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Prevalance of Enteric Parasites among Hiv Seropositive Patients and Evaluation of Different Concentration Techniques

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Abstract

Introduction: Human immunodeficiency virus (HIV) infection is a global pandemic, with cases reported virtually from every country. The gastrointestinal involvement in HIV/AIDS is almost universal, and intestinal parasitic infection remains as an important cause of morbidity and mortality in developing countries. If the number of parasites in the stool specimens are low, examination of a direct wet mount may not detect parasites, hence the stool should be concentrated either by sedimentation or floatation techniques. Therefore the present study was undertaken to determine the prevalence of enteric parasites among HIV seropositive patients with and without diarrhea and to evaluate different concentration techniques for their detection. *Material and Method:* A total of 510 HIV seropositive patients were included in the study. Stool specimens were collected in wide mouthed, clean, dry, plastic containers with tight fittings lids. Microscopic examination was done by direct saline and iodine mount. All the specimens were subjected to formol ether centrifugal sedimentation technique and zinc sulphate centrifugal floatation technique for concentration of parasitic ova and cysts. Sheather's sugar floatation technique was performed for detection of coccidian parasites. The data obtained was analyzed by applying appropriate statistics wherever needed. *Result and Observations:* Out of 510 HIV seropositive patients, intestinal parasites were detected in 132 (25.88%) cases. A total of 149 intestinal parasites were isolated from 132 patients of which 116 patients showed single parasite and 16 showed mixed infections. Detection rate of intestinal parasites by saline and iodine mount was 17.06%. The rate of detection of intestinal parasite by formol ether sedimentation technique and zinc sulphate centrifugal floatation technique was 26.67 % and 10.20 % respectively. A total of 78 (15.29%) coccidian parasites were detected by Sheather's sucrose floatation technique. *Conclusion*: Routine screening of the stool samples of all HIV seropositive patients with diarrhoea and without diarrhoea should be done for prompt patient care, to prevent the fulminant form of the disease. Along with saline and iodine mount, formol ether centrifugal sedimentation technique and Sheather's Sucrose floatation technique can be used to increase diagnostic sensitivity.

Keywords: HIV; Diarrhoea; Concentration Technique; Coccidian Parasites.

Introduction

Human immunodeficiency virus (HIV) infection is a global pandemic, with cases reported virtually from every country [1]. HIV has posed a major challenge to public health in the present time. Since 1981, when it first began to spread widely, HIV has caused the deaths of 25 million people worldwide. Globally an estimated 35.3 (32.2–38.8) million people were living with HIV in 2012 with 2.3 (1.9-2.7) million new HIV infections [2].

The gastrointestinal involvement in HIV/AIDS is almost universal, and a significant disease occurs in 50-90% of the patients while diarrhoea can be a presenting manifestation or life threatening complication in HIV patients [3]. The aetiological spectrum of the enteric pathogens includes bacteria, parasites, fungi and viruses [4]. Intestinal parasitic infection remains as an important cause of morbidity and mortality in developing countries [5]. The broad spectrum of the diseases which are caused by intestinal parasites in HIV patients range from asymptomatic infestations to severe life threatening diarrhoea, dehydration and mal-absorption [6].

If the number of parasites in the stool specimens are low, examination of a direct wet mount may not detect parasites, hence the stool should be concentrated either by sedimentation or floatation technique [7]. Various concentration techniques are used for detection of parasites such as formol ether centrifugal sedimentation technique, zinc sulfate centrifugal floatation technique. Therefore the present study was undertaken to determine the prevalence of enteric parasites among HIV seropositive patients with and without diarrhoea. An attempt was also made to evaluate different concentration techniques for their detection.

Aim and Objectives

1. To study the prevalence of intestinal parasitic infections among HIV seropositive patients.
2. To compare different concentration techniques for detection of intestinal parasites.

Material and Method

The present study was conducted in the Department of Microbiology at S.R.T.R. Govt. Medical College Ambajogai for period of two years from Oct. 2012 to Sep. 2014. Ethical clearance from institutional ethical committee was obtained.

A total of 510 HIV seropositive patients were included in the study. HIV seropositive patients, who had received antiparasitic treatment for diarrhoea in past 3 weeks, were excluded from study. Stool specimens were collected in wide mouthed, clean, dry, plastic containers with tight fittings lids. The patients were asked to collect their stool sample preferably in the morning. No attempt was made to isolate bacteria and viruses.

