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OPPORTUNISTIC INTESTINAL PARASITIC INFECTIONS IN HIV SEROPOSITIVE PATIENTS

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ABSTRACT

Human immunodeficiency virus (HIV) seropositive patients usually suffer from opportunistic intestinal parasitic infections leading to high mortality & morbidity, knowledge about the etiological agents in HIV can often guide the treatment. The aim of this study was to identify the opportunistic parasites in HIV associated diarrhoeal and non-diarrhoeal patients and to study the importance of stool examination in early detection of these parasites for appropriate therapy. Stool samples from 75 HIV seropositive cases with diarrhoea, and 35 HIV seropositive cases without diarrhoea, were examined by different microscopic methods for the detection of opportunistic intestinal parasites. Statistical analysis was done using Chi square test. The overall prevalence of opportunistic parasitosis in this study was 21.8%. The predominant opportunistic parasites detected in diarrhoeal patients were Cryptosporidium parvum (C. parvum) 17 (22.67%), followed by Isospora belli 2 (2.67%) and among non-diarrhoeal patients only 2 cases (5.72%) were positive for C. parvum. Out of 24 parasites detected, majority of the parasites were found in the age group of > 50 years i.e. 8 (33.33%) followed by the age group of 0 years -10 years 7 (29.16%). Modified Ziehl Neelsen method was observed to be the most sensitive method to detect coccidian parasites. It is recommended that stool samples of HIV seropositive patients should be checked routinely for Opportunistic intestinal parasites like C. parvum in order to institute appropriate treatment.

KEYWORDS: *HIV seropositive patients, Opportunistic intestinal parasites, Cryptosporidium parvum, Isospora belli.*

INTRODUCTION

Opportunistic infections pose one of the most important public health problems in Human immunodeficiency virus (HIV) seropositive patients because of decreased immune status. [1] The most common presenting feature in HIV infected individuals is diarrhoea. [2] Several intracellular intestinal protozoa cause opportunistic infections in AIDS and are the major cause of uncontrollable and debilitating diarrhoea in developing countries. These parasites are associated with the morbidity and mortality in AIDS patients worldwide, these outcomes would be expected to be higher in developing countries due to higher prevalence of infections in the general population. [3] It has been estimated that 30 to 60% of HIV infected individuals in developed countries and 90% of individuals with HIV in developing countries develop diarrhoea sometime during their clinical course. [4] Studies have shown that HIV infected patients with chronic diarrhoea and occult enteropathogens often have a longer duration of AIDS, a greater mean weight loss & a significant shorter survival than those without pathogens. [5]

Studies in India [6, 7] have highlighted the emergence of important gastrointestinal coccidian parasites like *Cryptosporidium, Isospora belli (I. belli), Cyclospora & Microsporidium species* in HIV infected individuals. These parasites can cause uncontrollable and debilitating diarrhoea in developing countries. As these infections can be treated with appropriate therapy, early identification of these etiological agents of diarrhoea in HIV infected patients is pivotal. This can save the patients from going to the incidence of mortality. Hence the present

study was undertaken to identify opportunistic intestinal parasitic infections in HIV infected individuals in our setup and to study the importance of stool examination in early detection of these parasites for appropriate therapy.

MATERIALS AND METHODS

A cross sectional study was conducted on 110 HIV seropositive patients attending inpatients and outpatients of *JJM Medical College and* Chigateri General *Hospital, Davanagere, Karnataka.* HIV tests were performed after pretest counseling with informed and written consent and in accordance with the institutional ethical guidelines. HIV seropositive patients were defined as one who had tested positive for HIV antibodies as per National AIDS Control Organization guidelines. HIV seronegative patients were excluded from this study. The patients having passage of three or more loose stools a day was taken as having diarrhoea. Of 110 patients, 75 were suffering from diarrhoea and 35 were without diarrhoea. Fresh stool samples were collected in clean, dry, wide mouthed plastic containers with tight fitting lids.

Stool samples were examined by saline wet mount, iodine preparation, modified Ziehl Neelsen (MZN) [8, 9] stain and Lactophenol cotton blue (LPCB) [10] staining. Saline wet mount was employed for the demonstration of motile trophozoites, larvae, cysts, ova etc. Iodine preparation was used particularly for nuclear characters and glycogen mass. MZN and LPCB were employed to stain and detect oocysts of Cryptosporidium, I. belli and other coccidian parasites in stool.

