.s
Male/female ratio	41/66		45/51		n.s
Number of patients diagnosed AIDS ^b	80	(66%)	65	(53%)	0.0499*
Total Number of <i>C. Parvum</i> infections	13 ^d	(11%)	3 ^c	(3%)	0.0099%**
In patients with diagnosis AIDS	11 ^e		3		
In patients HIV-positive but not AIDS ^b	2		0		
In HIV negative patients	0		0		
<i>C. parvum</i> infection detected with 2nd stool sample	1		1		
Total number of <i>I. belli</i> infections	14 ^d	(12%)	3	(3%)	0.0056**
In patients with diagnosis AIDS	10		2		
In patients HIV-positive but not AIDS	4		1		
In HIV negative patients	0		0		
<i>I. belli</i> infections detected with 2nd stool sample	3		1		

a Fisher's Exact test (2-sided) *P-value=significant; **P-value=very significant; n.s= not significant

b Including three patients with HIV test not done, but meeting the WHO AIDS case definition

c Including one patient only positive in monoclonal antibody immuno-fluorescent assay.

d Including one patient with a mixed infection of *C. parvum* and *I. belli*.

e Including one patient with HIV test not done, but meeting the WHO AIDS case definition.

No differences were established between the groups with regard to the number of samples, gender, age, urban or rural residency or the number of HIV positive patients. The number of patients with the diagnosis AIDS was higher in the diarrhoea patients group than in controls. In stools of 16 patients *C. parvum* oocysts were seen. Fourteen of them were found to be positive after examination of the first stool sample and the other two were detected after examination of the second sample. In the stool of one patient oocysts were detected by use of the monoclonal antibody test only. Fourteen patients with cryptosporidiosis met the WHO case definition for AIDS; two patients with *C. parvum* infections in the diarrhoea patient group were designated as HIV-positive but not AIDS.

Stool samples of 17 patients showed oocysts of *I. belli*. Four (24%) of them became positive after investigation of the second sample. Twelve patients with *I. belli* infection met the WHO case definition for AIDS; five were designated as HIV-positive but not AIDS. *C. parvum* and *I. belli* infections were only detected in HIV positive patients. One patient had oocysts from both parasites in the stools. In 26 of 121 (22%) patients with diarrhoea *Cryptosporidium* or *Isospora* infection was present. The prevalence of both infections was very significantly higher in the cohort of patients with diarrhoea than in the control group, 13/108 versus 3/119 ($p=0.0099$) for *C. parvum* and 14/107 versus 3/119 ($p=0.0056$) for *I. belli*.

DISCUSSION

This study shows that *C. parvum* and *I. belli* infections are important causes of diarrhoea in medical in-patients at QECH as in 22% of patients infection with either parasite was present. Both infections were only found in HIV positive patients. The two parasites are recognised as a serious threat in HIV infected persons and it has been suggested that these infections have a more profound effect in African HIV-infected individuals than those reported in HIV-infected patients in industrialised countries(13). The 11% of *C. parvum* and the 12% of *I. belli* gastro-enteritis in HIV positive adults that was found in this study is in accordance with the range found in other studies(5, 14). The significance of the parasites in the aetiology of diarrhoea was also emphasised in four recent studies of patients with HIV- 1 or HIV-2 infection from sub-Saharan Africa. These studies showed infection rates of 5, 8.2, 9 and 25 % for *C. parvum* and 1.1, 2, 4.4 and 11% for *I. belli* (15-17).

The contribution of examination of a second stool sample was considerable: 13% (2 of 16) for *C. parvum* and 24% (4 of 17) for *I. belli* were detected only after a second stool sample was obtained. This higher diagnostic yield indicates the relative sensitivity of examination of one specimen versus the study of multiple samples in a patient cohort with a high clinical index of suspicion(18). For routine clinical practice in sub-Saharan Africa one should consider to base the diagnosis of cryptosporidiosis and

isoporiasis in HIV-infected patients on the examination of at least two stool specimens if the first specimen is negative. A small number of HIV patients of the control group had an asymptomatic infection with either *C. parvum* or *I. belli* (both 3%). It was not clear if these patients would become symptomatic in the future. Transient or asymptomatic *Cryptosporidium* infections tend to be associated with higher CD4 counts and survival was found to be related to clinical presentation(19, 20). Also the extent to which these asymptomatic infections contribute to transmission of the disease is unknown.

Season and urban or rural living conditions can influence the prevalence of *Cryptosporidium* and *Isospora* infections. A seasonal influence has been reported on the number of *Cryptosporidium* infections(21). Our study was performed during the dry season and therefore the number of infections might be different in the rainy season. Differences in sanitation and water supply or contact with animals may also play a role in prevalence of *C. parvum*, since the transmission is thought to be through ingestion of food or water contaminated with sporulated oocysts. Differentiation of isolates into the various described genotypes of *Cryptosporidium* may elucidate the infection routes(22, 23). Most patients were from the vicinity of the hospital, but no differences were found in the number of infections between patients from rural and urban residency. For *I. belli* humans are supposed to be the only reservoir. Further research is needed to elucidate the role of the patients in transmission of their infections to other household members.

The number of patients diagnosed with AIDS was higher in the cohort of patients with diarrhoea than in the control group. None of the other parameters did differ between the groups. Diarrhoea is frequently found in HIV infection and it has been shown that CD4 counts are significantly lower in individuals with diarrhoea than in those without (2,3). In the present study the facility to obtain CD4 counts was not available, but since the presentation with diarrhoea on admission directed the inclusion process we assume that lower CD4 counts would have been found in the cohort of patients with diarrhoea than in controls. Therefore, the higher number of AIDS patients in the diarrhoea group was not an unexpected finding and merely a reflection of the difficulties one encounters in these studies to define appropriate controls.

However, the observed association between *C. parvum* or *I. belli* infection and diarrhoea (13/108 versus 3/119, $P = 0.0099$ and 14/107 versus 3/119, $P = 0.0056$, respectively) clearly identify these two parasites as an important cause of diarrhoea. Therefore the need for stool examination for parasites in HIV infected patients with diarrhoea is emphasised. In the medical wards of QECH stool examination is often not available. Patients with diarrhoea receive supportive treatment and sequential empirical treatment with co-trimoxazole, albendazole and metronidazole, according to the patients response. Our results show that examination of stool specimen provide

data for a more appropriate management in patient care. This study also provides support for the UNAIDS recommendation for co-trimoxazole prophylaxis for *I. belli* infection in HIV-infected patients (9).

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