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PREVALENCE OF INTESTINAL COCCIDIAN PARASITES IN IMMUNOCOMPROMISED PATIENTS IN A TERTIARY CARE CENTRE IN ASSAM

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ABSTRACT
Purpose: *To determine the prevalence of intestinal coccidian parasites in immunocompromised patients with or without diarrhoea at our centre.* ***Materials and Methods:*** *Stool samples were collected from 62 immunocompromised patients with diarrhoea and they were examined for the presence of coccidian parasites by microscopy. Also, 42 control samples from immunocompromised patients without diarrhoea were collected and examined.* ***Results:*** *The prevalence of intestinal coccidian parasites among the 62 immunocompromised patients with diarrhoea was found to be 12.9%. Overall, Cryptosporidium (12.9%) was the most frequently encountered pathogen in the immunocompromised patients with diarrhoea followed by Ancylostoma duodenale (6.5%), Cyclospora (4.8%), Ascaris lumbricoides (4.8%) and 1.6% each of Isospora and Trichuris trichiura. In the control group comprising the immunocompromised patients without diarrhoea, no opportunistic infection was seen by any of the coccidian parasites. The association of coccidian parasites with diarrhoea was significant (P < 0.01) as compared to non coccidian parasites (P > 0.1) .Among all the coccidian parasites, only Cryptosporidium spp. showed significant association with diarrhoea (P < 0.02).* ***Conclusion:*** *This study concluded that among the various intestinal coccidian parasites causing opportunistic infections in immunocompromised patients at our centre, Cryptosporidium spp. was the most predominant coccidian parasite strongly associated with diarrhoea.*

KEY WORDS
Intestinal coccidian parasites, Immunocompromised, Cryptosporidium, Diarrhoea

INTRODUCTION

Intestinal parasitic infections are an important public health problem in the tropical areas, particularly in developing countries like India, where the humid climate, the insanitary environment, and poor socioeconomic conditions aggravate the problem. Parasitic infections are a major cause of morbidity in developing countries and are increasingly gaining importance in developed countries among certain populations, particularly in those with Human Immunodeficiency Virus (HIV) seropositivity and Acquired Immunodeficiency Syndrome (AIDS) [1].

Diarrhoea is a common complication in immunocompromised patients namely AIDS, organ transplants, patients with malignancies and those having malnutrition but occur with greatest frequency in patients with AIDS [2]. In immunocompromised patients, progressive decline in their immunological responses makes them extremely susceptible to a variety of common and opportunistic infections. In recent years, numerous studies have outlined the emergence of important gastrointestinal protozoa mainly belonging to subclass coccidia like *Cryptosporidium* spp. , *Isospora belli*, and *Cyclospora cayetanensis* [3,4,5]. Since the diarrhoeal illness due to intestinal coccidian parasitic etiology among the immunocompromised patients is on the rise during recent times, the present study was conducted to find out the prevalence of intestinal coccidian parasites among the immunocompromised patients attending our centre.

MATERIALS AND METHODS

Study design

A case control prospective study was carried out amongst a total of 104 patients comprising of 62 immunocompromised patients with diarrhoea (cases) and 42 immunocompromised patients without diarrhoea (controls) admitted in the various departments of a tertiary care hospital in Assam. The samples were processed in the Department of Microbiology in a period of one year between September 2012 to August 2013.

Data collection

All the patients enrolled in the study were interviewed using standard questionnaire to collect clinical data after their due consent in accordance with institutional ethical guidelines. Particulars of the patients, observations and findings were recorded in a predesigned proforma and these were compiled and analysed at the end of the study.

WHO definition of the diarrhoea was taken for study purpose. Diarrhoea is defined as the passage of three or more loose or liquid stools per day (or more frequent passage than is normal for the individual). It can be further categorized into two groups, acute (< 2 weeks) and chronic or persistent (> 2 weeks) diarrhoea [6].

Inclusion criteria

1) HIV seropositive individuals, clinically diagnosed protein energy malnutrition children, cancer patient on chemotherapy and diabetic patients were included in the study.

2) Symptomatic patients presenting with diarrhoeal illness were included as the cases and patients without diarrhoea were included as the controls.

3) The cases were further categorized into two groups, acute and chronic. Those presenting with symptom of diarrhoea for less than 2 weeks were categorized as acute and those presenting with diarrhoea for more than 2 weeks were categorized as chronic.

