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A STUDY ON BURDEN OF CRYPTOSPORIDIOSIS IN HIV/AIDS PATIENTS ATTENDING ART CENTER OF A TERTIARY CARE HOSPITAL AND THEIR CLINICAL PRESENTATION, SOCIODEMOGRAPHIC PROFILE AND IMMUNE STATUS.

Dr. Bipasa Chakraborty*	MD, Assistant Professor, Dept. Of Microbiology, RGKMCH, Kolkata, West Bengal. *Corresponding Author
Prof. Mitali Chatterjee	MD, Professor and Head, Dept. of Microbiology, RGKMCH, Kolkata, West Bengal.
Prof. Maitreyi Bandyopadhyay	MD, Professor, Dept. Of Microbiology, RGKMCH, Kolkata, West Bengal.
Prof. Reena Roy Ghosh	MD, Professor, Dept. Of Microbiology, RGKMCH, Kolkata, West Bengal.
Prof. Prabir Kumar Mukhopadhyay	MD, Dean Of Student's Affairs And Professor, Dept. Of Microbiology, RGKMCH, Kolkata, West Bengal.
Prof. Manas Kumar Bandyopadhyay	MD, MSVP And Professor, Dept. Of Microbiology, RGKMCH, Kolkata, West Bengal.

ABSTRACT

BACKGROUND: Parasitic diarrhea among HIV/AIDS patients is a major opportunistic illness resulting in significant morbidity and poor quality of life. Cryptosporidiosis is one of its commonest cause. Disease severity ranges from asymptomatic infection to chronic profuse diarrhea. Its prevalence varies depending on local endemicity and immune-status. As there are few studies, this study was done to estimate the prevalence and evaluate the epidemiological and clinical features of cryptosporidiosis and measure the immune status of these HIV/AIDS patients attending ART center of a tertiary care hospital.

METHODS: This descriptive, observational, cross-sectional study was done from February 2020-January 2021 with 162 adult patients attending ART center after counseling and consent. Sociodemographic data and medical history were obtained following standard questionnaire at the time of stool collection. Formol ether sedimentation technique was performed with every stool sample followed by microscopical examination after preparing normal-saline wet-mount, iodine-mount, modified acid-fast and Kinyoun's cold stained smears. *Cryptosporidium spp*. oocysts were identified as red, acid-fast, rounded, 4-6µm diameter, sometimes with visible sporozoites. Flowcytometry was used for CD4+T-cell count.

RESULTS: Out of 162 samples, *Cryptosporidium spp* was detected in 31, with 19.13% prevalence. Seven *Cyclospora spp* were identified including one mixed infection with Cryptosporidium. Twenty-three(14.19%) patients presented with chronic diarrhea with watery stool, from which fourteen *Cryptosporidium spp* identified. Statistical analysis by Graph-Pad-Prism showed significant association between cryptosporidiosis and chronic diarrhea (P<0.0001). In our study there was also high rate of asymptomatic cryptosporidiosis (54.83%). Even among low CD4+T-cell count(<200) asymptomatic cases were 45.45%. However significant correlation was found between low CD4+T-cell counts and cryptosporidiosis(P<0.0001). Socio-demographic characteristics studied between patients with and without cryptosporidiosis showed no significant differences in age(P=0.9737), sex(P=0.7392), marital status(P=0.3815), sexual orientation(P=0.0502), education(P=0.1561), income(P=0.5068) and WHO-staging of the disease(P=0.1650).

CONCLUSIONS: History of chronic diarrhea and low CD4+T-cell counts were significantly associated with prevalence of cryptosporidiosis.

KEYWORDS : Cryptosporidiosis, HIV/AIDS, Diarrhea, Sociodemographic Profile, CD4+T Cells

INTRODUCTION:

