PREVALENCE OF CRYPTOSPORIDIOSIS IN HIV/AIDS PATIENTS IN A TERTIARY CARE HOSPITAL IN WEST BENGAL

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ABSTRACT

BACKGROUND

Cryptosporidium is one of the most common intestinal pathogen throughout the world and is the most common waterborne pathogen associated with diarrhoea in AIDS patients. These parasites draw attention as an emerging pathogen with spread of AIDS pandemic. We undertook a study to find the prevalence of Cryptosporidiosis in HIV/AIDS and its correlation with CD4 count.

METHODS

In this study of one year duration 80 HIV seropositive patients were included (Fifty with diarrhoea and thirty without diarrhoea). Control group consisted of 80 HIV seronegative patients. Thorough history was taken for all enrolled subjects regarding name, age, sex, HIV status, presence/absence of diarrhoea, socio-economic status, source of drinking water and water for other purposes. For the study group history of the presenting complain, associated opportunistic infection, history of tuberculosis and other drug history was taken. A thorough history of the nature of diarrhoea was taken. CD4 count of the enrolled patients was carried out. Stool samples collected from each enrolled patient were examined microscopically by different methods for the presence of *cryptosporidium* and other ova, cysts and parasites.

RESULT

Infection was commoner in males being highest in the age group of 30-39 years; 72% acquired HIV infection heterosexually. Chronic diarrhoea was seen in 46.25% of HIV patients whose mean CD4 count was 176.7 cells/µL. The prevalence of Cryptosporidium in the study group was 12.5%. In HIV positive diarrhoeal group, Cryptosporidium oocysts was found in 20% patients. Isospora was detected in one HIV patient. In the study population, 48.75% of patients had severe immunosuppression with CD4 count ≤200 cells/µL; 70% of *cryptosporidium* isolation was from severely immunocompromised hosts with CD4 count <100 cells/µL. The duration of diarrhoea was inversely proportional to the CD4 count; 80% of patients suffering from cryptosporidiosis used municipality water for drinking.

CONCLUSION

Cryptosporidiosis is a well-known opportunistic infection in HIV patients, particularly those with chronic diarrhoea and the risk increases with a low CD4 count. However, sexual activities and use of improperly stored or treated drinking water is also a risk factor.

KEYWORDS

Cryptosporidium, Diarrhoea, HIV, CD4 Count.

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INTRODUCTION

The waterborne pathogen *Cryptosporidium* is one of the most commonly identified intestinal pathogen throughout the world and is the most common waterborne pathogen associated with diarrhoea in people with AIDS. Genus *Cryptosporidium* causes Cryptosporidiosis. It belongs to phylum Apicomplexa, group Alveolata. Cryptosporidiosis is an important public health problem worldwide. Tyzzer in 1907 first recognised *Cryptosporidium* in gastric gland of asymptomatic laboratory mice.[1] Human cryptosporidiosis was first reported in 1976 by Nime.[2] From 1976 to 1982, only eight cases of *Cryptosporidium* infection was reported in man,[3] five of which were in immunosuppressed patients. Since then there has been a rapid increase in the number of such reports from almost all parts of the world. These parasites draw attention as an emerging pathogen with spread of AIDS pandemic.

Cryptosporidium infection of the gastrointestinal epithelium is self-limiting in immune competent persons but potentially life-threatening in immunocompromised persons, especially those with HIV/AIDS. Therapy of cryptosporidiosis is a challenge in immunocompromised patients. The host immune response is important in limiting the duration and severity of infection. Disease severity ranges from asymptomatic to severe, intractable diarrhoea depending upon the immune status, nutrition and age. It causes watery to mucoid diarrhoea with abdominal pain in normal hosts worldwide, persistent diarrhoea in children in developing

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countries and chronic diarrhoea in immunocompromised hosts including patients with AIDS.[4,5,6] In the immunocompromised individuals, disease is not always restricted to gastrointestinal tract, but can disseminate to other sites including respiratory tract. The majority of human infections are caused by *C. hominis* and the cattle genotype of *C. parvum*. Other Cryptosporidium species that occasionally infect immunocompetent humans are *C. meleagridis*, *C. felis* and *C. canis*. Species that have been reported only in immunocompromised individuals are *C. muris or C. andersoni*.

