

Study of intestinal parasites among the immunosuppressed patients attending a tertiary-care center in Northeast India

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Abstract

Background: Intestinal parasitic infestations cause a variety of clinical conditions, ranging from asymptomatic infestations to life-threatening situations. This study will highlight the importance of screening for intestinal parasites in immunosuppressed patients and increasing awareness of occurrence of intestinal parasites in this population.

Objective: To understand the prevalence and demonstration of intestinal parasitic infestations among the immunosuppressed patients and determine the association of intestinal parasite and clinical presentation among these patients.

Materials and Methods: A total of 149 immunosuppressed patients were included in the study. The prevalence of intestinal parasitic infections was diagnosed by microscopic examination of stool specimens. Smears were stained by Kinyoun's modified acid-fast stain. Stool samples were also examined after Sheather's sugar floatation and formol-ether concentration techniques.

Result: Of the 149 patients included in the study, parasitic infestation was present in 53.02%. The highest prevalence of parasitic infestation was found in patients with cancers 80% (12 of 15). Overall, 106 (71.1%) patients showed gastrointestinal symptoms, of which 63(59.4%) patients were positive for intestinal parasites. The most common parasite isolated was *Entamoeba histolytica/Entamoeba dispar*. The parasite prevalence with use of routine method was found to be 37% and with Sheather's sucrose floatation and formal-ether sedimentation method was found to be 43% and 52.3%, respectively.

Conclusion: This study thus highlights the importance of testing for intestinal parasites in immunosuppressed patients and emphasizes the necessity awareness among clinicians regarding the occurrence of these parasites in this population and health education of the population for food hygiene.

KEY WORDS: Intestinal parasites, immunosuppressed, concentration technique, gastrointestinal symptoms

Introduction

Intestinal parasitic infestations cause a variety of clinical conditions, ranging from asymptomatic infestations to life-threatening situations. Majority of the symptoms are related

to gastrointestinal (GI) tract. Parasitic infections that cause self-limited diarrhea in immunocompetent patients may cause profuse diarrhea in immunocompromised individuals, generally accompanied by loss of weight, anorexia, malabsorption syndrome, and, in some cases, fever and abdominal pain.^[1] Intestinal parasites are among the most common human infections distributed worldwide with prevalence rates as high as 40.59% in developing countries.^[2] In India, occurrence of intestinal parasites proclaimed from various workers reveal wide variations from 11.3% to 90%.^[2] By definition, a compromised host is the one in whom normal defense mechanisms are impaired (eg. AIDS), absent (eg. congenital deficiencies), or bypassed. These patients are becoming more common in medical facilities and represent a growing problem in terms of

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diagnosis and subsequent management. Individuals with defective immune system exhibit high susceptibility to infections with nonvirulent and minimally pathogenic organisms.^[3]

Immunodeficiency diseases can be classified as primary and secondary. The primary immunodeficiencies may be either congenital or acquired and are currently classified according to the mode of inheritance and whether the defect involves T cells, B cells, or both. AIDS caused by human immunodeficiency virus is a secondary immunodeficiency disease. Other examples of secondary immunodeficiency diseases are protein-losing enteropathy, lymphoreticular malignancy, and patients on immunosuppressive drugs.^[4] Impaired cell-mediated immunity results in progressive decline of immunological response making them susceptible to variety of common and intestinal infections leading to increased morbidity and mortality.^[5]

In the early 1980s, it appeared that the importance of parasitic infections was declining in immunocompromised cancer patients. However, in recent years, the frequency of these diseases has risen owing to the increased use of corticosteroid in cytotoxic regimens.^[6]

A lot of these pathogens, especially the intracellular protozoa that chiefly influence the small intestine, produce their most devastating effects in patients with immune deficiency. Parasitic infections generally are asymptomatic in otherwise healthy individuals; however, their manifestations in immune compromised individuals are more severe and devastating. In any parasitic infection in immunosuppressed host, certain organisms tend to produce greater pathological sequelae in these patients.^[3]