Parasitic diarrhea among human immunodeficiency virus (HIV) and acquired immunodeficiency syndrome (AIDS) patients is a major opportunistic illness and public health problem resulting in significant morbidity, poor quality of life with poor nutrition and weight loss. The prevalence of intestinal parasitic infections varies from patient to patient depending on local endemicity, seasonal variations, and their immune status.[1] Prevalence of HIV among adult people in India is around 0.26% according to annual report of National AIDS Control Organization (NACO) 2016-2017.[2] Diarrhea being the most common complaints, is associated with almost 50% of HIV/AIDS patients in developed countries and almost 100% in developing countries during their disease course.[1] Etiological agents include opportunistic pathogens causing chronic diarrhea and non-opportunistic pathogens causing acute diarrhea.[3] Several etiological intestinal parasitic agents are associated with acute and chronic diarrhea of these patients. Commonly reported are C*ryptosporidium spp, Cyclospora spp, Cystoisospora belli, Microsporidium spp, Giardia intestinalis, Entamoeba histolytica* and rarely *Strogyloides stercoralis.* In India profound morbidity due to chronic diarrhea leading to weight loss and wasting syndrome is very common.[3] The World Health Organisation (WHO) has defined diarrhea wasting syndrome with reactive HIV serology report as AIDS defining illness.[4] Cryptosporidiosis is one of its commonest cause and disease severity ranges from asymptomatic infection to chronic profuse diarrhea.

As there are very few studies regarding their prevalence, epidemiological and clinical features of Cryptosporidiosis and their relationship with CD4+T-cell counts and HIV/AIDS status, this study was done to estimate the prevalence of Cryptosporidiosis by examining stool samples from HIV/AIDS patients with or without diarrhea attending ART center of a tertiary care hospital of Eastern India and to evaluate their epidemiological and clinical features and to determine the relationship of CD4+T-cell counts with the prevalence of Cryptosporidiosis among these patients.

METHODS:

This descriptive, observational, cross-sectional study was done in department of Microbiology in a tertiary care hospital of Eastern India from February 2020 to January 2021 after obtaining approval from the Institutional Ethical Clearance Committee. HIV/AIDS patients with reactive HIV serology report who are registered and had attended our hospital's ART center in this time period with or without diarrhea were included in this study after proper counseling and consent. Stool

samples were collected from 162 adult patients of age 18 years or older, willing to participate in this study. All paediatric age group patients were excluded from our study. [Sample size was calculated as per formula, $n=z^2pq/d^2$ ($z=1.96$, expected prevalence $p=12$, $q=88$, margin of error $d=5$)]

Sociodemographic data and clinical history were obtained following standard questionnaire at the time of stool collection with special emphasis on history of diarrhea present or absent. Diarrhea as per WHO guidelines was defined as passage of three or more loose or liquid stools per day, in excess than the usual habit for that person. Acute diarrhea was defined when diarrheal episode lasted for <14 days, persistent diarrhea when >14 days but <30 days and chronic diarrhea when >30 days.

Three stool samples from each patient were collected separately on three different days, in a wide mouthed sterile plastic universal container aseptically in the ART center and transferred immediately to Microbiology parasitology laboratory for processing. Normal saline wet-mount and Iodine mount preparation were done. To increase parasitic yield, concentration method was done on all stool samples by formol ether sedimentation technique, followed by microscopical examination of the sediment. [5, 6] After decanting, the sediments were examined by preparing normal-saline wet-mount, iodine-mount, and smears were prepared and stained with modified acid-fast stain using 1% and 3% sulfuric acid using heat and Kinyoun cold acid fast staining method without using heat but using 4% basic fuchsin and 8% phenol as modification and all the slides were meticulously examined for ova, cyst, parasite and oocyst of C*ryptosporidium spp*. *Cyclospora spp*. and *Cystoisospora belli*. [5, 6, 7, 8, 9] Fluorescence microscopy was done for detecting autofluorescence for *Cyclospora* oocysts. [8,10] Also CD4+T-cell count were done by Flowcytometry (Partec CyFlow Counter) in ART center after collection of EDTA blood from every patient and association between CD4+T-cell count and cryptosporidiosis were studied. [11,12]Sociodemographic data like age, sex, marital status, sexual orientation (MSM/homosexuals or heterosexuals), education, income and WHO-staging were collected from every patient and analysed.