The specimens were examined by naked eye for colour, consistency, presence of blood, mucus and adult or segments of worms. Microscopic examination was done by direct saline and iodine mount. All the specimens were subjected to formol ether centrifugal sedimentation technique and zinc sulphate centrifugal floatation technique for concentration of parasitic ova and cysts. From formol ether centrifugal sedimentation technique, smears were prepared to identify the coccidian parasites by using modified Ziehl-Neelsen stain and examined with oil immersion objective. Sheather's sucrose floatation technique was performed for detection of coccidian parasites. The data obtained was analyzed by applying appropriate statistics wherever needed.

Result and Observations

In the present study, 510 HIV seropositive patients were screened for intestinal parasites amongst which, males (54.12 %) were more as compared to females (45.88%). Maximum numbers of the patients were seen in the age group of 31-40 years followed by 21-30 years. Out of 510 HIV seropositive patients, intestinal parasites were detected in 132 (25.88%) cases. A total of 149 intestinal parasites were isolated from 132 patients of which 116 patients showed single parasite and 16 showed mixed infections. In our study, most commonly observed intestinal parasite was *Cryptosporidium parvum* followed by *Isospora belli* and least commonly observed parasites were *Hymenolepis nana* and *Taenia spp*.

In our study, detection rate of intestinal parasites by saline and iodine mount was 17.06%. Among 87 isolates, *Cryptosporidium parvum* was most commonly observed parasite followed by *Entamoeba histolytica*.

A total of 136 intestinal parasites were detected either alone or in mixed infection among 119 patients by formol ether centrifugal sedimentation technique. 16 (3.14%) patients showed mixed parasitic infections and the combination of *Cryptosporidium parvum* and *Isospora belli* was most frequently observed. Overall *Cryptosporidium parvum* was most frequently

observed parasite in mixed combination.

In our study, 52 (10.20%) intestinal parasites were detected among 51 patients by zinc sulphate centrifugal floatation technique. In only one patient mixed parasitic infection was seen with a combination of *Giardia lamblia* and *Ascaris lumbricoides*. The rate of detection of intestinal parasite by formol ether sedimentation technique and zinc sulphate centrifugal floatation technique was 26.67 % and 10.20 % respectively and difference was found to be statistically significant.

A total of 78 (15.29%) coccidian parasites were detected by Sheather's sucrose floatation technique, among which 56 were *Cryptosporidium parvum* and 22 were *Isospora belli*. Detection rate of *Cryptosporidium parvum* and *Isospora belli* was more by Sheather's sucrose floatation technique as compared to formol ether centrifugal sedimentation technique but difference was statistically non significant.

Table 1: Distribution of intestinal parasites among HIV seropositive patients (n=510)

Intestinal Parasites	Single	%	Mixed	%	Total	%
Cryptosporidium parvum	46	9.02	10	1.96	56	10.98
Isospora belli	15	2.94	07	1.37	22	4.31
Entamoeba histolytica	18	3.53	02	0.39	20	3.92
Giardia lamblia	09	1.76	04	0.78	13	2.55
Ancylostoma duodenale	10	1.96	01	0.20	11	2.16
Ascaris lumbricoides	07	1.37	04	0.78	11	2.16
Strongyloides stercoralis	05	0.98	05	0.98	10	1.96
Hymenolepis nana	03	0.59	00	0.0	03	0.59
Taenia spp.	03	0.59	00	0.0	03	0.59
Total	116	22.75	33	6.47	149	29.22

Table 2: Various intestinal parasites detected by saline and iodine wet mount (n=510)

Intestinal parasites	Isolates	
	Number	Percentage (%)
Intracellular Protozoans		
Cryptosporidium parvum	26	5.10
Isospora belli	09	1.76
Extracellular Protozoans		
Entamoeba histolytica	16	3.14
Giardia lamblia	13	2.55
Helminths		
Ancylostoma duodenale	05	0.98
Ascaris lumbricoides	06	1.18
Strongyloides stercoralis	08	1.57
Hymenolepis nana	02	0.39
Taenia spp.	02	0.39
Total	**87**	**17.06%**

Table 3: Various intestinal parasites detected by formol ether centrifugal sedimentation technique (n=510)

Intestinal Parasites	Single	%	Mixed	%	Total	%
Intracellular Protozoans						
Cryptosporidium parvum	39	7.65	10	1.96	49	9.61
Isospora belli	11	2.16	07	1.37	18	3.53
Extracellular Protozoans						
Entamoeba histolytica	18	3.53	02	0.39	20	3.92
Giardia lamblia	08	1.57	04	0.78	12	2.35
Helminths						
Ancylostoma duodenale	10	1.96	01	0.20	11	2.16
Ascaris lumbricoides	07	1.37	04	0.78	11	2.16
Strongyloides stercoralis	05	0.98	05	0.98	10	1.96
Hymenolepis nana	02	0.39	00	0.00	02	0.39
Taenia spp.	03	0.59	00	0.00	03	0.59
Total	103	20.20	33	6.47	136	26.67

