

Prevalance of Intestinal Parasites Especially Cryptosporidium in HIV Seropositive Patients & its Corelation with CD4+ Count & HAART Therapy in Tertiary Care Hospital

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Abstract: Enteric Parasites are related to gastrointestinal disturbances especially diarrhoea among HIV seropositive patients. The manifestations were more severe before institution of HAART therapy. Present study was done to investigate the relationship between intestinal parasitic infections & CD4+ T cell count & diarrhoea in HIV seropositive patients on HAART therapy. Retrospective study was done on 1067 stool samples collected from HIV seropositive patients from April 2014 to august 2018. Stool samples were collected & processed using direct wet mount, formal ether concentration & modified Ziehl Neelsen staining technique. Blood samples were collected & analysed for CD4+ T cell counts by flow cytometry. **Results:** The overall prevalence of intestinal parasites was 23.4% (250 of 1067), highest being of Cryptosporidial oocysts 21.1% followed by Giardia 1.1%, Entamoeba spp 0.75% & Hook worm eggs were detected in 0.47% of cases. There was decrease in parasitic detection over the period. The maximum parasitic isolation was in the patients whose CD4 cell counts were below 200 cells/ μ l. This study showed the significant prevalence of intestinal parasites in HIV seropositive patients. Although the study is limited in scope, however, it does reflect the importance of evaluating the prevalence of intestinal parasites in HIV positive/ AIDS patients especially at the local level & provide vital information for health professionals who are managing these patients. This could lead to improvement in patients' management and care.

Keywords: Prevalence, parasitic infection, HIV-positive seropositive patients, diarrhoea, patients

1. Introduction

Human Immunodeficiency Virus (HIV) has become a major public health problem. AIDS is the end stage of Human Immunodeficiency Virus infection, which leads to decrease in the immunity level in affected persons. Due to the impaired immunity, these persons become more liable to infection with opportunistic microorganisms (Aruna Aggarwal et al., 2005).

Opportunistic infections in HIV infected patients vary from region to region (Ayyagari A et al., 1999). As per National AIDS Control Organisation (NACO) data, tuberculosis is the most common infection in AIDS patients followed by candidiasis and cryptosporidiosis (NACO, 1999). Parasitic infections are more common in developing countries than developed countries (FrammSR et al., 1997). The etiological agents of gastroenteritis include infections with bacteria, parasites, virus and fungi (Mitra AK et al., 2001). Intestinal parasites have been reported major cause of severe chronic diarrhea in HIV infected patients (Janoff EN et al., 1988). Among all the parasites coccidian parasites, Cryptosporidium parvum, Isospora belli, Cyclospora cayetanensis and Entamoeba histolytica are the most

common etiological agents causing diarrhea in HIV infected persons worldwide (Ekejindu IM et al., 2010; Hammouda NA et al., 1996).

Cryptosporidium parvum is an apicomplexan parasite causing self limiting diarrhoea in immunocompetent patients, but causes gastroenteritis/diarrhoea and malnutrition, which ultimately leads to significant morbidity and mortality, particularly in people living with human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS). Studies have shown that about 30–60 % of AIDS patients in developed countries and 90 % in developing countries experience diarrhea [1]. Infection is mainly transmitted by oocysts which are excreted in human & animal faeces both domestic & wild animals. Main source of infection is usually contaminated food or water & commonest mode of infection is by ingestion of contaminated food or water. Even direct person to person transmission & through contaminated surface can also occur.

The present study was done to evaluate the prevalence of Cryptosporidiosis in HIV patients & its corelation with immune status of HIV seropositive patients. Early institution of HAART in such patients help in maintaining the immune

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status & helps in control of opportunistic infections.

A major problem of public health concern is the fact that the oocysts are only 4 to 6 μ m in diameter, much too small to be easily removed by sand filters used to purify drinking water [13]. In addition the oocyst may remain viable for 3 to 6 months in moist environment and is extremely resistant to disinfectants such as chlorine.

2. Materials & Methods

The retrospective study was done at GMC Asr From April 2014 to Aug. 2018 for the period of about four & half years. All the patients who were HIV seropositive in our ICTC, GMC Amritsar, by using Strategy 3 as per NACO guidelines, having diarrhea & whose CD4+ count was below 200 cells/ μ l. were included in the study. All patients not fulfilling the criteria were excluded from the study.