Although, the natural history of parasitic diseases may be altered by co-infection of HIV, parasites may in turn facilitate the progression from asymptomatic infection to AIDS. Antiretroviral therapy has dramatically decreased the cryptosporidiosis prevalence and has a tremendous impact on diarrhoea related morbidity in HIV/AIDS patients in countries where antiretroviral agents are widely available. In AIDS patients, depletion of circulating T cells lead to increased susceptibility to opportunistic intestinal protozoal infections. Immunocompetent individuals can clear the parasites spontaneously, but AIDS patients (immunocompromised) fail to do so and suffer from prolonged diarrhoea.

Here, we undertook a study to find the prevalence of Cryptosporidiosis in HIV/AIDS patients in a Tertiary Care Hospital in West Bengal.

AIMS AND OBJECTIVES

1. To find out the prevalence of cryptosporidiosis among normal healthy subjects and HIV/AIDS patients.
2. To find out correlation between CD4 count and cryptosporidiosis.

MATERIALS AND METHODS

The present study is a cross-sectional study undertaken in the Department of Protozoology and Virology in the School of Tropical Medicine, Kolkata, from April 2010 to March 2011. Due clearance was obtained from the Institutional Ethical Committee. HIV positive patients in whom recent CD4 count was available and from whom informed consent could be obtained were included in the study. Patients taking antacids, non-absorbable anti-diarrhoeal preparations, mineral oils and other oily materials, patients suffering from malaria and with a history of taking antimalarial drugs within previous 2-3 weeks, those undergoing barium enema procedures within last one week and those undergoing invasive gall bladder investigations with dye within last three weeks were excluded from the study.

A total of 80 HIV seropositive patients attending the virology OPD of the School of Tropical Medicine, Kolkata and admitted in various wards of the adjoining Carmichael Hospital for Tropical Disease, Kolkata were included in the study and were selected as follows: Group 1: Consecutive fifty HIV seropositive patients with diarrhoea and Group 2: First thirty HIV seropositive patients without diarrhoea. Control subjects were recruited from two different sites (i) 50 patients having diarrhoea, but HIV seronegative were recruited from ID and BG Hospital, Beliaghata, Kolkata and (ii) First 30 patients without diarrhoea and HIV seronegative were recruited from the School of Tropical Medicine, Kolkata and adjoining Carmichael Hospital for Tropical disease, Kolkata. All study and control subjects were subjected to thorough history taking regarding name, age, sex, HIV status, presence/absence of diarrhoea, socio-economic status, source of drinking water and water for other purposes are noted in a designed proforma.

For the study group history of the presenting complaint, CD4 count, associated opportunistic infections, history of tuberculosis and other drug history was taken. A thorough history of the nature of diarrhoea was taken: frequency of stool (Times/day), duration (Days/months), presence of blood and mucous in stool and presence of nausea, vomiting, abdominal pain and fever. Three stool samples were collected from each enrolled patient and were examined in the Department of Protozoology by the following methods.

1. Direct wet mount preparation with saline and iodine.
2. Smear preparation after formal ether sedimentation and concentration.
3. Modified acid fast stain (Kinyoun) of the smear.

The preparations were examined for the presence of *cryptosporidium* and other ova, cysts and parasites.

CD4 count of the enrolled patients was carried out by FACS count (Becton Dickinson). Viral load was not measured due to economic constraints. Each time the participant provided a stool sample, the most recent CD4 count was recorded for analysis. Stool samples from patients without CD4 information were excluded from the analysis.