Table 4: Various intestinal parasites detected by zinc sulphate centrifugal floatation technique (n=510)

Intestinal parasites	Single	%	Mixed	%	Total	%
Extracellular protozoa						
Entamoeba histolytica	18	3.53	00	0.0	18	3.53
Giardia lamblia	12	2.35	01	0.20	13	2.55
Helminths						
Ancylostoma duodenale	10	1.96	00	0.0	10	1.96
Ascaris lumbricoides	07	1.37	01	0.20	08	1.57
Hymenolepis nana	03	0.59	00	0.0	03	0.59
Total	**50**	9.80	02	0.40	52	10.20

Table 5: Coccidian parasites detected by Sheather's sucrose floatation technique (n=510)

Coccidian Parasites	No. of Isolates	%
Cryptosporidium parvum	56	10.98
Isospora belli	22	04.31
Total	**78**	**15.29**

Table 6: Comparative results of formol ether centrifugal sedimentation and zinc sulphate centrifugal floatation technique (n=510)

Intestinal parasites	Formol ether centrifugal sedimentation technique		Zinc sulphate centrifugal floatation technique	
	Total	%	Total	%
Intracellular Protozoans				
Cryptosporidium parvum	49	9.61	0	0
Isospora belli	18	3.53	0	0
Extracellular Protozoans				
Entamoeba histolytica	20	3.92	18	3.53
Giardia Lamblia	12	2.35	13	2.55
Helminths				
Ancylostoma duodenale	11	2.16	10	1.96
Ascaris lumbricoides	11	2.16	08	1.57
Strongyloides stercoralis	10	1.96	0	0
Hymenolepis nana	02	0.39	03	0.59
Taenia spp.	03	0.59	0	0
Total	136	26.67	52	10.20
Z= 10.083, P= <0.001, significant at p <0.005				

Table 7: Comparative findings of formol ether centrifugal sedimentation and Sheather's sucrose floatation technique for detection coccidian parasites (n=510)

Coccidian parasites	Formol ether Centrifugal sedimentation Technique		Sheather's sucrose floatation technique	
	Total	%	Total	%
Cryptosporidium parvum	49	9.60	56	10.98
Isospora belli	18	3.53	22	4.31
Total	67	13.13	78	15.29
X^2 =0.003 p< 0.05, not significant				

Discussion

Acquired immunodeficiency syndrome (AIDS) ranks among the most dreaded diseases affecting mankind, causing dysfunction of both limbs of the immune system, resulting in overwhelming and fatal opportunistic infections. Gastrointestinal involvement primarily in the form of diarrhoea is a universal problem affecting almost 90% of HIV infected patients in developing countries [8]. The etiological spectrum of the enteric pathogens which cause diarrhoea includes bacteria, parasites, fungi and viruses [9]. The parasites can cause self-limiting diarrhoea of short duration in healthy individuals, but in the immunocompromised host including AIDS patients, the diarrhoea is usually chronic and sometimes, life-threatening [10]. The coccidian parasites are foremost among the enteric parasites in HIV seropositive patients with diarrhoea [11].

In our study, prevalence of intestinal parasites among HIV seropositive patients was 25.88% (132/510). The results comparable to our study were shown

by Mehta K et al [12], Gupta M et al [13], Gupta S et al [14] and Mohandas K et al [15] while Babatunde S K et al [16], Dwivedi KK et al [8], and Basak S et al [17] have documented higher prevalence. The prevalence of intestinal parasitic infections among HIV seropositive patients ranged from 17.3% to 87.8% in different parts of the world. The difference in the pattern of prevalence of intestinal parasites seen in the present study and others may be attributed to the regional variability (demographic and ecological factors) of the pathogen, behavioural activities, diagnostic methods used, asymptomatic shedding of oocysts and the use of prophylactic drugs etc.

The diagnosis of intestinal parasites in stool was established by identification of ova and cysts by variety of techniques including direct wet mount preparation, sedimentation and floatation method.

In our study, the detection rate of intestinal parasites by saline and iodine mount was 17.06% while Parameshwarappa KD et al [18] (38%) and Mergani MH et al [19] (28.19%) found higher detection rate by saline and iodine mount in their study. If the number of organisms in the stool specimens is low, examination of a direct wet mount may not detect parasites, hence the stool should be concentrated. Eggs, cysts and larvae are recovered after concentration procedures whereas trophozoites get destroyed during the concentration procedure. This makes direct wet mount examination obligatory as the initial phase of microscopic examination [7].