When the parasites were scanty in the stool routine microscopy or when the stool samples were negative for parasites, concentration techniques were used to concentrate the parasites or to identify the missed one. Both floatation and sedimentation techniques were used for the concentration of the parasitic eggs/ova/cysts. After concentration techniques the stool samples were re examined by saline wet mount, iodine preparation, MZN and by LPCB method. Statistical analysis was done using Chi square test.

RESULTS

Out of 110 HIV seropositive patients, 24 (21.8%) were found to be infected with opportunistic intestinal parasites. Out of 75 diarrhoeal cases studied, the parasites were detected in 22 (29.34 %) cases and in 35 non-diarrhoeal HIV cases; the parasites were detected in 2 (5.72%) cases. (**Table -1**) Statistical analysis was done using Chi square test about diarrhoeal Vs non-diarrhoeal cases, P value was 0.01.

Out of 110 patients studied, 19 (17.28%) were infected with *Cryptosporidium parvum* (C. parvum) [**Figure - 1**] followed by *Isospora belli (I. belli)* [**Figure – 2**] 2 (1.8%). Out of 75 diarrhoeal cases studied, 17 (22.67%) were of *C. parvum* and of 35 non-diarrhoeal cases studied, 2 (5.72%) cases were found to be infected with *C. parvum*. (**Table 1**) Statistical analysis was done using Chi square test about *C. parvum* in diarrhoeal Vs non-diarrhoeal cases, P value was 0.028. *C. parvum* was significantly more common in all HIV cases to cause diarrhoea.

Of 24 parasites detected in total, majority were *C. parvum* 19 (79.16%) followed by *I. belli* 2 (8.33%). Out of 22 parasites detected from 75 diarrhoeal HIV seropositive cases, majority i.e. 17 (77.27%) were of *C. parvum* followed by *I. belli* 2 (9.09%). (**Table 1**) Mixed parasitic infection was observed in 2 cases, of which one case was of *C. parvum & I. belli* [***Figure* - 3**] and other was of *Cyclospora* [**Figure -4**] *& I. belli*.

Out of 24 parasites detected, majority of the parasites were found in the age group of > 50 years i.e. 8 (33.33%) followed by the age group of 0 years -10 years 7 (29.16%). (**Table -2**) In the age group of above 50 years, out of 11 cases studied, *C. parvum* was detected in 6 cases (54.5%) followed by *I. belli and Strongyloides stercoralis (S. stercoralis)* 1(9.1%) each. In the age group of 0 years -10 years *C. parvum* was observed in 5 (35.7%) cases followed by *I. belli* 1 (7.1%) and *Cyclospora* 1 (7.1%). (**Table 2**)

Modified Ziehl Neelsen (MZN) technique was found to be the most sensitive microscopic method to detect the opportunistic intestinal parasites in HIV patients. (**Table 3**) The parasites detected by MZN were *C. parvum* (19) followed by *I. belli (*2) and *Cyclospora* (1). However by LPCB technique the parasites detected were *I. belli* (2) (Table 3) [**Figure – 5**] & *Cyclospora* (1) [**Figure – 6**]. *C. parvum, I. belli and Cyclospora* were not detected by Saline wet mount and Iodine preparation.

Figure 1: Modified Ziehl Neelsen stain showing oocysts of *Cryptosporidium parvum*

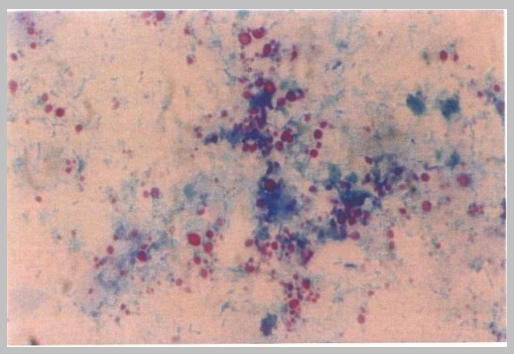

Figure 2: Modified Ziehl Neelsen stain showing oocyst of *Isospora belli*

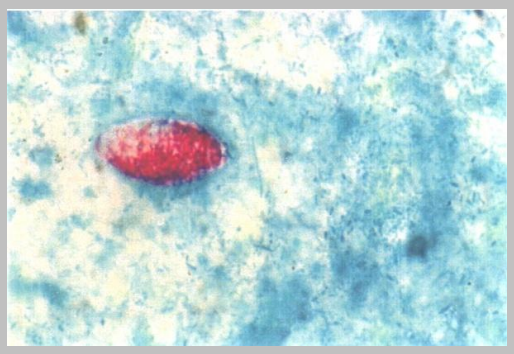

Figure 3: Modified Ziehl Neelsen stain showing mixed infection of both *Cryptosporidium parvum and Isospora belli*