Exclusion criteria

Patients from whom the fresh stool samples could not be obtained or if they refused to participate in the study were excluded from this study.

Sample Collection

From each patient, faecal specimen was collected in a sterile plastic container and transported to the laboratory within one hour for parasitological study. All the patients were advised to give two freshly voided stool specimens for two consecutive days but those patients who submitted at least one stool specimen were also included in the study.

Laboratory Diagnosis

Presence of intestinal parasites in the patients were confirmed by examination of stool by fresh wet mounts, formal ether concentration technique and modified Ziehl Neelsen (Z.N) staining. Fresh and concentrated stool specimens were examined as saline wet mounts to detect motile trophozoites, larva, cysts and ova etc. Iodine wet mounts were used particularly for staining of nuclei and glycogen mass of cysts of common intestinal protozoan parasites. Air dried smears from fresh stool samples were fixed and stained by modified Z.N. stain to detect the coccidian parasites. Formol ether concentration technique was used to concentrate the parasites. After concentration technique, the stool samples were again examined by saline wet mount, iodine preparation and modified Z.N.stain.

All stained slides were reviewed at least 200 fields using light microscopy at 100X (oil). All positive slides were confirmed by a second observer. The coccidian parasites were identified by their modified acid fast staining, size, and overall morphological features.

Data analysis

Statistical analysis was done using Chi-square test to evaluate association between diarrhoea and parasitic infections. Values were considered to be statistically significant if P <0.05 was obtained.

RESULTS

Out of a total of 104 patients comprising the study population, enteric parasites were detected in 23 (22.1%) stool samples, of which 8(7.7%) were opportunistic and 15 (14.4%) were non opportunistic. The overall prevalence of intestinal coccidian parasites among the 62 immunocompromised

patients with diarrhoea (cases) was found to be 12.9%. No coccidian parasites were found in the control group.

In 62 immunocompromised patients symptomatic for diarrhoea, 35 had acute diarrhoea and 27 had chronic diarrhoea. Among the 35 patients of acute diarrhoea, only 2 patients (5.7%) showed infection with coccidian parasites. On the other hand, 6 patients (22.2%) out of 27 patients with chronic diarrhoea were detected with coccidian parasitic infection. However, the association of coccidian parasites with the chronicity of diarrhoea was found to be non significant (P >0.05).

Among 8 cases of coccidian parasitic infestation, 5 cases presented with only *Cryptosporidium* infestation (21.7%) and 3 cases with mixed infections. All the 3 cases of mixed infection were present in patients with chronic diarrhoea (**Table-1**).

Table 1: Parasite profile of study population

Name of the Parasites	Number*
Ascaris lumbricoides	4(17.4%)
Ancylostoma duodenale	9 (39.1%)
Trichuris trichiura	2 (8.7%)
Cryptosporidium spp	5 (21.7%)
Cryptosporidium spp + Cyclospora + Ascaris lumbricoides	1(4.3%)
Cryptosporidium spp + Cyclospora	1(4.3%)
Cryptosporidium spp + Cyclospora + Isospora	1(4.3%)

*Figures within bracket shows the percentage distribution of parasites

Table 2: Parasites identified in Diarrhoeal (Cases) and Non-Diarrhoeal (Control) patients

Study group	Diarrhoeal*	Non-diarrhoeal
No. of patients	62	42
Cryptosporidium spp. **	8(12.9%)	-
Cyclospora	3(4.8%)	-
Isospora	1(1.6%)	-
Ascaris lumbricoides	3(4.8%)	2(4.8%)
Ancylostoma duodenale	4(6.5%)	5(11.9%)
Trichuris trichiura	1(1.6%)	1(2.4%)

*The association of coccidian parasites with diarrhoea was significant, P <0.01; ** P <0.02

Table 3: Prevalence of Coccidian Cases according to clinical profile

Immunocompromised Groups	Total Cases	%	Coccidian Cases	Prevalence (%)
HIV / AIDS	24	38.7	3	12.5
Malignancy	22	35.5	3	13.6
PEM	11	17.7	2	18.2
Diabetes	5	8.1	0	0
Total	**62**	**100**	**8**	**12.90%**

