



Research Article

STUDY OF INTESTINAL PARASITIC INFECTIONS AMONG HIV INFECTED PATIENTS AT A RURAL MEDICAL COLLEGE, AMBAJOGAI, MAHARASHTRA

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Abstract- HIV pandemic is spreading rapidly all over the world. India and china reported the maximum number of cases within Asia. Most common parasitic diseases causing diarrhoea in HIV infected patients are cryptosporidiosis, isosporiasis, cyclosporiasis and microsporidiosis etc. The prevalence of HIV infection differs from place to place and the pattern of parasitic infections also varies as well. This study is conducted to assess the pattern and prevalence of parasitic infections in HIV positive patients at a rural medical college of Maharashtra. The most common HIV infected age group was 21-30 years both in case of males as well as in females, followed by the age group of 31-40 years. Most common parasite isolated was *Cryptosporidium parvum* followed by *Giardia lamblia*. More than 1/3rd of the stool samples were positive for intestinal parasites. Most patients presented with complaints of diarrhoea. Diarrhoeal disease is very prevalent in HIV infected patients. Our study focuses on the aim to increase awareness about the high prevalence of intestinal parasitic infections in HIV infected patients. So routine stool examination is necessary to check for presence of intestinal parasites, that will help in implementing control measures and will reduce the morbidity and mortality in HIV infected patients.

Keywords- Intestinal parasites, *Cryptosporidium parvum*, *Giardia lamblia*, Human Immunodeficiency Virus, Diarrhoea

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Introduction

The most disastrous disease of recent time is HIV infection [1]. HIV infection is showing a rising trend in whole world, especially in developing countries like Africa, Asia. India and China reported the highest number of HIV cases in Asia [2]. As HIV infection decreases the CD4count which causes irreparable damage to the immune system, and the infected individual gets susceptible to various kind of opportunistic infection [3]. There may be resurgent disease like tuberculosis or it may be completely new diseases like Pneumocystis, Toxoplasma, cryptosporidiosis, isosporiasis, cyclosporiasis and microsporidiosis [4,5]. 30-60% of the HIV infected in developed countries and 90% in developing countries presents with diarrhoea due to intestinal parasitic infections [6]. HIV pandemic spreading rapidly all over the world, approximately 14000 new cases occurring daily [7]. A high incidence of parasitic infections is seen in immunocompromised patients such as those with AIDS. These parasites never seemed to cause any major problem earlier because people who acquired them had the defence mechanisms to fight them off. Parasites like Pneumocystis, Toxoplasma, *Isospora* and *Cryptosporidium* can really cause extensive damage in immunocompromised patients [8]. As the prevalence and most common parasitic infections in HIV positive patients differ from place to place so this study was conducted to assess the pattern and prevalence of parasitic infestations in rural area of Maharashtra.

Material and Methods

The study was carried out in the Department of Microbiology at SRTGMCH from January 2018 to June 2018. 152 samples were taken from HIV positive patients attending the ART clinic, presenting for the first time or with recurrent attacks of loose motions along with passage of mucus and/or blood, vomiting, abdominal pain, weight loss was also investigated. Detailed clinical history was noted. Past history of diarrhoea, drug treatment, radiological procedures (Barium studies) or any major illness were noted. Patients who were on antiparasitic drugs were

excluded from our study. All stool specimens were collected in a suitable, clean, wide mouthed plastic container. Liquid stools, soft stools, hard stools were examined or preserved within 30 minutes, 1 hour, 24 hours of passage respectively. Examination of fresh specimens was necessary for observing motility of protozoan parasites [9]. Stool was grossly examined for its consistency, color and odour, presence of blood or mucus or macroscopic presence of roundworm, pinworm, or tapeworm proglottids [9]. Saline and Iodine mounts were prepared and were examined systematically with low power objective; if suspicious objects were noticed switch to high power objective to observe details. Saline preparation was employed to demonstrate worm eggs, larvae, protozoal trophozoites as well as RBCs and WBCs. Iodine mount was employed to demonstrate the cysts under high power objective. The stained cysts were examined for nuclei, glycogen mass, chromatid bodies [10]. Protozoan cyst stained with iodine show yellow- gold cytoplasm, brown glycogen material, and pale refractile nuclei. For detection of parasites, a combination of methods was applied as different methods serve different purposes. All specimens were further processed by concentration method: Formal ether sedimentation technique. Modified Zeihl Neelsen staining was done for detection of *Isospora* and *Cryptosporidium* etc. After the complete processing of stool specimen was over 3 parts of 10% formalin were added to one part of feces for preservation. After treatment with 10% formal saline, screw capped tops were fitted tightly and the containers were stored at room temperature [9,10].