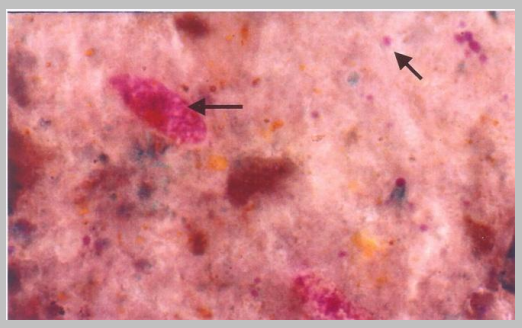

Figure 4: Modified Ziehl Neelsen stain showing oocyst of *Cyclospora*

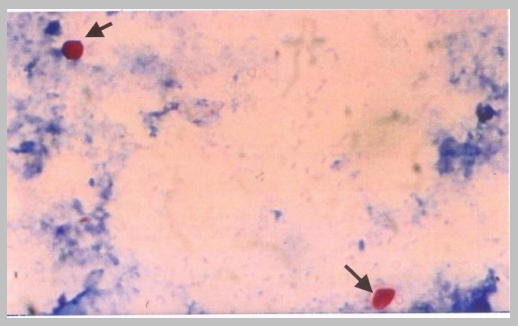

Figure 5: Lactophenol cotton blue staining showing *Isospora belli*

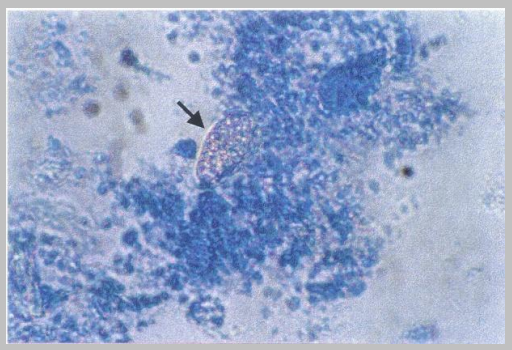

Figure 6: Lactophenol cotton blue staining showing *Cyclospora*

TABLE-1
Parasites identified in diarrhoeal and non- diarrhoeal patients

	No. of cases	*C. parvum*	*I. belli*	*Cyclospora*	*S. stercoralis*	*A. duodenale*	Total
Diarrhoeal	75	**17 (22.67%)**	**2 (2.67%)**	1 (1.34%)	1 (1.34%)	1 (1.34%)	**22 (29.34%)**
Non-diarrhoeal	35	**2 (5.72%)**	**-**	-	-	-	**2 (5.72%)**
Total	**110**	**19 (17.28%)**	**2 (1.8%)**	**1 (0.9%)**	**1 (0.9%)**	**1 (0.9%)**	**24 (21.8%)**

TABLE – 2
Depicting different parasites identified in different age groups

Age (years)	Parasite identified						Total
	No. of cases	*C. parvum*	*I. belli*	*Cyclospora*	*S. stercoralis*	*A. duodenale*	
0-10	14	5(35.7%)	1(7.1%)	1(7.1%)	-	-	7(50%)
11-20	3	1(33.3%)	-	-	-	-	1(33.3%)
21-30	21	2(9.5%)	-	-	-	-	2(9.5%)
31-40	45	4(9.0%)	-	-		1(2.2%)	5(11.1%)
41-50	16	1(6.3%)	-	-	-	-	1(6.3%)
>50	11	6(54.5%)	1(9.1%)	-	1(9.1%)		8(72.7%)
Total	110	19	2	1	1	1	24

TABLE -3
Parasites identified by different methods of examination

Method of examination	*C. parvum*	*I. belli*	*Cyclospora*	*S. stercoralis*	*A. duodenale*
MZN	19	2	1		
LPCB	-	2	1		
Iodine preparation	-	-	-		1
Saline wet mount	-	-	-	1	1

DISCUSSION

HIV manifested by diarrhoea is the major cause of morbidity and mortality in developing countries [11]. Among these, coccidian intestinal parasitic diseases are more common in HIV infected individuals [12]. These coccidian parasites cause life threatening profuse diarrhoea in immunosuppressant individuals like AIDS [13]. Early identification of these agents of diarrhoea is pivotal for appropriate therapy and to reduce morbidity and mortality associated with it.