Table 4: Age profile of patients with Coccidian parasites

Age in Years	Clinical profile			Total	%
	HIV / AIDS	Malignancy	PEM		
0-10	-	1	2	3	37.5
11-20	-	1	-	1	12.5
21-30	1	-	-	1	12.5
31-40	1	1	-	2	25
41-50	1	-	-	1	12.5
Total	**3**	3	**2**	**8**	**100**

Among the immunocompromised patients with diarrhoea, the overall parasitic infection showed *Cryptosporidium* (12.9%) as the most frequently encountered pathogen, followed by *Ancylostoma duodenale* (6.5%), *Cyclospora* (4.8%), *Ascaris lumbricoides* (4.8%) and 1.6% of *Isospora* and *Trichuris trichiura* each. In the control group the maximum prevalence was of *Ancylostoma duodenale* (11.9%) followed by *Ascaris lumbricoides* (4.8%) and *Trichuris trichiura* (2.4%) (**Table-2**).

The association of coccidian parasites with diarrhoea was significant ($P < 0.01$) as compared to non coccidian parasites ($P > 0.1$) .Among all the coccidian parasites, only *Cryptosporidium spp.* showed significant association with diarrhoea ($P < 0.02$) while the association of *Cyclospora* ($P > 0.1$) and *Isospora* ($P > 0.1$) with diarrhoea were found to be insignificant. The prevalence of coccidian cases according to clinical profile of the patients has been shown in **Table-3**.

The age distribution of patients with intestinal coccidian parasitic infection ranged from 1.5 to 45 years. The maximum number of patients positive for coccidian parasites were in their first decade of life (n=3, 37.5%). The prevalence of coccidian parasites is same in both the sexes (50% each) (**Table-4**). The CD4 cell counts of HIV seropositive patients in whom intestinal coccidian parasites were isolated showed that majority of patients (n=2, 66.7%) had counts <50 cells/µl.

DISCUSSION

Opportunistic infection by coccidian parasites is a major health problem in immunocompromised patients worldwide. Though the illness caused due to these organisms is usually self limiting, they can cause life threatening infections including diarrhoea in immunocompromised patients [8]. Hence, this study was undertaken to study the prevalence of enteric coccidian parasites in immunocompromised patients at our centre.

In the present study the maximum numbers of patients positive for coccidian parasites were in their first decade of life. Previous indian studies reported that most of the HIV patients with coccidian parasitosis were in the age group of 31-40 years [5, 9]. The maximum number of patients in our study was in their first decade of life due to high prevalence of coccidian parasites among malnourished children.

Various Indian studies reported a male preponderance of cryptosporidiosis [4, 10]. The difference in our finding of sex distribution and those of other studies is due to the prevalence of cryptosporidiosis among malnourished children. If we exclude malnourished children from our study, we also observe the male predominance (60%) when compared to female (40%).

Overall, *Cryptosporidium spp.* was the most prevalent enteric coccidian parasite (12.9%) followed by *Cyclospora* (4.8%) and *Isospora* (1.6%) among the 62 immunocompromised patients with diarrhoea. In this study, among all the three coccidian parasites, only the *Cryptosporidium spp.* showed significant

association with diarrhoea ($P < 0.02$). This is in accordance with the earlier studies from north India in which *Cryptosporidium spp.* was found to be the most common enteric coccidian parasite [10, 13].

In the present study, 3 (12.5%) out of 24 individuals having HIV / AIDS were found to be *Cryptosporidium* positive. Our findings are comparable to various previous studies from United States, Thailand and India reporting prevalence of Cryptosporidium in HIV / AIDS patients [7, 5, 10, 11, 12,].

All the 3 retropositive patients suffering from coccidian parasitosis had CD4 T cell count below 100/µl. This finding is in agreement with the previous studies which have also reported increased prevalence of coccidian infection in patients with CD4 T cell count less than 200/µl(R)[13,14].

Several studies have investigated the epidemiology of *Cryptosporidium* infection in patients with malignant disease and reported variable prevalence. Prevalence of *Cryptosporidium spp.* in patients with malignancy in our study (13.6%) corresponds closely to the findings of a study from Turkey[15]. Studies even reported prevalence findings less than that found in our study [16,17]. However, a high prevalence has been reported in a study from Karnataka [18].

Cryptosporidium is more common and more severe in malnourished children. Our prevalence of *Cryptosporidium spp.*(18.2%) and *Cyclospora* (9.1%) in malnourished children mimics various other studies done in developing countries[19,20,21,22].