Results

The samples from the male patients predominated over the samples from the female patients. Out of 152 patient's sample, 104 (68.42%) were from male patients and 48 (31.58%) were from female patients. 64 patients (42.11%) belonged to the age group of 21- 30 years, 45 (29.61%) were males and 19

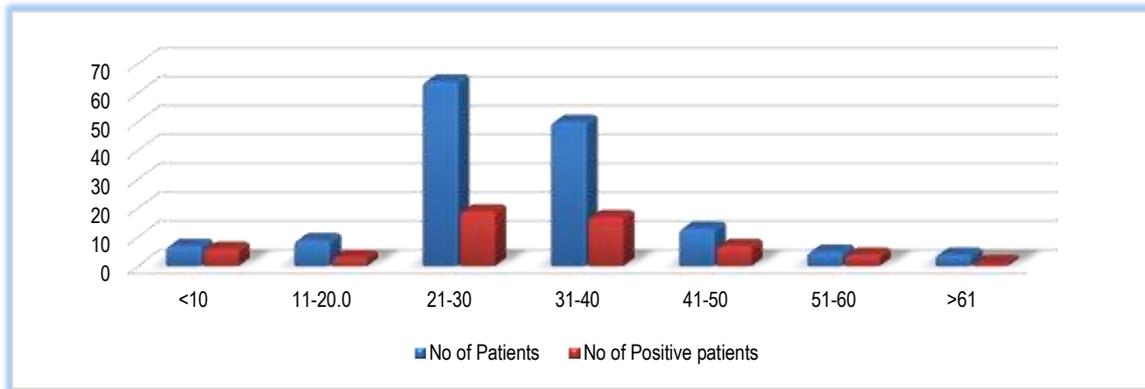


Fig-1 Age and sex distribution of Patients and positive samples in HIV patient

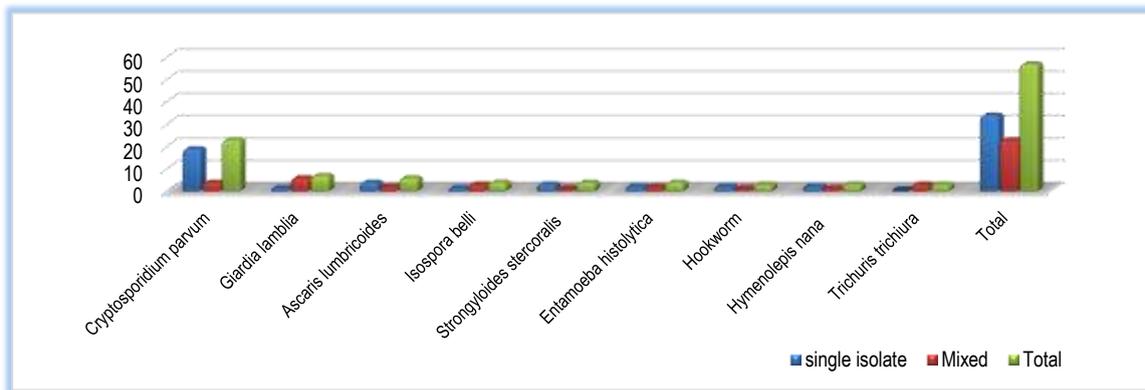


Fig-2 Intestinal parasites detected by Formol ether sedimentation technique in HIV patient

(12.50%) were females, followed by 50 (32.89%) patients were in 31- 40 years age group, 33 (21.71%) were males and 17 (11.18%) were females. Lowest number of patients was seen in the age group of 61 years and above (2.63%). From the pediatric age group of 0- 10 years, a total of 07 (4.60%) patients were seen, 03 (1.97%) were males and 04 (2.63%) were females [Fig-1].

