

PREVALENCE OF CRYPTOSPORIDIAL DIARRHOEAS AMONG HUMAN IMMUNODEFICIENCY VIRUS PATIENTS

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ABSTRACT

Background: Cryptosporidiosis is a major cause of diarrhoea in HIV infection in developing countries. AIDS and protein energy malnutrition are severely impairing the immune system, in such patients cellular immunity is low and implies that cellular immunity is the major component in eliminating the infection. Despite numerous clinical trials with several antimicrobial agents, no therapy has been shown to be curative for cryptosporidium. **Aim:** To assess the Prevalence of Cryptosporidial diarrhoeas among HIV positive patients. **Materials and Methods:** A prospect cross sectional study was carried out in the department of Skin and STD of Vinayaka Mission Medical College Hospital, Salem for 3 years from 2009-2012. Both outpatients and inpatients attending the STD Department for treatment of opportunistic infection of HIV – AIDS were included in the study. Five grams of formed or about 10 ml of unformed stool was collected and sample were examined within one to two hours of collection. The smears were prepared from stool samples and stained by modified Ziehl-Neelsen. The results of the study are analysed by using SPSS version 16. **Results:** Out of the total 100 patients who were screened for cryptosporidium 37 were shown positive for it. Out of 68 males 28 (41.1%) were found to be positive for cryptosporidia and out of 32 females 9(28.1%) were found to be positive. So compared to females the males were more affected and that was found to be statistically significant. Patients with CD4 amount <200 were found to be more affected by cryptosporidium than patients with CD4 count more than 200 and this difference was found to be statistically significant. **Conclusion:** Given the severity and lack of effective specific treatment of cryptosporidium in AIDS patients, emphasis should be given to its prevention and control.

Key words: HIV/AIDS, Cryptosporidium, CD4 counts

INTRODUCTION

Among acquired immunodeficiency syndrome (AIDS) patients in developing countries as many as 95% may have bacteria. Despite the spread of human immunodeficiency virus (HIV) infection in India and the high prevalence of diarrheal disease, there is

little information available on the epidemiology of diarrheal disease among people with HIV infection. In a review of HIV related opportunistic infections in Northern India, chronic diarrhea was the second most common.^[1] Parasitic infections with *Isospora belli*, *Entamoeba histolytica* and *Cryptosporidium parvum* have been reported as being the most frequently identified organisms in India.^[2] *C. parvum* is a small parasite measuring about 3-5 µm in diameter. The life cycle of *C. parvum* is completed within the small intestine and colon of the host with the developing stages associated with the luminal surface of the intestinal epithelial cells where it remains intracellular but extracytoplasmic.

Cryptosporidiosis is a major cause of diarrhea in HIV infection in developing countries. AIDS and protein energy malnutrition are severely impairing the immune system, in such patients cellular immunity is low and implies that cellular immunity is the major component in eliminating the infection.^[3]

Cryptosporidium, an intracellular protozoan has changed from that of a rare largely asymptomatic disease, to an important cause of diarrhea in animals and humans worldwide.^[4]

Reported Cryptosporidiosis prevalence is 3-4% in the USA,^[5] 3.5-22.44% in Brazil^[6] and about 50% in Africa and Haiti.^[7] In HIV infected patients, *Cryptosporidium* is the most frequent microbial cause of diarrhea, usually causing chronic bulky and intermittent diarrhea with liquid non-bloody stools, accompanied by pain and abdominal colic, and a noticeable loss of weight.^[8]

Despite numerous clinical trials with several antimicrobial agents, no therapy has been shown to be curative for *Cryptosporidium*. The infection can be contracted from other humans through sexual contact and nonsexual contact through ingestion of contaminated food or drinking water.^[9] As most of the data were pertaining to western countries and only very few Indian studies had been done in this area this study was undertaken to

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assess the prevalence of cryptosporium infection among the HIV patients in South India.

Aim

The aim is to assess the prevalence of cryptosporidial diarrheas among HIV positive patients.

MATERIALS AND METHODS

A prospective cross-sectional study was carried out in the Department of Skin and Sexually Transmitted Diseases (STD) of Vinayaka Mission Medical College Hospital, Salem for 3 years from 2009 to 2012. Both outpatients and inpatients attending the STD Department for Treatment of Opportunistic Infections of HIV – AIDS were included in the study. A total of 100 patients were enrolled in that period of study. A face to face interview along with medical examination and investigation were carried out with relevant case records to all the patients who were included in the study. All the 100 patients were provided a wide mouthed, clean, dry, properly labeled plastic container for collection of stool samples. Five grams of formed or about 10 ml of unformed stool was collected and samples were examined within one to 2 h of collection. The smears were prepared from stool samples and stained by modified Ziehl–Neelsen.

Detection of *Cryptosporidium* oocysts in the concentrated stool was done using the modified cold Ziehl–Neelsen staining technique.^[10] 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 10-15 min. After rinsing again in tap water, the slide was counterstained with 0.4% methylene blue 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 blue background [Figure 1].

The results of the study are analyzed by using SPSS version 16 and Chi-square test was used to assess the association of various factors.