Stool samples were collected in clean, wide mouth plastic container & transferred to Microbiology Lab immediately after collection. Stool samples were examined for presence of trophozoites, cysts & oocysts using direct, formal ether sedimentation and modified ZN staining technique.

CD4+T cell count was also done by Flowcytometry by FACS counter for evaluation of immune status of HIV infected patients.

3. Result

Out of all 1067 patients, there was male preponderance, 770 (72.2%) were male patients & 297 (23.8%) were female patients. (Table 1) 799 (74.9%) patients presented with symptoms of diarrhea & rest 268 (25.1%) were asymptomatic. Out of 1067 patients, 225 (21.1%) were positive for cryptosporidial oocysts. Asymptomatic carriage was seen in 26 (2.4%) patients out of total 225, rest 199 detected positive for cryptosporidial oocysts presented with symptoms of diarrhea. (Table 3)

Other intestinal parasites detected were Giardia intestinalis in 12 patients (1.1%), Entamoeba histolytica in 8 patients (0.75%), Hook worm eggs in 5 patients (0.47%)

Year wise positivity for cryptosporidial oocyst was seen in descending order being 61 (29.4%) in 9 months period of 2014, 60 (24.1%) in 2015, 42 (18.4%) in 2016, 37 (16.6%)

in 2017 & 25 (15.5%) in 8 months duration of 2018. (Table 1)

CD4+ counts showed increasing trend over the period of four & half year duration. Mean CD4+ count was 51 cells/ μ l. in 2014, 67 cells/ μ l. in 2015, 71 cells/ μ l. in 2016, 123 cells/ μ l. in 2017 & 148 cells/ μ l in 2018. (Table 1). Maximum positivity was seen in 26-35 years age group (122), followed by 36-45 years age group (46), 46-55 years age group (26), 16-25 years age group (21) & minimum positivity in 56-65 years age group (10). (Table 4)

4. Discussion

With the emergence of AIDS, diarrhoea especially parasitic diarrhoea has increased significantly. Because of suppressed immune status of patient, morbidity & mortality associated with diarrhoea is also significant. The line of treatment is different for different parasitic infections & necessitates a definitive diagnosis and study of the etiological agents causing diarrhea, especially when it can be fatal in this vulnerable group of individuals.

Present study has found the prevalence of Cryptosporidium infection in HIV seropositive patients presenting with & without diarrhea & its correlation with CD4+ cell count. All patients from Jan. 14 were on HAART therapy.

Overall average prevalence noted is 21.1% with minimum of 15.5% in 2018 & maximum of 29.4% in 2014. Similar results were seen in studies carried out by **Soumendra Nath Maity et al (2015)** ; Basak et al (2010); Sadraei et al (2005); Tuli et al (2008) and Mohandas et al (2002). Prevalence of 18.4% in HIV patients was also reported in a similar study done in our department (Aruna et al 2005)

During this period, the patients CD4 cell count has improved from Mean value of 51 cells/ μ l. in 2014 to 148 cells/ μ l. in 2018. Our findings also matches with other studies done in India. Decreasing prevalence of Cryptosporidiosis & improved immune status of seropositive patients is mainly because of free easily available antiretroviral treatment to the patients. Before 2009, HAART therapy was started at CD4 count below 200 cells/ μ l., thereafter before 2013, it was 350 cells/ μ l. & after that all patients with CD4 count below 500 cells/ μ l are put on treatment. As per latest guidelines of 2017, all patients are to be treated irrespective of CD4+ cell count.

Table 1: Sex & Symptom wise distribution of Patients

Period	Mean CD4+ Cell count	Males		Females		Total	
			Positive for cryptosporidial oocysts		Positive for cryptosporidial oocysts		Total Positivity
April 14 – Dec. 14	51 cells/ μ l.	146 (70.5%)	40	61 (29.5%)	21	207	61 (29.4%)
Jan. 15 – Dec. 15	67 cells/ μ l.	181 (72.9%)	41	67 (27.1%)	19	248	60 (24.1%)
Jan. 16 – Dec. 16	71 cells/ μ l.	166 (72.8%)	31	62 (27.2%)	11	228	42 (18.4%)
Jan. 17 – Dec. 17	123 cells/ μ l.	161 (72.1%)	27	62 (27.9%)	10	223	37 (16.6%)
Jan. 18 – Aug. 18	148 cells/ μ l.	116 (72.1%)	17	45 (27.9%)	8	161	25 (15.5%)