The prevalence of cyclosporiasis in HIV infected patients with diarrhoea in our study has been found to be 8.3% and it corresponds to the study from Rajasthan where *Cyclospora* was isolated from 8.6% cases of HIV positive patients with diarrhoea [23]. Another study from south India reported 5.8% prevalence of *Cyclospora* in HIV infected patients with diarrhoea which is nearly similar to our study [24].

In the present study, *Isospora* was isolated from 4.2% of HIV-positive patients with diarrhoea. While some studies even reported higher prevalence of *Isospora belli* than *Cryptosporidium spp.* [5, 25, 26], in this study lower isolation rate was detected as compared to earlier studies which may be due to asymptomatic shedding of oocysts and treatment with trimethoprim sulphamethoxazole for other infections in AIDS cases.

No coccidian parasite could be isolated among the diabetes patients suffering from diarrhoea in our study. There are reports of three patients with diabetes suffering chronic diarrhoea due to cryptosporidiosis till date [27, 28]. A study from Egypt reported that of various immunocompromised patients group examined, the prevalence of cryptosporidiosis was lowest in those with diabetes [29].

From the above discussion, it is evident that the accurate diagnosis of coccidian parasites is a major concern. The study suggests that screening of stool samples needs to be done routinely among immunocompromised population for the presence of coccidian parasites by modified acid-fast staining. There were some limitations in our study as majority of the patients admitted at our hospital had already received antibiotics prior to their visit and therefore the number of symptomatic patients were less.

CONCLUSION

It may be concluded from our study that coccidian parasites have significant association with diarrhoea in immunocompromised patients. Their screening is not done even in known immunocompromised patients in most hospitals as many clinicians are still not aware of their clinical importance. Microbiologists can play a pivotal role in sensitising the clinicians about the significance of intestinal coccidian parasites in immunodeficient patients. The timely screening of immunocompromised patients with diarrhoea for intestinal coccidia may help in better management of these patients.

Therefore, it is suggested that steps should be taken to prevent the occurrence of these parasitic infections in immunocompromised patients by drinking safe water and maintaining proper hygiene. Early diagnosis and treatment of coccidian parasitic infections will go a long way in improving the quality of life of immunocompromised patients.