In our study, 21.8 % HIV seropositive patients were found to be infected with opportunistic intestinal parasites. In India like our study prevalence of intestinal parasitic infection in AIDS ranges from 20 % to 30% [14, 15]. Slightly higher prevalence of intestinal parasitosis in HIV infected patients is reported from other countries (33-50%) [16].

The prevalence of intestinal parasites was significantly higher in HIV patients with diarrhoea 29.34% than in those without diarrhoea 5.72%. Statistical analysis was done using Chi square test about diarrhoeal Vs non-diarrhoeal cases, P value was 0.01 which is significant. Hence intestinal parasites were significantly responsible for producing diarrhoea among HIV patients. In India the prevalence of intestinal parasites recorded from a patients suffering from diarrhoea with HIV ranges from (20-36%). [14,17]. We found two *C. parvum* from HIV seropositive patients who were not suffering from diarrhoea which suggests *C. parvum* may be asymptomatic carrier parasite in HIV seropositive individuals but it can act as a potential pathogen.

The most predominant parasite detected in this study was *C. parvum* 19 (17.28%). *C. parvum* was detected in 22.67 % cases suffering from diarrhoea. *C. parvum* was found to be significantly more common opportunistic intestinal parasite to cause diarrhoea in HIV patients. *C. parvum* was reported as predominant pathogen with significant association to diarrhoeal cases by various authors also [14, 15, 18]. *C. parvum* is ubiquitous, highly resistant to disinfectants and infective in low doses. *C. parvum* is an important life threatening infection in immunocompromised persons.

I. belli was the next common parasite encountered with 2.67% diarrhoeal cases. This lower prevalence may be because of asymptomatic shedding of oocysts of *I. belli* and treatment with trimethoprim – sulphamethoxazole for other infections in AIDS cases. In India prevalence of *I. belli* varies from 2.5 % to 50% [6, 15, 17, 19] and abroad 0.78% to 15% [20]. However in some studies *I. belli* was reported to be the predominant parasite followed by *C. parvum.* [17, 21]

In this study, *Cyclospora and Strongyloides stercoralis* (*S. stercoralis*) was also found in one patient each. *Cyclospora* is recently described as emerging enteric pathogen in HIV individuals which can cause outbreak of diarrhoea [22]. Various authors have reported about *Cyclospora and Strongyloides stercoralis* as a pathogen in HIV infected patients. [6, 15, 17, 23, 24] Mixed infections {(with *C. parvum, I. belli*) and (*Cyclospora, I. belli*)} was seen in one patient each. Both the patients were suffering from diarrhoea. Co-infection with *C. parvum, I. belli and S. stercoralis* in AIDS is also reported by various authors [7, 25].

All intestinal opportunistic parasites in HIV infection occur through faeco oral route, hence sanitary disposal of faeces, personnel hygienic measures like hand washing, safe drinking water supply and safe food is recommended. As these intestinal parasitic infections are common, these infection should be suspected in any HIV infected patients with diarrhoea.

In our study, above 50 years age group (33.33%) was found to be highly affected with intestinal parasites followed by 0 years -10 years age group (29.16%). This may be because these groups when infected with HIV are more prone to develop infection compared to adult age group. Uppal et al reported 78% intestinal parasites in 0 years -10 years age group [14].

In this study Modified Ziehl Neelsen (MZN) stain was observed to be the most sensitive microscopic method to detect opportunistic intestinal coccidian parasites. MZN was useful to detect *C. parvum, I. belli and Cyclospora* which is in accordance with Sehgal et al [8] and Garcia et al [9]. LPCB was found to be useful to detect and identify *I. belli and Cyclospora*, which is similar with the study of Parija et al [10]. Stool microscopy with MZN & LPCB offers many advantages; it is a simple, easily affordable and most specific confirmatory technique and is the gold standard in the diagnosis of intestinal parasitic infections.

We recommend MZN method in stool microscopy to detect opportunistic intestinal coccidian parasites in HIV patients. Earlier demonstration of these parasites will enable clinicians to give effective treatment and save the patient from going to incidence of mortality.

CONCLUSIONS

In this study, opportunistic intestinal parasites were significantly responsible for diarrhoea

among HIV patients. *Cryptosporidium parvum* was the predominant opportunistic intestinal parasite in HIV patients. Hence stool samples of HIV seropositive patients should be checked routinely for opportunistic intestinal parasites like *C. parvum*. Above 50 years age group was found to be highly affected with intestinal parasites followed by 0 years to 10 years age group. Modified Ziehl Neelsen method was the most sensitive microscopic technique to detect opportunistic intestinal coccidian parasites like *C. parvum and I. belli.*

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