During processing of stool samples all standard precautions were followed with donning and doffing of proper personal protective equipments (PPE) and using a biosafety cabinet for the procedures. During laboratory procedures, swabs, wooden spoons, guaze pieces, faecal debris and stool preparation filtrate and other wastes generated during stool processing were discarded in plastic container containing 1% sodium hypochlorite solution. Also 10% formalin was added to all the universal containers containing the stool specimen and kept overnight for disinfection and to render oocysts non-viable before discarding. [7] Any surface contamination were cleaned with detergent and disinfected with 3% hydrogen peroxide solution. [7,13]

C*ryptosporidium spp*. oocysts in wet mount are highly refractile, round with double walled cyst and in modified acid fast stain, there are acid-fast, red-coloured, rounded, 4-6µm diameter, sometimes with visible sporozoites, 4 in number within the oocysts. [7] *Cyclospora* oocysts are 6-10µm diameter. The oocysts of *Cyclospora* are acid-fast, they are notoriously variable in their degree of acid-fastness, even with 1% sulphuric acid as the decolourizer. Typically, even in the same specimen, their appearance ranges from nonstaining structures (common) to intensely staining dark red (rare)—a phenomenon well-illustrated in several publications. [8, 14] Fluorescence microscopy were done for detecting autofluorescence for *Cyclospora* oocysts with blue excitation (450 to 490 nm) giving green autofluorescence. [10]

Data interpretation and statistical analysis from questionnaires and laboratory reports were done using Excel spread sheet (Microsoft Corporation), descriptive biostatistics and Graph Pad Prism version 5.00 (Graph Pad software, San Diego, CA, USA). All statistical tests were considered significant if the *P*-value was ≤0.05.

RESULTS:

From our study 31 *Cryptosporidium spp* were identified out of 162 HIV/AIDS patient's stool samples with overall 19.13% prevalence of Cryptosporidiosis. (Figure-1,2) Twenty-three (14.19%) patients presented with chronic diarrhea with watery stool. From these 23 chronic diarrhea patients, 14 *Cryptosporidium spp* oocyst were identified indicating a higher prevalence of 60.86% Cryptosporidiosis in chronic diarrhea patients. On the other hand, 17 out of 31 Cryptosporidiosis cases or 54.83% were asymptomatic. Seven *Cyclospora spp* oocyst (4.32%) were also identified including one mixed infection with *Cryptosporidium spp*. (Figure-3,4) Statistical analysis by Graph-Pad-Prism showed significant association between Cryptosporidiosis and chronic diarrhea ($P<0.0001$, by Chi square test, $X^2=30.17$). (Table-1). Socio-demographic characteristics of these patients are studied and there was no significant association between cryptosporidiosis and age ($P=0.9737$), sex ($P=0.7392$), marital status ($P=0.3815$), sexual orientation ($P=0.0502$), education ($P=0.1561$) and income ($P=0.5068$). (Table-2). WHO-staging of the disease also had no significant association with cryptosporidiosis ($P=0.1650$). (Table-1). In patients with low CD4+T cells < 200 cells/µL, 50% prevalence of Cryptosporidiosis was seen (11cases out of 22 such patients) whereas in patients with CD4+T cells > 200 cells/µL, 14.28% prevalence of Cryptosporidiosis was detected (20 cases out of 140 such patients). Low CD4+T-cell count had significant association with prevalence of Cryptosporidiosis ($P<0.0001$ by Chi square test, $X^2=26.88$). (Table-1). Eight patients had pulmonary tuberculosis, one had genital herpes, one was HBsAg reactive and three VDRL reactive(>1:8dilution).

DISCUSSIONS:

Cryptosporidiosis is one of the common opportunistic infections among HIV/AIDS patients and also included as an AIDS-defining illness. In India according to few studies and reports, prevalence of cryptosporidiosis ranges from 4.7% -56.5%. [15] In our study overall prevalence of Cryptosporidiosis was 19.13% but much higher 60.86% prevalence of Cryptosporidiosis was seen among only chronic diarrhea patients. Similar findings were seen in a study by Gupta S *et al*, where 30% HIV patients presented with chronic diarrhea and 55.8% of them were detected with intestinal parasites. [16] Among them 76.35% were coccidian parasites comprising of *Cryptosporidium spp* (23.6%) and *Cystoisospora belli* (50%), unlike our study finding where no *Cystoisospora belli* was detected among the study participants. [16]While studies from South India by Kaniyarakkal V *et al* and Swathiranjan CR *et al* have recorded *Cystoisospora belli* as most common opportunistic coccidian parasitic infections among HIV/AIDS patients, studies from North India, by Mohandas *et al* have reported 10.8% prevalence of *Cryptosporidium spp* as the most common cause. [1,17, 18] Similar studies from Eastern India by Saha S *et al* have also showed 12.5% prevalence of cryptosporidiosis as most common cause of parasitic diarrhea among these patients similar to our study. [19] So prevalence of cryptosporidiosis and other coccidian enteric parasites varies depending on regional and local endemicity.

