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Intestinal Protozoal and Helminthic Parasitic Infections in Immunocompromised Individuals

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Intestinal parasitic infections are increasing day by day in individuals with impaired mechanisms of immune system. Diarrhoea and malnutrition are the main predisposing factors in tropical countries. This study was undertaken to know the intestinal protozoal and helminthic parasitic infections in immunocompromised individuals during the year 2001.Fresh stool samples from 74 immunosuppressive individuals were collected and processed by direct examination and concentration techniques in Microbiology lab. Direct examination was performed by wet cover slip preparation and staining by modified Ziehl-Neelsen(Z-N) and Safranine-Methylene blue stains. 6 intestinal parasitic infections were detected from 74 samples which accounted for 8.1% in our study. *Cryptosporidium* infection(2) was predominant among protozoal infections followed by 2 *Asaris*,1 each of *Taenia* and *Giardia spp*. Males 60(81.1%) were predominantly affected compared to females 14(18.9%). 21-30 years age group was commonly affected in our study. HIV with Tuberculosis 18(24%) was the most predominant factor followed by malnutrition. Modified Z-N showed best results followed by Safranine-Methylene blue stain. Formal ether concentration technique showed good results followed by Zinc floatation technique.

Key words: Immunocompromised, *Cryptosporidium parvum*, Diarrhoea.

The intestinal parasitic infections are increasing in immunocompromised individuals because of decrease in defence mechanisms in them. The term immunocompromised host refers to the individual, who has one or more defective body's natural defence mechanisms that lead to life threatening infections. It may be due to tuberculosis, diabetes, Human Immunodeficiency Virus infection/Acquired Immunodeficiency Syndrome, malignancy *etc*. The diagnosis of parasitic infections of such patients continues to present multiple challenges. The prevalence of infections varies widely but appears to be the highest in the immunocompromised hosts[1].

The intestinal parasitic infections vary with the age groups and with different immunosuppressive states. They are encountered in the extreme age of life and in our country, from children with diarrhoea[2].

All these parasites cause morbidity and mortality in AIDS patients worldwide and these outcomes would be expected to be appreciably higher in developing countries due to the higher prevalence of infections in the general population[3].

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In the present study, an attempt has been made to detect the intestinal parasitic infections in immunocompromised individuals, using various staining and concentration techniques, to know the prevalence of different intestinal parasites in various age groups and in different immunosuppressive states and to assess the morbidity and mortality rate to give an effective treatment for opportunistic infections.

MATERIAL AND METHODS

The study group consisted of outpatients and inpatients of Medical, Surgical, Obstetrics and Gynaecology, Skin & STD and other departments of Vijayanagar Institute of Medical Sciences, Bellary. The study group also consisted of patients from Tuberculosis hospital, Freedom Foundation unit and Certified school.

Case selection

The clinically suspected immunocompromised individuals presenting with acute and chronic diarrhoea not responding to any routinely used antimicrobial agents were taken as cases. A total of 74 cases of individuals suffering from various immunosuppressive conditions, and 50 immunocompetent individuals with diarrhoea as controls were enrolled in the study from Jan 2001 to Dec 2001.

Specimen collection

The fresh stool samples were collected in wide mouthed, clean, dry plastic containers with tight fitting lids. The specimens after receiving were labelled properly. The specimens were processed by examination of stool sample.

Macroscopic examination

The colour, odour and consistency of the stool sample were noted. The presence of blood or mucous, live worms or segments of worms were also observed.

Microscopic Examination

Saline and Iodine preparations were done and examined under the low and high power objectives for motile trophozoites, larva, cysts, ova, pus cells, RBCs etc and nuclear characteristics & glycogen mass respectively[4].

The smears were prepared, stained by different Staining Techniques Modified Ziehl-Neelsen staining[5], lacto phenol cotton blue stain[6], Safranine methylene blue staining[7], Modified kinyoun acid fast stain[8] and examined under the oil immersion objective to look for pink-red stained oocysts about 4-5m in diameter[9-11].