RESULTS AND ANALYSIS

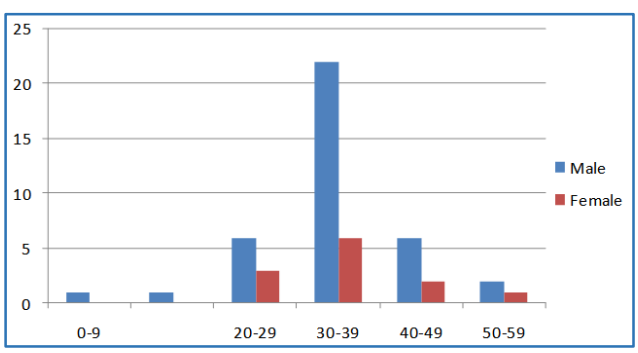

Fig. 1: Age & Sex Distribution among HIV Positive Patients with Diarrhoea

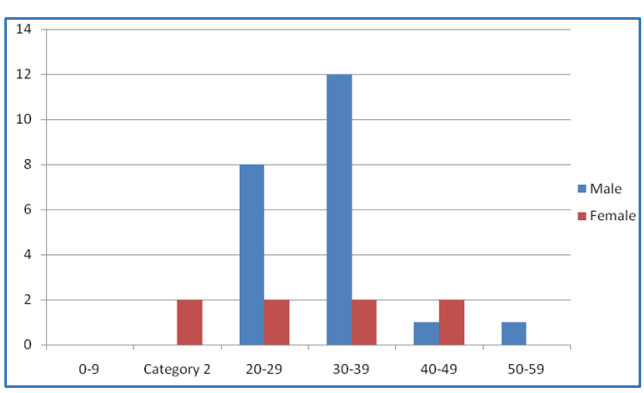

Fig. 2: Age & Sex Distribution among HIV Positive Patients without Diarrhoea

The male:female ratio of the 80 HIV positive patients was 3:1. Most of the recruited patients were in the age group of 30-39 years.

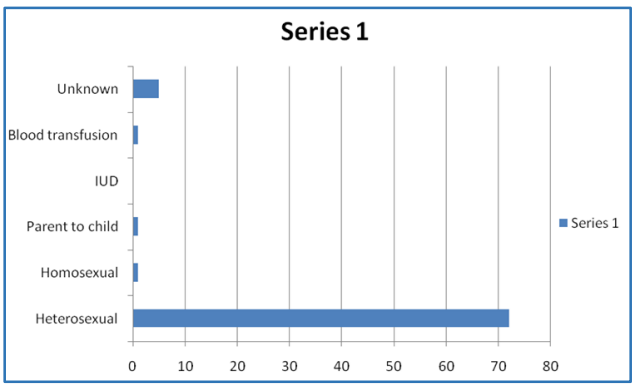

Fig. 3: Mode of Acquisition of HIV/AIDS Infection

72% of the patients acquired HIV infection by heterosexual contact.

Stool Examination

	HIV Positive (Study Group)		HIV Negative (Control Group)	
	With Diarrhoea (50)	Without Diarrhoea (30)	With Diarrhoea (50)	Without Diarrhoea (30)
No. of Patients showing Some Parasites	23	6	7	5
No. of Patients showing Cryptos-poridium	10	-	1	-

Table 1: Prevalence of Parasites in the Stool of the Study and Control Group

Therefore, the prevalence of *Cryptosporidium* in the study group was 12.5% (10 out of 80) and in the control group was only 1.25% (1 out of 80).

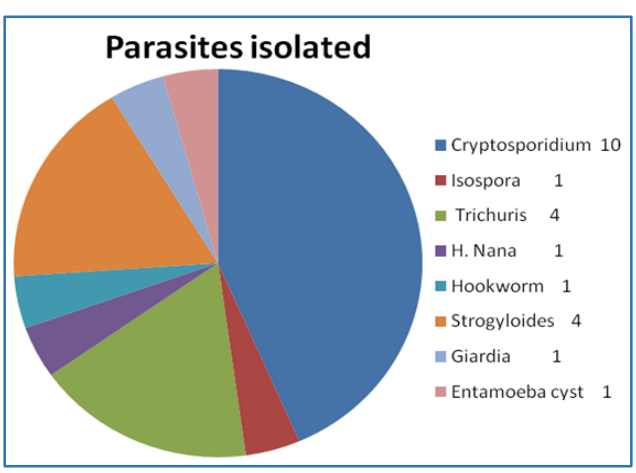

Fig. 4: Parasites Isolated in HIV Positive Patients with Diarrhoea

A variety of other parasites was also isolated from HIV positive patients with diarrhoea. Isospora was isolated from one patient.