Table-1 Age and sex distribution of patients with HIV/AIDS and diarrhea

Age in years	Males	%	Females	%	Total	%
< 10	3	1.97%	4	2.63%	7	4.60%
Nov-20	7	4.61%	2	1.32%	9	5.93%
21-30	45	29.61%	19	12.50%	64	42.11%
31-40	33	21.71%	17	11.18%	50	32.89%
41-50	9	5.92%	4	2.63%	13	8.55%
51-60	4	2.63%	1	0.66%	5	3.29%
61 and above	3	1.97%	1	0.66%	4	2.63%
Total	104	68.42%	48	31.58%	152	100%

No. of patients (HIV positive): 152

Table-2 Various clinical presentations of patients, [No of patients (n): 152]

SN	Signs and symptoms	No of cases	% (Total subjects)
1	Diarrhoea/ Loose motion	72	47.37
2	Abdominal discomfort/ Pain in abdomen	34	22.37
3	Weight loss	12	7.89
4	Anaemia	12	7.89
5	Miscellaneous	11	7.24
6	Routine/ No specific symptoms	4	2.63
7	Chronic pain abdomen	4	2.63
8	Loss of appetite	3	1.97
	Total	152	100

Out of the 152 HIV infected patients, 72 (47.37%) patients presented with Diarrhoea/loose motion followed by 34 (22.37%) patients presented with abdominal discomfort/pain in abdomen. 12 (7.89%) patients presented with Weight loss and 12 (7.89%) patients presented with anemia. A total of 11 (7.24%) patients presented with miscellaneous symptoms.

Different enteric parasites isolated from stool samples of HIV infected individuals are shown in [Table-3]. *Cryptosporidium parvum* was most commonly observed intracellular protozoan parasite i.e. in 23 (15.13%) samples, either alone in 19(12.50%) samples or as mixed infestation in 4 (2.63%) samples.

Table-3 Intestinal parasites detected in stool of HIV patients (by Formol ether sedimentation technique), Number of patients:152

Parasites detected	Isolated	%	Mixed	%	Total	%
<i>Cryptosporidium parvum</i>	19	12.5	4	2.63	23	15.13
<i>Giardia lamblia</i>	1	0.66	6	3.95	7	4.61
<i>Ascaris lumbricoides</i>	4	2.63	2	1.32	6	3.95
<i>Isospora belli</i>	1	0.66	3	1.97	4	2.63
<i>Strongyloides stercoralis</i>	3	1.97	1	0.66	4	2.63
<i>Entamoeba histolytica</i>	2	1.32	2	1.32	4	2.63
Hookworm	2	1.32	1	0.66	3	1.97
<i>Hymenolepis nana</i>	2	1.32	1	0.66	3	1.97
<i>Trichuris trichiura</i>	0	0	3	1.97	3	1.97
Total	34	22.37	23	15.13	57	37.5

The other intracellular protozoan parasite isolated was *Isospora belli* was observed in 04 (2.63%) samples, either alone in 1 (0.66%) sample or as mixed infestation in 03(1.97%) samples. Most commonly observed extracellular protozoan parasite was *Giardia lamblia*, was present in 07 (4.61%) samples, either alone in 1 (0.66%) sample or as mixed infestation in 6 (3.95%) samples. *Entamoeba histolytica* was present in 4 (2.63%) samples, out of which in 02 (01.32%) samples *Entamoeba histolytica* was present as mixed infestation. Among the helminths *Ascaris lumbricoides* was isolated from 6 (3.95%) samples. Hookworm was present in 3 (1.97%) samples, out of which in 01 (0.66%) sample Hookworm was present as mixed infestation. *Strongyloides stercoralis* was present in 4(2.63%) samples. *Trichuris trichiura* and *Hymenolepis nana* was observed in 3 (1.97%) samples each [Fig-2].