Concentration techniques were done using Floatation Techniques (Simple floatation technique[6], Zinc sulphate centrifugal floatation technique[6]) and Sedimentation techniques (Simple sedimentation technique[6],Formol-ether concentration technique[5])

RESULTS

Out of 74 cases of various immunosuppressive Conditions studied, the male predominance was observed 60(81.1%) followed by female population 14(18.9%) (Table-1). The highest prevalence of males in 21-30 years age group i.e., 24 (32.4%) followed by 31-50 years. Of the females studied, 21-30 years of age group showed the highest prevalence as 8 (10.8%) (Table-2). Individuals with different immunodeficiency conditions predominated highest in HIV infection with tuberculosis 18 (24.3%) followed by malnutrition 17 (23%) and HIV infection alone 14 (19%). Other immunodeficiency conditions in the individuals studied showed varied distribution (Table-3).

Table 1. Description & Sex wise distribution among study group& controls

Study Group	Description	No. of Individuals	Sex wise	
			Male	Female
cases	Immunodeficiency individuals with diarrhoea	74	60	14
controls	Immunocompetent individuals with or without diarrhoea	50	41	9
	Total	124	101	23

In the present study the overall prevalence of parasitic infections among various immunocompromised individuals is 8.1% *i.e.*6, out of which 2(2.7%) *Cryptosporidium,* 2(2.7%) *A. lumbricoides*, 1(1.4%) *Giardia.spp* and 1(1.4%) *Taenia* were identified, among them

Table 2. Age and sex distribution among study group

Age(Years)	Male		Female		Total	
	No	%	No	%	No	%
0-10	5	6.7	1	1.35	6	8.1
11-20	3	4.1	1	1.35	4	5.4
21-30	24	32.4	8	10.8	32	43.2
31-40	12	16.2	3	4.08	15	20.3
41-50	13	17.6	1	1.4	14	18.9
>50	3	4.1	-	-	3	41.
Total	60	81.1	14	18.9	74	

X2=1.53 df=5 P>0.05 Not significant

Table 3. Distribution of individuals in different immunodeficiency conditions

S. No.	Immunodeficiency conditions	No.	%
1.	Diabetes	5	6.8
2.	HIV infection with diabetes	4	5.4
3.	Tuberculosis	1	1.4
4.	HIV infection with tuberculosis	18	24.3
5.	Malnutrition	17	23.0
6.	HIV infection with malnutrition	2	2.7
7.	Long term corticosteroid therapy	5	6.8
8.	HIV infection	14	19
9.	Acquired Immunodeficiency syndrome	7	9.5
10.	Cancer chemotherapy	1	1.4

Table 4. Identification of different parasites in various immunodeficiency conditions

S. No.	Immunodeficiency condition	No.	*Parasites Identified				
			C. parvum	Giardia spp	Taenia	A.lumbricoides	Total
1.	Diabetes	5	-	-	-	-	-
2.	HIV infection with diabetes	4	-	-	-	-	-
3.	Tuberculosis	1	-	-	-	-	-
4.	HIV infection with tuberculosis	18	-	-	1 (5.6)	-	1 (5.9)
5.	Malnutrition	17	-	1 (5.9)	-	1 (5.9)	2 (11.8)
6.	HIV infection with malnutrition	2	-	-	-	-	-
7.	Long term steroid therapy	5	-	-	-	-	-
8.	HIV infection	14	1(7.1)	-	-	1 (7.1)	2 (14.2)
9.	AIDS	7	1 (14.2)	-	-	-	1 (14.2)
10.	Cancer chemotherapy	1	-	-	-	-	-