REFERENCES

1. Centers for Disease Control and Prevention. Revised classification for HIV infection and expanded surveillance case definition for AIDS. *Morb Mortal Wkly Rep* 1993; 41: 1 – 19.
2. Rotterdam H, Tsang P; Gastrointestinal disease in the immunocompromised patient. *Human Pathology* 1994;25:1123-1140.
3. Mukhopadhya A, Ramakrishna BC, Kang G. Enteric pathogens in Southern Indian HIV infected patients with and without diarrhoea. *Indian J Med Res* 1999; 109: 85 – 89.
4. Lanjewar DN, Rodrigues C, Saple DG, Hira SK, Dupont HL. *Cryptosporidium, Isospora* and *Strongyloides* in AIDS. Natl J India 1996; 9 (1): 17 – 9.
5. Kumar SS, Ananthan S, Lakshmi P. Intestinal parasitic infection in HIV infected patients with diarrhoea in Chennai. *Ind J Med Microbiol* 2002; 20: 88-91.
6. World Health Organization. Fact sheet on diarrhoeal disease. April,2013. Available at:http://www.who.int/mediacentre/factsheets/fs330/en/ (Last accessed on February 25, 2015).
7. Smith PD, Lane HC. Intestinal infections in patients with the acquired immunodeficiency syndrome (AIDS): etiology and response to therapy. *Ann Intern Med* 1988; 108: 328-33.
8. Meisel JF, Perera DR, Meligrilo C, Rubin CB. Overwhelming watery diarrhoea associated with cryptosporidium in an immunosuppresed patient. Gastroenterology 1976;70:1156-66.
9. Kashyap A, Singh MP, Ghoshal U.Occurrence of gastro-intestinal opportunistic parasites in immunocompromised patients in northern India. *Journal of Biology.*2013,vol.01,issue03,pp77-80.
10. Mohandas K, Sehgal R, Sud A, Malla N. Prevalence of intestinal parasitic pathogens in HIV – seropositive individuals in North India. *Jpn J Infect Dis* 2002; 55: 83 – 84.
11. Laughton BE, DruckmanDA. Prevalence of enteric pathogens in homosexual man with and without acquired immunodeficiency syndrome. *Gastroenterology* 1988; 94: 984 – 993.
12. Saksirisampant W, Eampokalap B, Rattanasrithong M, Lekanonsakaul S, Wisnswanitkit V, Nasingkarim. A prevalence of *Cryptosporidium* infection among Thai HIV infected patients. *J Med Assoc Thai* 2002; 85 *Suppl* 1: S 424-428.
13. Sadraei J, Rizvi MA, Baveja UK. Diarrhea, CD4+ cell counts and opportunistic protozoa in Indian HIV-infected patients.Parasitol Res 2005; 97:270-3.
14. Flanigan T, Whalen C, Turner J, Soave R, Toerner J, Havlir D,*et al*. Cryptosporidium infection and CD4 counts. Ann InternMed 1992; 116:840-2.
15. Tanyuksel M, Gun H, Doganci L. Prevalence of *Cryptosporidium spp.* in patients with neoplasia and diarrhoea. *Scan J Infect Dis* 1995; 27: 69-70.
16. Sreedharan A, Jayshree RS, Sridhar h. Cryptosporidiosis among cancer patients: an observation. *J Diarrhoeal Dis Res* 1996; 14: 211-213.
17. Menon BS, Abdullah MS, Mahmoud F, Singh B. Intestinal parasites in Malaysian children with cancer. *J Trop Paediatr* 1999; 45: 241 – 242.
18. Ballal M, Prabhu T, Chandra A, Shivnanada PG. *Cryptosporidium* and *Isospora belli* diarrhoea in immunocompromised host. *Indian J Cancer* 1999; 36 (1): 38 – 42.
19. MacFarlane DE, Horner- Bryce J. Cryptosporidiosis in well nourished and malnourished children. *Acta Pediatr Scnad* 1987; 76: 474 – 477.
20. Sallon S, Deckelbaum RJ Schmid II. *Cryptosporidium*, malnutrition and chronic diarrhoea in children. *Am J Dis Child* 1988; 142: 312-15.
21. Jaggi N, Rajeshwari S, Mittal SK, Mathur MD, Baveja UK. Assessment of the immune and nutritional status of the host in childhood diarrhoea due to *Cryptosporidium. J Common Dis* 1994; 26: 181 – 185.
22. Hoge CW, Shlim DR, Echeverria P. Cyanobacterium-like cyclospora species. *N Engl J Med*. Nov 11 1993;329(20):1504-5.
23. Nitya V, Nazeema P, Adil A. Enteric pathogens in HIV positive patients with diarrhea and their

correlation with CD4 + T-lymphocyte counts. Tropical parasitology,2012; 2(1): 29-34.

24. Venkatesh N R , Ukey PM ,Lava. Opportunistic intestinal parasitic infections in hiv seropositive patients ijpbs ,Volume2, Issue 1,jan-march, 2012, 224-230.
25. Gupta S, Narang S, Nunavath V, Singh S.Chronic diarrhoea in HIV Patients: Prevalence of coccidian parasites. Indian J Med Microbiol 2008:26:172-5.
26. Kulkarni SV, Kairon R, Sane SS, Padmawar PS, Kale VA, Thakar MR, Mehendale SM, Risbud AR,Opportunistic parasitic infections in HIV/AIDS patients presenting with diarrhoea by the level of immune suppression, Indian J of Medical Resources;2009 Jul;130(1):63-6.
27. Chan, A. W., I. A. MacFarlane, and J. M. Rhodes. Cryptosporidiosis as a cause of chronic diarrhoea in a patient with insulin-dependent diabetes mellitus. J. Infect. 1989. 19:293.
28. Perez T , S., G. Luna-Castanos, A. Matilla-Matilla, and L. Nieto-Cisneros. 1995. Chronic diarrhea and *Cryptosporidium* in diabetic patients with normal lymphocyte subpopulation. 2 case reports. *Gaceta Med. Mex*. 131:219–222.
29 .Abaza, S. M., L. M. Makhlouf, K. A. el-Shewy, and A. A. el-Moamly. Intestinal opportunistic parasites among different groups of immunocompromised hosts. J. Egypt. Soc. Parasitol. 1995. 25:713–727.

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