Table-4 Mixed parasitic infestations in different combinations (in HIV positive patients), No of patients (HIV positive):152

1	<i>Giardia lamblia</i> + <i>Ascaris lumbricoides</i>	2
2	<i>Giardia lamblia</i> + <i>Cryptosporidium parvum</i> + <i>Entamoeba histolytica</i>	1
3	<i>Giardia lamblia</i> + <i>Cryptosporidium parvum</i>	1
4	<i>Giardia lamblia</i> + <i>Entamoeba histolytica</i>	1
5	<i>Giardia lamblia</i> + <i>Trichuris trichiura</i>	1
6	<i>Isospora belli</i> + <i>Trichuris trichiura</i>	2
7	<i>Cryptosporidium parvum</i> + <i>Hymenolepis nana</i>	1
8	<i>Cryptosporidium parvum</i> + <i>Isospora belli</i>	1
9	<i>Strongyloides stercoralis</i> + Hookworm	1
	Total	11

[Table-3] shows mixed parasitic infestations in different combination, was observed in 11 (7.24%) out of 152 samples. The combinations showed *Giardia lamblia* was the most frequently found parasite. *Giardia lamblia* was observed in combinations, along with *Ascaris lumbricoides* in 2 samples, along with *Cryptosporidium parvum* and *Entamoeba histolytica* in 1 sample, along with *Cryptosporidium parvum* alone in 1 sample, along with *Entamoeba histolytica* in 1 sample and along with *Trichuris trichiura* in 1 sample. In 2 samples *Isospora belli* was observed along with *Trichuris trichiura*. *Cryptosporidium parvum* was observed along with *Hymenolepis nana* in 1 sample and along with *Isospora belli* in 1 sample. *Strongyloides stercoralis* was found along with Hookworm in 1 sample.

Table-5 Age and sex distribution of positive samples in HIV patients, No of positive samples: 57

Age in years	Males	%	Females	%	Total	%
<10	2	3.51	4	7.02	6	10.53
Nov-20	2	3.51	1	1.75	3	5.26
21-30	16	28.07	3	5.26	19	33.33
31-40	11	19.3	6	10.52	17	29.82
41-50	7	12.28	0	0	7	12.28
51-60	4	7.02	0	0	4	7.02
61 years and above	1	1.75	0	0	1	1.75
Total	43	75.44	14	24.56	57	100

[Table-5] shows age and sex distribution of positive samples. Parasitic infestations were more common in male patients i.e. 43(75.44%) patients as compared to female patients i.e. 14 (24.56%) patients. Age group between 21-30 years had highest positivity i.e. 19 (33.33%) patients, followed by the age group between 31-40 years i.e. 17 (29.82%) patients [Fig-1].

Discussion

The parasites causing diarrhoea in HIV infected person either can be known enteric parasite like, *Entamoeba histolytica*, *Giardia lamblia* and *Strongyloides stercoralis* or may be opportunistic parasite like, *Cryptosporidium*, Microsporidia, *Isospora* and *Cyclospora*. In immunocompetent patients it is less likely that they will suffer from parasitic infection but with the rising trend of HIV/AIDS the scenario changed. The Endemicity of a particular parasite in a local area is a responsible factor for the pattern and rate of parasitic infection in the immunocompromised patients. Samples were collected from 152 HIV positive patients and of them 37.5% samples were positive for intestinal parasite. Samples were collected predominantly from 21-30 years (42.11%) male (68.42%) patients. These findings are similar with Manish Kumar Mathur *et al.* [13]. Mostly parasitic infection is asymptomatic or it may cause self-limiting diarrhoea if immunity is preserved. But in immunocompromised individual parasitic infection can cause prolonged diarrhoea which can cause death if not treated timely. In our study the most common presenting complaint was diarrhoea. Other Indian studies like studies by Prasad *et al.* (2000) and Nilesh Chavan *et al.* also mentioned the same as most common presenting complaint [14,15]. 37.5% of the collected sample was positive for intestinal parasite in our study. Few other studies reported very high rate of intestinal parasitic infection like Pape *et al.* from Peru reported infection rate of 57.33%. In the year 1993, Cotte *et al.* in France revealed 70.6% prevalence, Brandonisio *et al.* in Italy reported the same to be 27.92% [16-18]. Multiple studies in India reported the prevalence of parasitic infection in HIV positive patients. Prasad *et al.* (2000) from Lucknow, Mohandas *et al.* (2002) from Chandigarh, Kumar *et al.* (2002) from Chennai reported prevalence >50%, 30% and 30% respectively [19,20]. There are multiple factors contributing to the difference in prevalence of parasitic infection like, habit of personal hygiene and sanitary practices, difference in regional distribution of the parasites, varied method of stool examination, patients with different level of immunity. Even the same patients may acquire multiple parasitic infections due to the above-mentioned causes. In our study the predominant parasite was *Cryptosporidium* (15.13%). In Brazil, Moura *et al.* (1989) reported prevalence of *Cryptosporidium* was 18.2% in HIV infected patients whereas Cimerman *et al.* (1999) reported the same as 7% [21,22]. In a study by Chacin *et al.* in Venezuela reported 41.3% prevalence [23]. Among Indian studies Anand *et al.* (1996) from Canchipur (Manipur), Prasad *et al.* (1995-98) from Lucknow, Mohandas *et al.* (2002) from Chandigarh, Kumar *et al.* from