***** Opportunistic and associated intestinal parasites
Note: Number in parenthesis indicates percentage

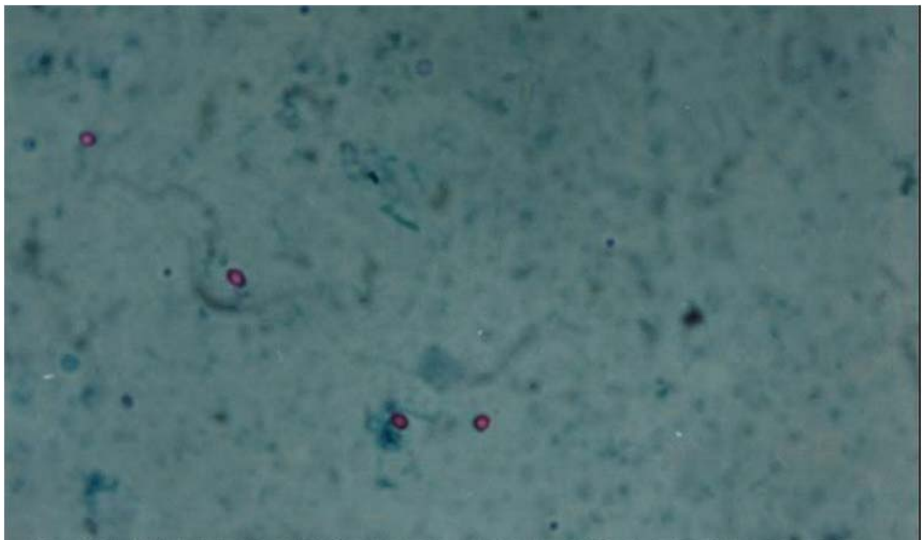

Fig. 1. Modified Ziehl-Neelsen Stain showing oocysts of *Cryptosporidium parvum*

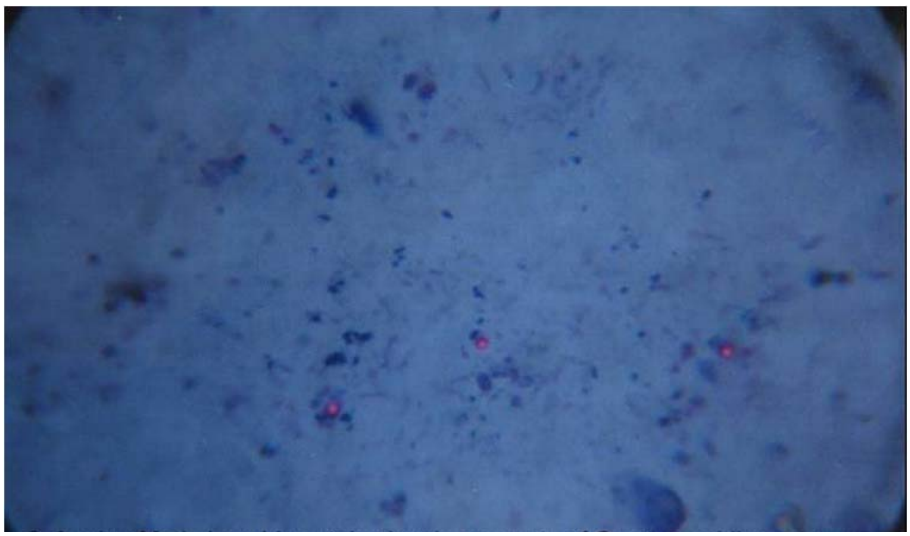

Fig. 2. Safranine Methylene blue stain showing oocysts of *Cryptosporidium parvum*

Cryptosporidium is the only opportunistic intestinal parasite and others *Giarida.spp, Taenia* and *A.lumbricoides* are the associated intestinal parasites (Table-4). Modified Ziehl-Neelsen stain shows oocysts of *Cryptosporidium.parvum*. (Fig. 1) and Safranine methylene blue stain shows oocysts of *Cryptosporidium.parvum.* (Fig. 2).

DISCUSSION

Opportunistic intestinal infections have played a critical role in determining symptomatic illness in immunocompromised individuals. However the parasites cause mild or self-limited diseases in immunocompetent individuals.

In our study, we have observed male predominance 60 (82.1%) and female population being 14 (18.9%). The male: female ratio is found to be 4:29:1. In a study by John F Lindo *et al*, they have also observed male predominance being 58% and the male: female ratio to be 1.3:1[3]. Other comparable studies are by Ayyagari A *et al.*, and a study from Tamil Nadu, where they have found male predominance in the HIV infected individuals and the male: female ratio found to be 20:3 and 2:11 respectively[12].