We used different concentration techniques i.e. formol ether centrifugal sedimentation technique, zinc sulphate centrifugal floatation technique and Sheather's sucrose floatation technique to detect the maximum number of intestinal parasites from patients.

In our study, the detection rate of intestinal parasite by formol ether centrifugal sedimentation technique was 26.67%. Comparable detection rates were reported by Abbas M et al [20] (19.35%), Puri J et al [21] (26.75%) and Mergani H et al [19] (30.16%) while higher rate of detection was reported by Parameshwarappa KD et al [18](56.88%) and Balakrishna J et al [22] (83%).

In the present study, 116 HIV seropositive patients showed single parasite and 16(3.14%) showed mixed infections. Similar to our study, Anand B et al [23] and Gupta S et al [14] in their study reported mixed infection in 3% and 3.53% of patients respectively, whereas Awole M et al [24], Kotgire S et al [25], Fekadu S et al [26] and Kashyap B et al [27] showed 14.06%, 8%, 10.6% and 11% mixed infections among HIV seropositive patients respectively. Mixed infections is a common observation in areas where various types of parasites are prevalent and also due to poor hygienic practices.

The combination of *Cryptosporidium parvum* and *Isospora belli* was most frequently observed. Overall *Cryptosporidium parvum* was most frequently observed parasite in mixed infections. Similar to our study, Amatya R et al [28] noted *Cryptosporidium parvum* as most commonly observed parasite in mixed combination. While Anand B et al [23] and Gupta S et al [14] in their study showed *Isospora belli* was most frequently observed parasite in mixed combination.

In the present study, by zinc sulphate centrifugal floatation technique, 52 intestinal parasites were detected from 51 patients and mixed infection was seen in only one patient with a combination of *Ascaris lumbricoides* and *Giardia Lamblia*. The detection rate of intestinal parasites by zinc sulphate centrifugal floatation technique was 10.20%. Mergani H et al [19] (26.88%), Abbas M et al [20] (36.64%) and Parameshwarappa et al [18] (55%) noted a higher detection rate of intestinal parasites as compared to our study. Oocysts of *Cryptosporidium parvum*, *Isospora belli*, eggs of *Taenia spp* and larvae of *strongyloides stercoralis* are not detected by zinc sulphate centrifugal floatation technique. These parasites were maximally detected in our study, so this may be the reason for low detection rate of intestinal parasites in zinc sulphate centrifugal floatation technique.

In the present study for detection of coccidian parasites, we performed Sheather's sucrose floatation technique, as it is recommended for the detection of coccidian parasites [7]. Sheather's sucrose floatation technique detected 78 (15.29%) coccidian parasites amongst which 56 (10.98%) were *Cryptosporidium parvum* and 22 (4.31%) were *Isospora belli*. Mergani H et al [19] and Scott et al [29] detected 3.60% and 18.1% *Cryptosporidium parvum* by Sheather's sucrose floatation technique respectively.

In our study, 67 coccidian parasites were detected by formol ether centrifugal sedimentation and 78 parasites were detected by Sheather's sucrose floatation technique. The Sheather's sucrose floatation technique detected slightly more number of coccidian parasites compared to formol ether centrifugal sedimentation technique. However, this difference was not statistically significant. So we can say that for the detection of coccidian parasites, formol ether centrifugal sedimentation technique followed by modified Z-N staining is as effective as Sheather's sucrose floatation technique. Similar findings were shown by Mergani H et al [19] and Scott et al [29] in their study for detection of *Cryptosporidium parvum*.

In the present study, oocysts of Cyclospora were not detected. This can be attributed to varying geographical distribution of parasites.

No intestinal parasites were detected in 166 patients with diarrhoea in the present study which may be attributed to other diarrheaogenic agents like bacteria, viruses and fungi. The identification of which was not done in our study. In our study, significant number of intestinal parasites were also seen in HIV seropositive patients without diarrhoea thus indicating that there may be asymptomatic infections which may be going undiagnosed, thereby increasing the morbidity and mortality which are associated with them.

Conclusion

Detection of intestinal parasites in HIV seropositive patients will help in proper management of these patients as drugs are available for the treatment of most of the infections. Hence, routine screening of the stool samples of all HIV seropositive patients with diarrhoea and without diarrhoea should be done for prompt patient care, to prevent the fulminant form of the disease. Along with saline and iodine mount, formol ether centrifugal sedimentation technique and Sheather's Sucrose floatation technique can be used to increase diagnostic sensitivity.

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