HIV Status	Acute Diarrhoea	Chronic Diarrhoea	Persistent Diarrhoea	Total
HIV Positive	9	37	4	50
HIV Negative	28	10	12	50

Table 2: The Presentation of Diarrhoea in the Study and the Control Group was as Follows

The mean CD4 count in HIV positive patients suffering from acute diarrhoea was 295.5 cells/µL, while those suffering from chronic diarrhoea was 176.7 cells/µL.

CD4 Range (Cells/ µL)	HIV Infected with Diarrhoea (N=50)	HIV Infected Without Diarrhoea (N=30)	% the Study Group
0-199	30	9	48.75%
200-500	18	17	43.75%
>500	2	4	7.5%

Table 3: Distribution of the CD4 Count in the Study Group was as Follows

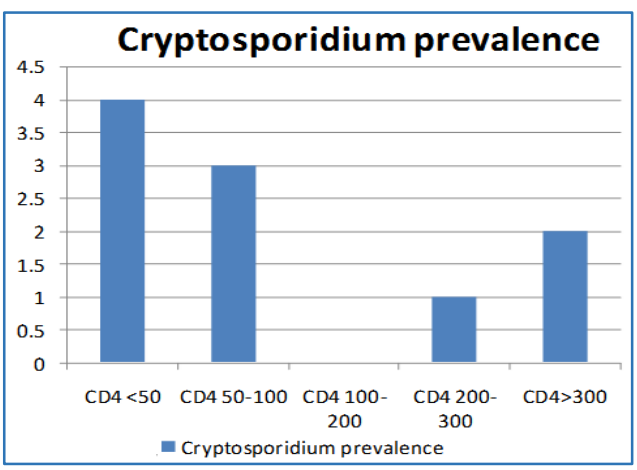

Fig. 5: Correlation between CD4 Level and cryptosporidium in HIV Positive Patients with Diarrhoea

7 out of 10 Cryptosporidium positive patients had a CD4 count <100 cells/µL.

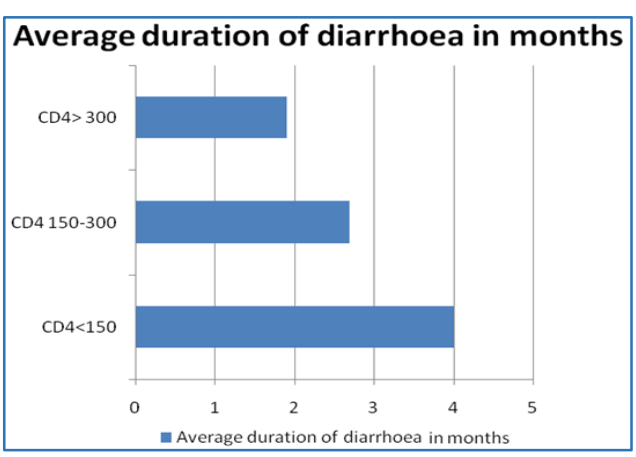

Fig. 6: Correlation between the Duration of Diarrhoea (in months) and CD4 Level in Cryptosporidiosis Patients

The average duration of diarrhoea in HIV patients with CD4 count <150 cells/µL was 4 months, whereas that in HIV patients with CD4 count >300 cells/µL was 1.9 months. The duration of diarrhoea was more in the lower CD4 range.

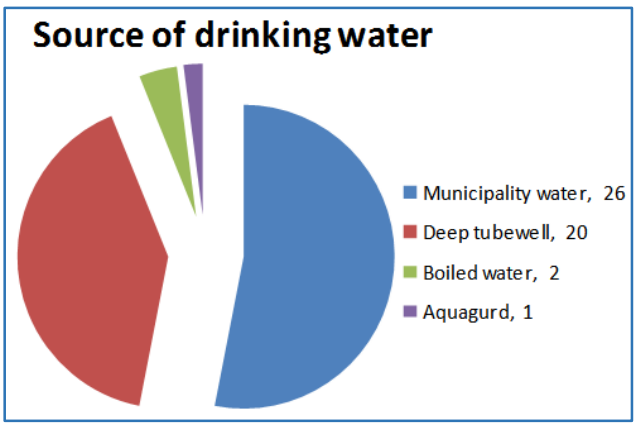

Fig. 7: Source of Drinking Water of the HIV Positive Patients with Diarrhoea

Most of the HIV positive patients with diarrhoea used municipality water for drinking purpose.