RESULTS

Out of the total 100 patients who were screened for *Cryptosporidium* 37 were shown positive for it. Almost 80% are from rural side and majority of them were

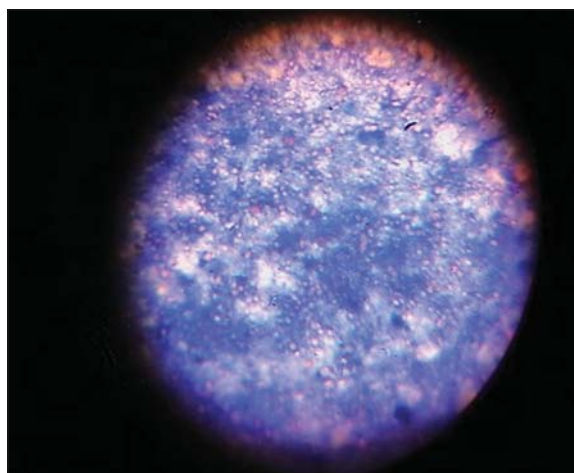


Figure 1: Oocysts of *Cryptosporidium parvum* in modified Ziehl–Neelsen staining (×1000)

daily laborers belonging to low socio economic status almost 75% of the patients had the history of using unprotected water supply with poor personal hygiene.

Table 1 shows the type of HIV transmission among patients with cryptosporidial diarrhea and it is seen from the table the most common transmission was through heterosexual contact which was almost the same as that of the HIV patients.

Table 2 shows the age and sex wise distribution of the study population and it was inferred from the table that most of the patients were in the age group 16-35 years which was the reproductive age group. Among the gender the males are found to be more affected than the females. Out of 68 males 28 (41.1%) were found to be positive for *Cryptosporidium* and out of 32 females 9 (28.1%) were found to be positive. So compared to females the males were more affected and that was found to be statistically significant.

Table 3 shows the distribution of *Cryptosporidium* based on CD4 count and it was found that patients with CD4 count <200 were found to be more affected by *Cryptosporidium* than patients with CD4 count more than 200 and this difference was found to be statistically significant.

DISCUSSION

Diarrhea is a common complication of HIV infections, inducing weight loss and cachexia and occurring in almost 90% of AIDS patients in developing countries.^[11] *Cryptosporidium* is a well-established cause of diarrhea

Table 1: HIV transmission route among patients with *Cryptosporidium* infection (n = 37) (males = 28 and females = 9)

HIV transmission route	n (%)		Total	P value
	Men	Women		
Heterosexual route	25 (89.2)	9 (100)	34 (91.8)	<0.001
Drug addiction	1 (3.5)	0	1 (2.7)	
Transfusion	1 (3.5)	0	1 (2.7)	
Congenital	1 (3.5)	0	1 (2.7)	

HIV: Human immunodeficiency virus

Table 2: Age and sex wise distribution of HIV patients with cryptosporidial diarrhoea

Age group	Male (%)	Female (%)	Total (%)	P value
6-15	1 (3.5)	0	1 (2.7)	<0.005
16-25	7 (25)	2 (22.2)	9 (24.3)	
26-35	13 (46.4)	4 (44.4)	17 (45.9)	
36-45	3 (10.7)	2 (22.2)	5 (13.5)	
46-55	4 (14.2)	1 (11.1)	5 (13.5)	
Total	28 (100)	9 (100)	37 (100)	

HIV: Human immunodeficiency virus

Table 3: Patients with cryptosporidial diarrhea based on CD4 count

CD4 count	Patients with cryptosporidial diarrhea	Percentage	P value
<200	30	81	<0.005
201-400	6	16.2	
>400	1	2.7	

among HIV infected patients worldwide with prevalence of infection ranging from 3% in developed countries to 50% in developing countries.^[10]

Many western countries had observed the prevalence of *Cryptosporidium* diarrhea as 8% in a study from the UK,^[12] 50% from Haiti^[13] 10% in Italy and France^[14] and 5% in a North American study^[15] which was in contrast of the present study which had shown the prevalence of *Cryptosporidium* as 37%. This divergence may be a reflection of different periods studied, environmental conditions or therapeutic fashions. A study done in Nigeria have quoted that severe cryptosporidiosis was being associated with CD4 <100 which was in par with the present study where almost 82% of our patients CD4 count was less than 200. Patients are more likely to contract *Cryptosporidium* infection with the lowering of the immune status as evidenced by the association of low CD4 counts and cryptosporidiosis.

The present study documents that infections with intestinal protozoan parasites are common in HIV

seropositive individuals. Figures from various studies demonstrate the striking Geographical variations with respect to the prevalence of intestinal parasites in HIV infected patients.^[16] Ayyagari *et al.*^[17] has reported a high prevalence of *I. belli* from the Northern parts of India, where as Kumar *et al.*^[18] has reported *C. parvum* as the commonest parasite which was associated with HIV infections in Southern parts of India whereas our study had projected *C. parvum* as the most common coccidian parasite which was associated with HIV positive cases. Studies conducted by Basak *et al.*,^[19] Tuli *et al.*^[20] and Mohandas *et al.*^[21] have also reported the similar results.

An African study^[22] had stated that the prevalence of cryptosporidiosis was higher among the HIV infected children aged 5 years or less than in those HIV infection persons aged above 5 years old which was in contrast with our study which had shown the prevalence was more among the age group of 15-45 years.

A study by Shrihari *et al.*^[23] has mentioned that a high prevalence of intestinal parasitic infections in HIV positive individuals may be due to contaminated water supply and lack of personal hygiene, which is common in a rural scenario which is in par with our study where 85% of them are from rural side and in that 75% had poor water sanitation and poor personal hygiene.

CONCLUSION

The prevalence of *Cryptosporidium* in our study was 37%. Given the severity and lack of effective specific treatment of cryptosporidiosis in AIDS patients, emphasis should be given to its prevention and control. *C. parvum* is major water borne protozoan pathogen; water contamination should be investigated to protect public health from the risk of transmission of the pathogen. Along with early diagnosis and treatment and other steps should be taken to improve the water supply, sanitation and to provide health education to prevent the morbidity and mortality which are associated with HIV infected individuals.

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