Some of the common parasites found in immunosuppressed patients are *Giardia lamblia*, *Entamoeba histolytica*, *Strongyloides stercoralis*, *Cryptosporidium parvum*, *Cyclospora cayeatanensis*, *Isospora belli*, and *Microsporidia* spp.^[3]

There is paucity of reports regarding prevalence of parasites in this part of Northeast India. In view of this, the study was conducted to understand the prevalence of intestinal parasitic infestations among the immunosuppressed patients, to demonstrate the intestinal parasite profile in immunosuppressed patients, and to determine the association of intestinal parasite and clinical presentation.

Materials and Methods

This study was carried out in the Department of Microbiology, for a period of 1 year. Stool samples received from different departments were included in the study as per inclusion criteria.

The stool samples received from the patients with the following immunosuppressive (IS) status like HIV infection, malignancy, patients on IS therapy including persons who underwent transplantation, diabetes mellitus (DM) patients with retinopathy/neuropathy/nephropathy, severe anemia (<6g/dL), protein energy malnutrition (grade III PEM), tuberculosis (TB), and chronic diseases (e.g. chronic kidney disease, chronic obstructive pulmonary disease, etc.) were included in the study.

A clinical data in reference to the duration and frequency of diarrhea, weight loss, loss of appetite, associated abdominal symptoms, vomiting, and fever were obtained from the individual patient's record.

Specimen Collection and Transportation

Patients were instructed to avoid contamination of the stool specimen with urine or water.

The specimen was sent to the Microbiology Department for further processing on the same day. The specimen was labeled properly and processed immediately.

Examination of Stool Sample^[3]

Macroscopic Examination

The specimen was examined by naked eye for color, consistency, presence of blood, mucus, adult worms, or segments of worms and recorded accordingly.

Microscopic examination

Microscopic examination of the stool specimen was performed by the following techniques:

Direct wet smear: Saline preparation and iodine preparation.

Concentration techniques:^[7] Formol-ether sedimentation and Sheather's sugar floatation technique were employed for all specimens for the concentration of the parasitic ova and cysts.

Permanent staining techniques:^[3] Specialized stain such as Kinyoun's acid-fast stain was performed for coccidian parasites.

Statistical Analysis

Significance was evaluated by Fisher's exact test and/or χ^2 -test and "p" value less than 0.05 was considered as significant.

Result

A total of 149 immunosuppressed patients were enrolled in the study, of which 49% of the patients were male and 51% were female subjects, and the highest number of patients [46 (30.8%)] belonged to the age group of 1–10 years. Parasitic infestations were present in 79 (53.02%) patients with male and female ratio of almost 1:1 [Figure 1] and highest prevalence belonging to age group 21–30 years [Table 1]. The most common parasite isolated was *E. histolytica/E. dispar* [31 (33.7%)], followed by *Ascaris lumbricoides* [22 (23.9%)], *G. lamblia* [19 (20.65%)], *Trichuris trichiura* [10 (10.87%)], hookworm [6 (6.52%)], *S. stercoralis* [3 (3.26%)], and *Taenia* spp. [1 (1.08%)].

The highest prevalence of parasitic infestation was found in patients with cancers [80% (12 of 15)], followed by severe anemia [68.42% (13 of 19)], PEM [59.20% (29 of 46)], tuberculosis (41.67%), HIV positive (40%), other chronic diseases (38.50%), and diabetes mellitus (17.64%). The most common infestation in HIV-positive patients, cancer patients, diabetes mellitus patients, severe anemia patients, and in patients with

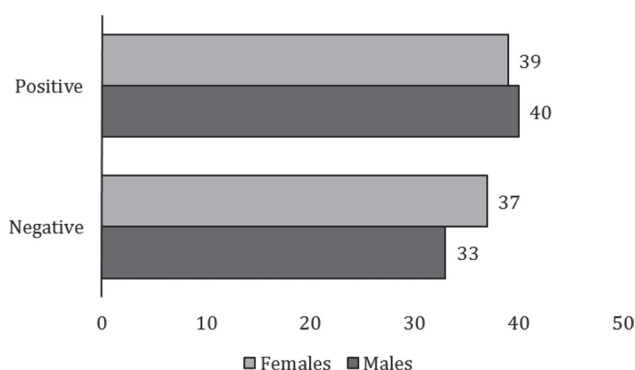


Figure 1: Sex-wise prevalence of intestinal parasites.