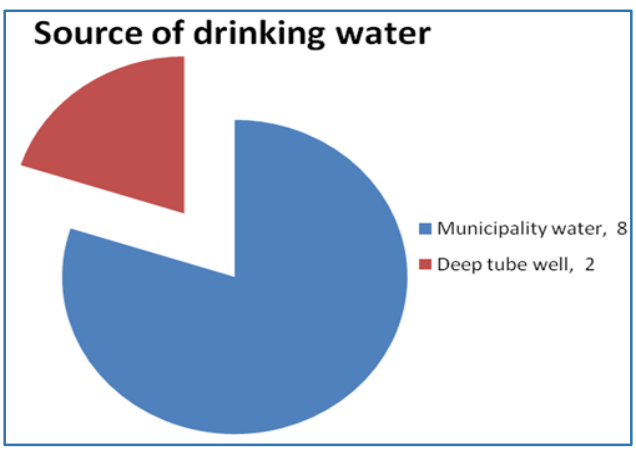

Fig. 8: Source of Drinking Water of the HIV Positive Patients with Diarrhoea showing Cryptosporidium in Stool

8 of the 10 patients showing Cryptosporidium in stool used Municipality water as a source of drinking water.

DISCUSSION

The present cross-sectional study was designed to have a comprehensive idea of the prevalence of cryptosporidiosis in HIV infected persons reporting at the School of Tropical Medicine, Kolkata, with an aim to find out correlation between CD4 count and cryptosporidiosis.

In the present study, infection in the study group was commoner in males with a highest rate in the age group of 30-39 years (Fig. 1 & 2). This is similar to a study conducted by Aziz A et al in Jaipur where the male-to-female ratio was 3:1, 69.2% of the study population were males and 30.8% were females and most patients were in the age group of 31-40.[7] In our study, 72% acquired HIV infection heterosexually (Fig. 3). Rest of the patients acquired HIV by other modes of transmission. Sexual behaviour is a known risk factor for acquiring cryptosporidiosis. Chances of cryptosporidiosis were more in oro-anal sexual practices.

Present study showed that 80% of patients suffering from cryptosporidiosis used municipality (Fig. 8) water as a source of drinking water. Municipality used ground water or treated water for supply to the locality. Efficacy of water treatment plants depend on the source of water, temperature of treatment plant, amount of disinfectant used, etc. Post treated water can also be contaminated due to leak or break in supply system. In homes, water can also be contaminated due to inappropriate storage methods.[8]

The present study had the aim to isolate Cryptosporidium associated with diarrhoea in both HIV positive and HIV negative patients. In HIV infected diarrhoea group, 9 patients presented with acute diarrhoea and 37 patients (46.25%) presented with chronic diarrhoea (Table 2). In HIV positive diarrhoeal group, Cryptosporidium oocysts were found in the stool of 10 patients (20%), whereas in the non-diarrhoeal group no Cryptosporidium was isolated. In HIV negative diarrhoeal group Cryptosporidium oocysts was found in one patient (2%). However, the overall prevalence of Cryptosporidium in the study group was 12.5% (10 out of 80) and in the control group was only 1.25% (1 out of 80) (Table 1).

In Indian study, Cryptosporidium has been isolated from all parts of the country in stool samples of HIV infected persons. In a study in Southern India, Cryptosporidium has been isolated in Chennai as 13.72% and 8% in diarrhoeal and non-diarrhoeal patients respectively.[9] In Northern India isolation rate in HIV infected diarrhoeal patients in two different studies was 11%[10] by Prasad et al and 10.8%[11] by Mohandas et al. In Mumbai, Joshi et al isolated Cryptosporidium in 8.5% cases.[12] Some higher rates of isolation have been found in some studies like in Karnataka 46.7%,[13] in asymptomatic HIV infected individuals by Anand et al as 65.8%,[14] by Panda et al as 23%[15] and by Agarwal et al as 33.3%.[16] In world literature, isolation of *cryptosporidium* from HIV infected patients has been found to vary in different countries like 12.8% in Thailand,[17] 10.5% in South Korea,[18] 41.3% in Venezuela,[19] 39.7% in Epthiopia,[20] 1.5% in Iran,[21] 10.4% in Bogota-Cambodia,[22] 3% in Netherland[23] and 15-16% in Maryland USA.[24,25]