Table 2: Intestinal Parasites detected in HIV seropositive patients

Total patients (Year wise)	Cryptosporidium oocyst detected	Giardia spp	Entamoeba histolytica	Hook worm eggs
207 (2014)	61	3	2	1
248 (2015)	60	2	2	2
228 (2016)	42	3	1	Nil
223 (2017)	37	2	1	1
161 (2018)	25	2	2	1
1067	225 (21.1%)	12 (1.1%)	8 (0.75%)	5 (0.47%)

Table 3: Distribution of *Cryptosporidium* infection in relation to clinical symptoms among HIV seropositive patients

Total patients	Symptomatic	+ve for oocysts	Asymptomatic	+ve for oocysts
207	158	54	49	7
248	200	55	48	5
228	172	36	56	6
223	150	33	73	4
161	119	21	42	4
Total-1067	799 (74.9%)	199 (18.7%)	268 (25.1%)	26 (2.4%)

Cryptosporidium infection was highly associated with diarrhoea (Table 2) Infection was highest among patients with diarrhoea duration between 3-5 days (74.9%) Diarrhoea is the major symptom in cryptosporidiosis which was confirmed in this study as other similar studies have done (Ajjampur et al, 2008. & Gupta S et al, 2008). Reason for this may be because in HIV infection, Diarrhoea is a major sign of progression to AIDS.

Regarding the duration of diarrhoea, patients with diarrhoea that lasted between 3-5 days had higher prevalence of *Cryptosporidium*. This may be because cryptosporidiosis causes short self limiting watery diarrhoea which can resolve as immune status recovers and most patients enrolled in this study were on HAART which helps boost immune status. A no. of studies have reported a decline in morbidity & hospitalisation after receiving HAART. **Wasma**

Table 4: Distribution of *Cryptosporidium* Infection According to different Age Group

Age in Years	2014		2015		2016		2017		2018		Total	
	No. of Patients	Positive	No. of Patients	Positive	No. of Patients	Positive	No. of Patients	Positive	No. of Patients	Positive	No. of Patients	Positive
16-25	21	4	26	6	22	5	20	4	19	2	108	21
26-35	94	37	117	35	103	23	97	15	74	12	485	122
36-45	63	11	75	13	71	6	69	11	42	5	320	46
46-55	23	6	25	5	27	6	30	5	21	4	126	26
56-65	6	3	5	1	5	2	7	2	5	2	28	10
Total	207	61	248	60	228	42	223	37	161	25	1067	225

5. Conclusion

The present study shows the decrease in prevalence of *Cryptosporidium* infection in HIV seropositive patients with increase in CD4+ cell count indicating its opportunistic nature. As asymptomatic carriage was also seen which indicates the source of infection in surroundings, personal hygiene measures are important for patients of high risk. Early initiation of HAART therapy may reduce the prevalence of *Cryptosporidium* infection by boosting immunity, as is followed as per new guidelines of treatment.

Prasituebsai et al who studied the children population in Asia reported decline in opportunistic infections though these OI s remain the important cause of morbidity & mortality

Maximum positivity was seen in age group of 26-35 years followed by 36-45 years. Persons in this age group are from reproductive age & highly active socially. High risk of HIV infection increases the chances of getting opportunistic infections especially Cryptosporidiosis.

Male preponderance was seen in the study (72.2%) with higher positivity in males. Females are more ignorant of their health & possibly less often report illness. Predominance of male cases may also be due to their migration to the major cities in search of work. Staying away from the families for longer periods and males being promiscuous by habit resulted in them, acquiring HIV infection.

There was a significant association between *Cryptosporidium* infection and CD4+T-cell count.

There was inverse relation between CD4+ cell count & *Cryptosporidium* detection. Prevalence was highest in patients with CD4+T-cell count less than 200 cells/ μ l. This finding is similar to that of Assefa *et al.* and Gupta *et al.* that showed that, the rate of parasitic infection decreases with increase in CD4+T-cell count. This indicates that there is low opportunity for this parasite to get established as the patients CD4 +T-cell count increases.

However detection of other intestinal parasites, such as *E histolytica*, *Giardia* & Hook worm eggs was much less in this study. Previous studies conducted in the department showed the higher detection of these parasites. The patients were taking empirical treatment in the form of metronidazole & other supportive therapy only. Detection of these pathogens, though very less number indicates the poor hygienic measures & poor quality of life.

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