In our study we have found age group between 21-30 years (mean 25 years). 24 (32.4%) individuals are males and 8 (10.8%) are females within the age group 21-30.

John F Lindo et al reported the mean age of HIV positive individuals with diarrhoea as 33.8 years [3]. Mukhopadhya A et al reported the mean age of HIV positive individuals with diarrhoea as 33.5 years[13], and Ayyagari et al reported 21-40 years of age (mean 30 years)[12].

A comparative study by G. Kang et al by comparing the five staining methods for the detection of *Cryptosporidium* oocysts in faecal specimens from the field, recommends that a preliminary screen of faecal samples be done by Mepcrine-Carbol fuchsin stain followed by confirmation by a modified Ziehl-Neelsen stain[14]. In the present study also, modified Ziehl-Neelsen stain has been found to be a better method of permanent staining method, which provided a clear distinct morphological assessment and bright appearance of oocyst.

In the present study the overall prevalence of parasitic infections among various immunocompromised individuals is 8.1% i.e., 6 out of which 2(2.7%) *Cryptosporidium*, 2(2.7%) *A.lumbricoides*, 1(1.4%) *Giardia.Spp* and 1(1.4%) *Taenia* were identified, among them C*ryptosporidium* is the only opportunistic intestinal parasite and others *Giarida.spp, Taenia* and *A.lumbricoides* are the associated intestinal parasites. Ashish Mukhopadhya et al reported that enteric parasitic pathogens are common in Southern Indian HIV infected patients[13]. They reported 15% *Cryptosporidium, Giardia.spp* 15%. Nagamani et al reported *Giardia.spp* and *C.parvum* as the major parasites implicated in intestinal infections[15]. Jayshree *et al.*, reported 3.1% *Giardia.spp* and 0.3% *C.parvum in* malignant patients[16]. Our study reported 2.7% *C.parvum* and 1.4% *Giardia.spp* in HIV infected individuals, followed by malnutrition in which a case of *Giardia.spp* and *A.lumbricoides* (1.4% each) were reported. However no incidence of parasitic infections was reported from patients with malignancy.

Among other intestinal parasites, *Ascaris, Taenia.spp* and *Giardia.spp* were predominated in the present study. Sethi, Sehgal *et al* from Northern India reported a changing trend of intestinal parasitic infection and the study showed *Giardia.spp*, the most common parasite (1.9%), which is comparable to study from Southeast Asia 4% [17]. John F Lindo *et al* reported from Florida 1.9% *Giardia.spp* infection in HIV positive individuals and 1.9%, *A.lumbricoides* in HIV positive individuals[3]. This study correlates with the present study as we have reported 1.4% and 2.7% *Giardia.spp* and *A.lumbricoides* respectively.

Stool microscopy offers many advantages. First and foremost, demonstration of parasites in the stool confirms the diagnosis and is the gold standard in the diagnosis of intestinal parasitic infections.

CONCLUSION

The present study draws a conclusion that *C. parvum*, a protozoan parasite plays a major role in the epidemiology of opportunistic infection, and causing diarrhoea predominantly in HIV infected individuals among the immunocompromised conditions. Also the association of the parasites like *Giardia, Ascaris* and *Taenia.spp* was seen with the immunocompromised individuals preferably malnutrition.

The present study throws light into the fact that in India particularly in South India; *C.parvum* remains a predominant protozoan parasite in HIV infected individuals as compared to other immunodeficiency conditions. Hence it is necessary that any individual, who is HIV positive or has AIDS presenting with acute or chronic diarrhoea should be screened for *Cryptosporidium* with the most accurate method of staining technique. However other non opportunistic intestinal parasites may also be associated, therefore it is essential to screen the stool samples of the patients with

immunosuppressive conditions for such parasites that help to find out the exact clinical cause of diarrhoea. Since parasitic infection manifest severe life threatening persistent diarrhoea in AIDS patients, children with malnutrition, it is evident from our study that demonstrating these opportunistic parasites at the earliest will enable the clinician to give effective treatment and save the patient from the increasing incidence of morbidity and mortality that results from the opportunistic intestinal parasitic infections seen in immunocompromised individuals.

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