In our study the rates are much higher in the diarrhoeal group (20%), while no Cryptosporidium was isolated in the non-diarrhoeal group. In our study, the prevalence of Cryptosporidiosis in HIV infected patients (12.5%) agrees with results by Prasad et al[10] and Mohandas et al[11] and also with study reports Thailand, South Korea and Cambodia.[17,18,22]

However, the prevalence is lower than the studies done in other parts of the country. This could be probably due to use of more sensitive detection methods in other studies. Another most probable explanation for the low count may be the low prevalence of *cryptosporidium* in this locality.

Isospora belli, another opportunistic coccidian protozoan was detected in 2% of HIV infected diarrhoeal patients in our study (Fig. 4). This was consistent with the study by Mohandas et al, showing a detection rate of 2.5% in HIV seropositive patients.[11]

In the present study, CD4 cells were estimated for HIV infected patients. It ranged from 22 cells/µL to 847 cells/µL with a median of 57.5 cells/µL in diarrhoeal patients. Among them 39 patients out of 80 (48.75%) in the study population had severe immunosuppression with CD4 count ≤200 cells/µL, while 35 patients that is 43.75% had moderate immunosuppression with CD4 count between 201 to 499 cells/µL. Only 6 patients (7.5%) had mild disease with CD4 count ≥500 cells/µL (Table 3); 70% (7 out of 10) of *cryptosporidium* isolation was from severely immunocompromised hosts with CD4 count was less than 100 cells/µL (Fig. 5). This finding is consistent with a study

conducted by Tuli L et al, who concluded that the maximum isolation of Cryptosporidium (66.6%) was from HIV patients with CD4 count <200 cells/µL.[26] Dwivedi et al[27] observed that infection rate was 55.6% in HIV infected diarrhoeal patients with single parasitic infection who had a mean CD4 count of 161 cells/µL. Sarfati et al found the prevalence of opportunistic protozoa (Mostly Cryptosporidium, Isospora and microsporidium) was 32% in patients with less than 50 CD4 cells/µL in Cameroon.[28] In our study, the CD4 levels were inversely proportional to the duration of diarrhoea. The duration of diarrhoea increased with the decrease in the CD4 count. Similar results were also obtained by Tuli L et al.[26] In our study, the average duration of diarrhoea in HIV patients with CD4 count < 150 cells/µL was 4 months, whereas that in HIV patients with CD4 count >300 cells/µL was 1.9 months (Fig. 6).

HIV infection causes a gradual decline in the peripheral CD4 helper lymphocyte count. These lymphocytes are part of the body's immune system and play a key role in cell mediated immunity. But as HIV destroys these lymphocytes, HIV-infected patients become predisposed to opportunistic infections. As their CD4 lymphocyte count fall below 200 cells/µL, they become prone to a wide range of opportunistic infection including cryptosporidiosis.

This study had some limitations: Light microscope was used in this study for detection of parasites in stool, which is not as sensitive as modern methods like polymerase chain reaction which is not available in our institute. Also this study does not differentiate between species, because of the dearth of sensitive parasites detection techniques like polymerase chain reaction technique, Isoenzyme analysis and antigen detection techniques.

CONCLUSION

It is well known that immunocompromised patients infected with HIV are at higher risk for Cryptosporidiosis and that carriage of the parasite is associated with diarrhoeal disease in most cases. There is good evidence that risk of fecal carriage of cryptosporidiosis is directly related to the CD4 count. However, behavioural factors such as sexual activity (Most particularly having multiple sexual partners and engaging in oro-anal sex) also play a significant role. Prevalence was higher in patients using municipality water for drinking. Therefore, to reduce the prevalence of coccidian parasites among HIV infected persons practicing safe sex should be encouraged. Also maintenance of proper drinking water quality with appropriate storage facilities, proper sanitation and hygiene (Particularly washing of hands) should be practiced to reduce the disease burden in the community.

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