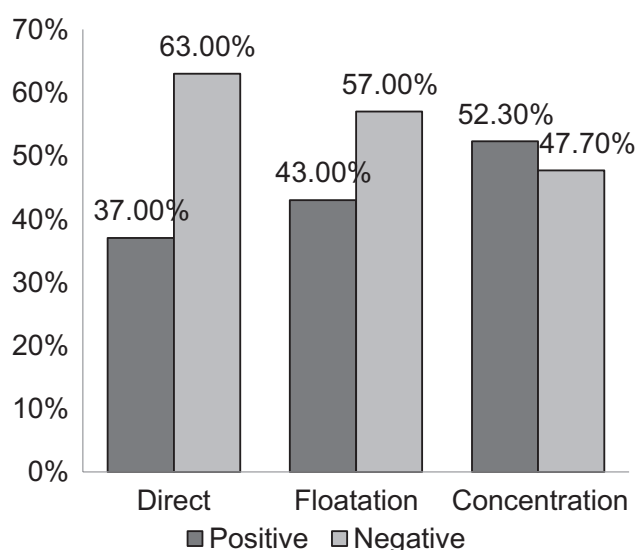


Figure 2: Comparison of direct, floatation and formol-ether concentration techniques for detection of different parasites.

IS drugs was found to be *E. histolytica/ Entamoeba dispar*. *A. lumbricoides* was the most common infestation in patients with PEM [Table 2].

Mixed infestations were seen in nine patients, where four (44.44%) showed infestation with *Ascaris + Entamoeba*, one (11.11%) showed infestation with hookworm + *Trichuris*, one showed infestation with *Entamoeba + Ascaris + Trichuris* (11.11%), two (22.22%) showed infestation with *Entamoeba + hookworm + Trichuris*, and one (11.11%) showed infestation with *Ascaris + hookworm + Trichuris + Strongyloides*.

On comparison of routine microscopy method with others, stool concentration method showed higher prevalence and isolation by the formal-ether sedimentation method (52.3%) [Figure 2]. This association when compared with routine microscopy was statistically significant ($p = 0.011$).

Overall, 106 (71.1%) patients presented GI symptoms. The association of GI symptoms was statistically significant

($p = 0.0183$). The prevalence of parasitic infestation was highest in patient complaining of diarrhea (65.5%). The study also showed that 43 (28.9%) patients who did not have any GI symptoms [16 (11%)] had intestinal parasites, indicating that they still harbor these opportunistic and nonopportunistic parasites and act as carriers in the community via contaminated water and open defecation practices [Table 3].

Discussion

The study showed association of intestinal parasitic infection in 79 (53.02%) among the 149 patients with different immunosuppressed status. The result of this study is comparable with the reports of Idris et al.^[8] and Rao et al.,^[9] where both reported a prevalence of 57% in their studies. However, when compared with our study, many reported a varied range of prevalence ranging from 23% to 97.4%.^[1,10,11] The difference among prevalence of parasites in the abovementioned studies may be owing to multifactorial reasons in different geographical locations.

In this study, the prevalence of intestinal parasites in immunosuppressed patients was found to be the highest (63.15%) in the age group 21–30 years, which was similar to the study by Al-Megrin.^[12] An increase in the infectivity rate in this group in our study may be owing to the cumulative effect of the factors such as exposure to outdoor life, poor socioeconomic and poor sanitary conditions, which demands broad-based community study to ascertain such association.