Chennai reported prevalence of 46.6%, 11%, 13% and 12% respectively [14,19,20,24]. Around the globe 10-20% diarrhoea is caused by *Cryptosporidium* in HIV infected patients [20]. Several studies reported prevalence of *G. lamblia* infection in a range of 1-11% [19]. Our study observed a similar rate of infection (4.61%). Similar to a study by Manish Kumar Mathur *et al.* [4]. Our study observed prevalence of *Isospora belli* infection was 2.63%. There is high reported prevalence of *Isospora belli* infection in HIV infected patients from several countries like, Brazil (9.9%), Zaire (12%), Zambia (16%) and Haiti (12%) [25]. In HIV infected patients with chronic diarrhoea *I. belli* reported to be a very common etiological agent [26]. The proper estimation of intestinal parasitic infection in HIV positive patients is a challenge because there may be asymptomatic shedding oocyst or patients may be treated with trimethoprim sulphamethoxazole for any other reason [20]. 3.95% samples were positive for *Ascaris lumbricoides*. Yonatan Kindie *et al.* reported prevalence of 20% in urban males and 6.67% in rural males [27]. Mixed parasitic infestations were observed in 11 (7.24%) out of 152 samples. 23 parasites (15.13% of the total samples) were isolated from 11 samples. The combinations showed *Giardia lamblia* was the most frequently found parasite in combinations, along with *Trichuris trichiura* in 1 sample and along with *Entamoeba histolytica* in 1 sample. Our study finding was similar to a study done by Jaishreepuri *et al.* in the same area. Dibua *et al.* reported in 2013, high incidence of mixed parasitic infestations in HIV positive patients [28].

Conclusion

To summarize the present study indicates that the coccidian parasites are the most important agents of diarrhoea among HIV positive individuals. Our study focuses on the aim to increase awareness about the high prevalence of intestinal parasitic infections in HIV infected patients. So routine stool examination is necessary to check for presence of intestinal parasites, that will help in implementing control measures and will reduce the morbidity and mortality in HIV infected patients.

Application of research: To evaluate prevalence and pattern of I intestinal parasitic infection among HIV infected patients in rural area of Maharashtra. So proper screening can be done and therapy can be started early to cut down the morbidity and mortality.

Research Category: Medical parasitology

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University: Maharashtra University of Health Sciences, Nashik, 422004

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Study area / Sample Collection: Department of Microbiology, SRT Rural Govt Medical College, Ambajogai, 431517

Conflict of Interest: None declared

Ethical approval: Ethical approval taken from SRT Rural Govt Medical College, Ambajogai, 431517, Maharashtra University of Health Sciences, Nashik, 422004, Maharashtra, India.

Ethical Committee Approval Number: SRTRGMC/Phar/IEC/229/17

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