From a study on HIV/AIDS patients by Wanyiri J W *et al,* about sociodemographic profile and clinical features of Cryptosporidiosis in Kenya, Cryptosporidium spp was the most common enteric pathogen and was identified from 34% of HIV/AIDS patients regardless of whether they had diarrhea or not, which was quite high prevalence. [20] They also found that there was no significant difference in CD4+ T-cell counts among *Cryptosporidium spp*. infected patients with or without diarrhea. [20]

An Indian study by Gupta K *et al* have clearly shown that HIV patients with CD4+T cells < 200 cells/µL were having 59.3% prevalence of enteric parasites and those with > 200 cells/µL were having much lesser 23.5% prevalence. [21] In our study also much higher ie 50% prevalence of Cryptosporidiosis was seen with HIV/AIDS patients with low CD4+T cells < 200 cells/µL and in patients with CD4+T cells > 200 cells/µL, 14.28% prevalence of Cryptosporidiosis was seen. Hence significant association was statistically found between low CD4+T cell counts and cryptosporidiosis by Chi square test ($X^2=26.88$, $P=<0.0001$). Study by Janagond A B *et al* also supported that with low CD4+T cell counts, cryptosporidiosis infection among HIV/AIDS patients was high. [22]

Four types of diarrhea are usually seen with cryptosporidiosis infections - chronic diarrhea, cholera like illness requiring intravenous oral rehydration treatment, transient diarrhea and intermittent diarrhea. [15] In our study 14.19% patients presented with chronic diarrhea. Presence of chronic diarrhea was the second most important association with cryptosporidiosis infection in our study ($P<0.0001$, by Fisher's exact test). Also in study by Gupta S *et al*, diarrhea was significantly associated with prevalence of opportunistic enteric parasites. [16] In our study there was high rate of asymptomatic cryptosporidiosis (54.83%). Even among low CD4+T-cell count (<200) asymptomatic cases were 45.45%. However low CD4+T-cell

count was significantly associated with prevalence of Cryptosporidiosis with or without diarrhea.

CONCLUSIONS:

Cryptosporidiosis infection is common among HIV/AIDS patients and may be asymptomatic or may present with diarrhea. History of chronic diarrhea and low CD4+T-cell counts were significantly associated with cryptosporidiosis infection. As asymptomatic cryptosporidiosis is high among these patients, so care must be taken during examination of stool. Stool microscopy is a very useful method for identification of *Cryptosporidium spp* and other coccidian parasites which needs expertise for interpretation from wet mount and modified acid fast stained smears, but don't need any costly reagents, kits or instruments for identification like ELISA or PCR. So for resource poor set up, it is a very cost effective way of identification. Burden of cryptosporidiosis varies from place to place. So prevalence studies can help estimate the actual burden of this disease among these patients. As cryptosporidiosis is an AIDS-defining illness and it can cause severe illness, so proper identication, reporting and appropriate treatment is essential.

TABLES:

Table-1: Evaluation of data on history of chronic diarrhea, WHO stage and CD4+T cell counts among total HIV/AIDS patients under study and those with Cryptosporidiosis and their association with Cryptosporidiosis:

Parameters studied		Total no of stool samples from HIV/AIDS patients (n=162)	Cryptosporidium oocyst detected (n=31)	% prevalence (overall prevalence =19.13%)	Chi square test X^2 (95%CI)	*P*-value
History of chronic diarrhea	Present	23	14	60.87%	Chi square test X^2=30.17, df=1	**<0.0001**
	Absent	139	17	12.23%		
WHO staging	1	125	22	17.60%	Chi square test for trend X^2=1.928, df=1	0.1650
	2	22	4	18.18%		
	3	10	3	30.00%		
	4	5	2	40.00%		
CD4+T cell count (cells/µL)	0-100	6	4	66.67%	Chi square test X^2 =26.88	**<0.0001**
	100-200	16	7	43.75%		
	200-350	29	10	34.48%		
	350-500	38	4	10.52%		
	>500	73	6	8.22%		