In our study, we have observed parasitic infections in male patients (50.63%) compared with female patients (49.37%), which do not indicate any gender preponderance among immunosuppressed patients. This observation is in concordance with that of Al-Megrin.^[12] Overall, the most common parasite isolated was *E. histolytica/E. dispar* [31(33.7%)]. The most common infestation in HIV-positive patients, cancer patients, DM patients, severe anemia patients, and in patients with IS drugs was found to be *E. histolytica/E. dispar*. In our study, only microscopy was employed to identify the parasites. Molecular methods for differentiation of *E. histolytica* from other nonpathogenic parasites such as *E. dispar* were not employed. The higher prevalence of *E. histolytica* can be explained owing to the favorable climate condition for survival of protozoan cyst outside the human host, absence of intermediate host, and lack of latency period to maturation when passed in feces.

The intestinal parasitic infections among cancer patients (80%) was found to be higher in our study when compared with similar studies^[1,8,10,13,14] *E. histolytica/E. dispar* was the most common parasite in cancer patients in our study, similar to studies by Guarner et al.^[13] and Rudrapatna et al.^[14]

In our study, patients with severe anemia showed a parasitic prevalence of 68.42%, which was comparable to many studies.^[9,15,16] *E. histolytica/E. dispar* was the most common parasite in patients with severe anemia, which is a unique feature of our study, as Dori et al.^[16] and Rao et al.^[9] found

Table 1: Age-wise prevalence of parasitic infestation in the study population

Age group (years)	Positive		Negative		Total	
	Number	Percentage	Number	Percentage	Number	Percentage
0–10	29	61.70	18	38.3	47	31.54
11–20	9	52.94	8	47.06	17	11.40
21–30	12	63.15	7	36.85	19	12.75
31–40	13	48.15	14	51.85	27	18.12
41–50	6	50	6	50	12	8.05
>50	10	37	17	63	27	18.12
Total	79	53.02	70	46.98	149	100

Table 2: Type of parasitic infestations in different IS states

Diagnosis	<i>E. histolytica/E. dispar</i>	<i>G. lamblia</i>	<i>Taenia</i>	<i>A. lumbricoides</i>	Hookworm	<i>T. trichiura</i>	<i>S. stercoralis</i>
HIV infection	4	1	0	2	0	0	0
Cancer	6	3	0	1	0	2	0
IS therapy	2	1	0	2	0	0	1
DM	2	0	0	0	0	1	0
Severe anemia	5	2	1	2	2	1	0
PEM	9	11	0	12	2	4	1
TB	2	0	0	2	1	1	1
Chronic diseases	1	1	0	1	1	1	0
Total (number)	31	19	1	22	6	10	3

Table 3: Comparison of presence of parasites and GI symptoms

	Parasite present	Parasite absent	Total
GI symptom present	63	43	106
GI symptom absent	16	27	43
Total	79	70	149

hookworm and Tsuyuoka et al. found *A. lumbricoides* to be the most common parasites isolated in their respective studies.

The prevalence of parasites among patients on IS therapy in this study was found to be 60%, which was similar to the studies by Abaza et al. (31.7%)^[10] and Idris et al. (50%)^[8]. In this study, *E. histolytica/E. dispar* and *A. lumbricoides* were the most common parasites isolated, which was different from the study conducted by Idris et al., who found *Blastocystis hominis* as the most common parasite isolated.^[10]

Among PEM patients, 59.2% showed prevalence of parasites, which was similar to findings by Bhandari et al.^[17] However, our study varied in the prevalence when compared with many studies.^[8,18] In our study, *A. lumbricoides* was the most common parasite isolated among PEM patients. Bechir et al.^[19] observed that *A. lumbricoides* and *E. histolytica/E. dispar* were the most common parasites isolated. However, Bhandari et al.^[17] observed that *E. histolytica*, *G. lamblia*, and *A. lumbricoides* were most commonly isolated in patients with grade 3 and grade 4 PEM.^[17] Thus, it was seen that the results of this study is comparable with most of the other studies.