Table-2: Evaluation of socio-demographic parameters studied among total HIV/AIDS patients under study and those with Cryptosporidiosis and their association with Crytosporidiosis:

Socio-demographic Parameters studied		Total no of stool samples from HIV/AIDS patients (n=162)	Cryptosporidium oocyst detected (n=31)	% prevalence (overall prevalence =19.13%)	Fisher's exact test / Chi square test X^2 (95%CI)	*P*-Value
Sex	Male	95	19	20.00%	Chi square test X^2 =0.110, df=1	0.7392
	Female	67	12	17.91%		
Age	18-24 yrs	37	8	21.62%	Chi square test X^2=0.2234, df=3	0.9737
	25-40 yrs	51	9	17.64%		
	40- 60 yrs	53	10	18.87%		
	>60yrs	21	4	19.05%		
Marital status	Married	105	18	17.14%	Chi square test X^2 =0.766, df=1	0.3815
	Unmarried	57	13	24.52%		
Sexual orientation	MSM/ Homosexual	6	3	50.00%	Chi square test X^2 =3.836, df=1	0.0502
	Heterosexual	156	28	17.95%		
Education	No formal education	20	6	30.00%	Chi square test X^2 =3.714, df=2	0.1561
	School	107	16	14.95%		
	college	35	9	25.71%		
Income (Rs)	<1000	40	5	12.50%	Chi square test X^2 =3.314, df=4	0.5068
	1000-5000	64	15	23.43%		
	5000-10000	29	4	13.79%		
	10000-20000	18	5	27.78%		
	>20000	11	2	18.18%		

FIGURES:

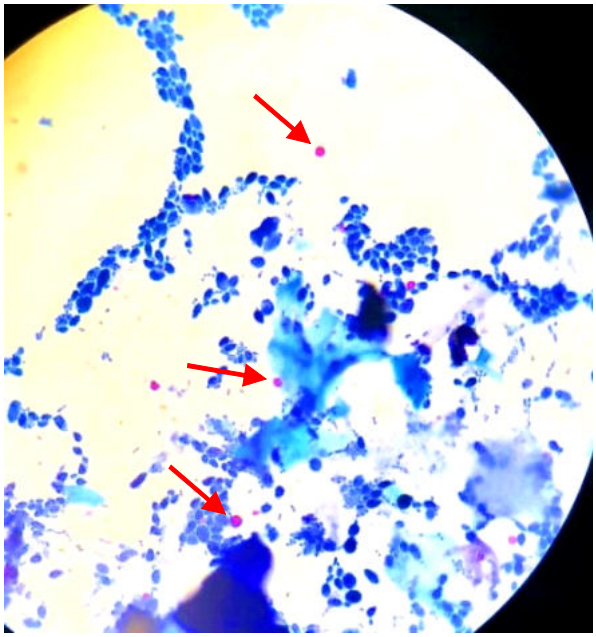

Figure-1: *Cryptosporidium sp* oocyst stained with modified acid fast stain, showing bright red oocyst (4µm diameter) pointed out by red arrows in a blue background having yeast cells. (1000X magnification)