The high prevalence of *Ascaris* may be explained by the fact that they cause absorption and retention of protein and nitrogen and by themselves ingesting, absorbing, and utilizing the host food. Heavy ascariid infestation can probably induce PEM in persons whose diet is otherwise inadequate.

The prevalence of parasites among HIV-positive patients in this study was found to be 40%, which was in accordance with many similar studies.^[20–22] However, the prevalence rate among HIV patients shows a wide variation from 11.4% to 62.7% in various studies.^[23–29] The wide variation of prevalence may be attributed to difference in geographical distribution of parasites, sanitary practices, and different selection criteria of cases. In this study, *E. histolytica/E. dispar* was the most common parasite isolated in HIV-positive patients, which was similar to studies by Assefa et al.,^[30] and Asma et al.^[31] *C. parvum* was the most common parasite isolated in many studies.^[28,32] *G. lamblia* was the most common parasite isolated in a study by Cimerman et al.^[20] and *I. belli* was the common parasite in the study by Gupta et al.^[21] The difference in predominant parasite isolated in different studies compared with our study was because these parasites were more encountered when CD4 T-cell count falls below 200 cells/ μ L. However, in our study, we included all HIV-positive patients irrespective of their CD4 T-cell count.

Parasites in Diabetic patients in this study was found to be 17.64%. This was similar to study by Akhlagi et al.^[33] However, there is a variation in prevalence ranging from 8% to 47%^[10,34] The most common infestation among diabetic patients was

found to be *E. histolytica/E. dispar* which differed from many studies where *G. lamblia*,^[35] *A. lumbricoides*^[36] to be the most common parasite isolated.

The prevalence of intestinal parasite in patients with GI symptoms was found to be 53.77%. This was comparable to most of the studies.^[1,12,13,24,25] Our study also revealed that 22 individuals showed no GI disorders such as diarrhea and pain in the abdomen, but they still harbor these opportunistic and nonopportunistic parasites and act as carriers in the community via contaminated water and open defecation practices.

In our study, we also found that 37% of stool samples were positive for parasites by direct microscopy, whereas with formol-ether sedimentation method, 52.3% of stool samples were positive for parasite. This finding is similar to that reported by Saxena *et al.*^[35] and Vinayak *et al.*,^[36] who reported that, by formol-ether sedimentation, 31% of stool samples were positive for helminthic ova and pathogenic protozoan cysts compared with 21% by direct saline and iodine preparation. This can be explained by the fact that the use of formalin fixes and preserves the fecal specimen, and ether decreases the specific gravity of small fecal particles, causing them to float in the suspension. The coarse nonabsorbent elements including eggs and cysts are left at the bottom, and ether also dissolves fat. The addition of these two chemicals and centrifugation improved the isolation rate.

This study thus reveals that intestinal parasites are prevalent among immunosuppressed patients in Meghalaya and rapid detection of such infestations is important for empirical therapy.

Conclusion

Our study was conducted on a limited number of sample size within a period of 1 year. However, the study supported the fact that immunosuppressed individuals are prone to infections with intestinal parasites.

Demonstration of intestinal infections in the immunosuppressed patients employing concentration method such as Sheather's sugar floatation technique and formol-ether concentration method are found to be essentially effective in our study. Such approaches are easier for reliable laboratory diagnosis for appropriate management of immunosuppressed patients with intestinal parasitic infection.

In our study, we found that prevalence of parasites was highest among cancer patients in comparison to other similar studies. However, this may not be significant because of the limited sample size.

Among HIV-positive patients, *E. histolytica/E. dispar* was the most common parasite isolated in contrast to many studies, where coccidian parasites were more commonly isolated in this group of patients. This difference may be because, in our study, HIV-positive patients were included irrespective of their CD4 T-cell counts.

This study thus highlights the importance of testing for intestinal parasites in immunosuppressed patients and emphasizes the necessity of awareness among clinicians regarding

the occurrence of these parasites in this population and health education of the population for food hygiene. The frequency and the danger of those opportunistic infections require their efficient diagnosis and appropriate management.

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