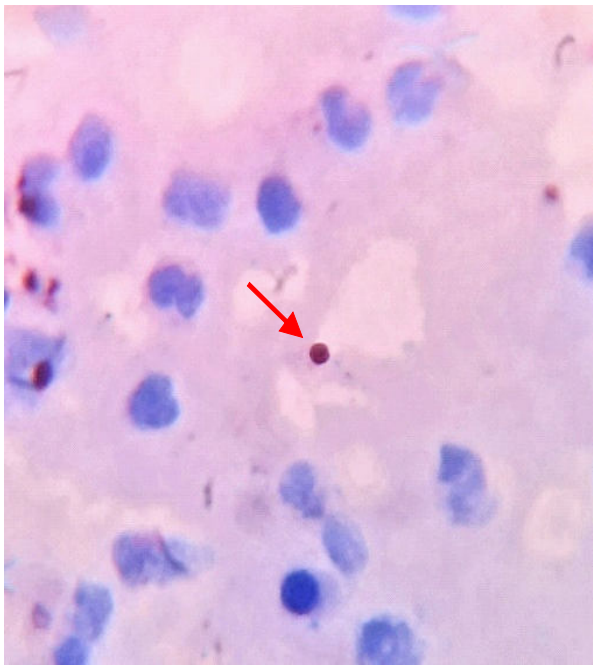

Figure-2: *Cryptosporidium sp* oocyst stained with modified acid fast stain, showing bright red oocyst (4µm) with sporozoites visible inside the oocyst. (1000X magnification)

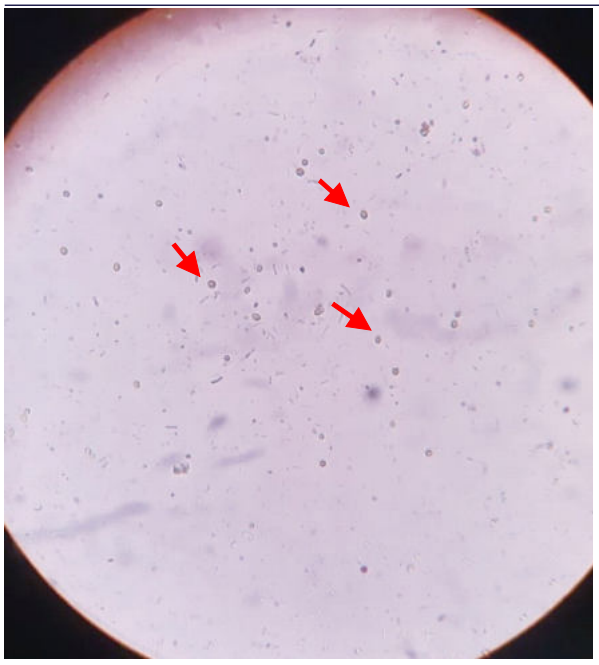

Figure-3: *Cryptosporidium sp* oocyst in unstained wet mount (saline mount), oocyst are 4 µm in diameter. (400X magnification)

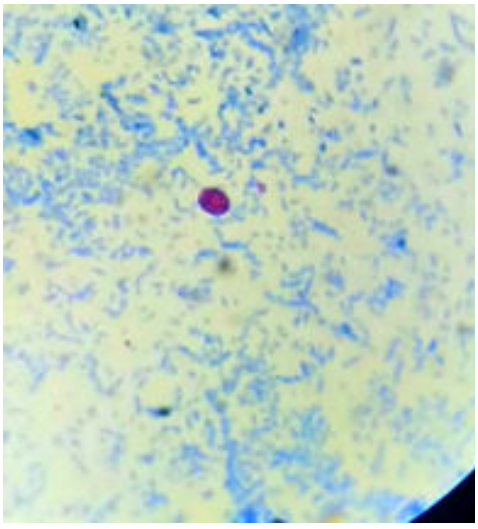

Figure-4: *Cyclospora cayetanensis* oocyst stained red by modified acid fast stain, oocyst is 7.5 µm diameter. (1000X magnification)

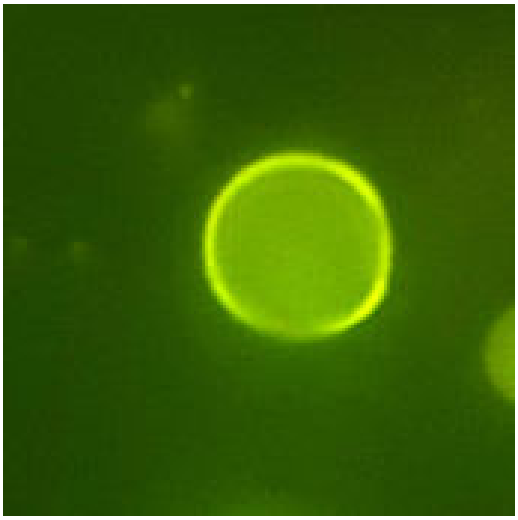

Figure-5: *Cyclospora cayetanensis* oocyst in UV microscopy showing autofluorescence at blue excitation (450-490 nm)

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