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Original Article

Microbiology Section

Study of Parasitic Infection in Immunosuppressive Patients and its Association with the Socio-demographic Status

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ABSTRACT

Introduction: In developing countries, immunosuppressive patients are at greater risk of parasitic infection which may cause morbidity and mortality. Socio-economic and environmental factors including lack of health hygiene in close contact with infected reservoir animal which plays an important role.

Aim: To determine the prevalence of parasitic infections and their association with socio-demographic status.

Materials and Methods: This was a cross-sectional study which was carried out at tertiary care hospital located in Central East India. Total 120 stool samples were collected from the immunosuppressive patients and were processed using direct wet mount preparation with saline and Lugol, formalin-ether concentration and Modified Ziehl-Neelsen stain. Different socio-demographic parameters were recorded. Statistical analysis was done using Graph Pad Prism version 8 Chi-square test. The p-value ≤0.05 was considered as statistically significant.

Results: Out of total 120 patients, 20 (16.7%) were found to be infected. Majority of the population were males (66.7%). Among the total positive samples, 75% (15) showed the presence of Protozoa in which 10 samples had *Entamoeba histolytica*. The presence of Helminths was found in 25% (5), in which three samples showed *Ascaris lumbricoides* and two had Taenia species (10%).

Conclusion: The prevalence of parasitic infection among immunosuppressive patients in the present study was 16.7%. *Entamoeba histolytica* was the most commonly observed parasite. There was no significant association between prevalence of parasitic infections and socio-demographic data variables.

Keywords: Amoebiasis, Formalin ether concentration technique, Helminth, Modified ziehl-neelsen stain

INTRODUCTION

Intestinal parasitic infection is observed as a major public health problem, especially in developing countries. With the increasing population of immunosuppressive people, it has become one of the major cause for morbidity and mortality is intestinal parasitic infections. It is observed as a major public health problem especially in developing countries. Parasitic infections like intestinal amoebiasis, giardiasis, ascariasis, tanieasis, crytosporidiasis and others result into diarrhea, gastro-intestinal and other systemic manifestations or the affected people may remain asymptomatic and may have carrier state. Socio-cultural milieu plays a significant role in these infections leading to adverse health effects [1]. In developing countries like India, socio-economic and environmental factors such as poor personal hygiene, poverty, lack of pure drinking water, malnutrition, poor health, sanitation, climate and overcrowding have been associated with parasitic infection [2,3]. Research shows that immune suppression, primary immunodeficiency and use of immunosuppressive drugs such as post-transplantation increases the risk of parasitic infection as chronic carriage stage [4]. However, a weak immune system can make a patient more prone to infectious diseases and parasitic infections [5]. Environmental changes, lack of clean water, diversity and density of population, lack of health hygiene close contact with infected animals is considered to be the most significant factors in the incidence and spread of parasite disease [6]. Globally, parasitic infections are common amongst the intestinal infections affecting annually about 3.5 billion people [7]. Moreover, parasitic infection may lead to poor immunity in infants, nutritional depletion, and loss of mucous membranes, lymphatic leakage and local hemorrhage [8]. Intestinal Parasitic Infections (IPI) are one of the public health problems in developing countries because of overcrowding, poor sanitation and low socio-economic status. Immunosuppressive individuals of poor and deprived communities of tropical and subtropical may have increased host susceptibility due to their pre-existing co-morbidities like HIV/AIDS, tuberculosis, diabetes and malnutrition. Health awareness, better personal hygiene and improved sanitation can offer prevention [9,10].

However, severity of the infection depends upon the species of parasite, type and course of infection, nature of interactions between the parasite and concurrent infections, nutritional and immunological status of host and different socio-economic factors [11]. According to World Health Organisation (WHO) amoebiasis caused by *Entamoeba histolytica*, is the most common parasitic infection in immunosuppressive patients with substantial morbidity and mortality followed by *giardia* caused by *giardia* spp and cryptosporidiosis caused by *Cryptosporidium* spp [12]. *Ascaris lumbricoides*, hookworm and *Hymenolepis nana* are the most common nematode and cestode causing infection [13].

Considering the importance of effective diagnosis and treatment of parasitic infected patients, direct microscopy for the detection of intestinal parasite disease is considered as useful and beneficial tool [14]. However, in immunosuppressed patients, low level of parasitemia can cause symptomatic consequences and severe complications, proper and specific laboratory diagnosis by serological and molecular based assays are important.

The aim of this study was to find out prevalence as well as association between parasite infections and their socio-demographic status in adult age group (>18 years) of immunocompromised individuals attending tertiary care hospital of Durg district, Chhattisgarh state.

MATERIALS AND METHODS

The cross-sectional study was carried out in Department of Microbiology at a tertiary care hospital. The research study was approved by Sumandeep Vidyapeeth Institutional Ethics Committee. [SVIEC/ON/PhD/17018]. Patients on immunosuppressant; fulfilling inclusion criteria, during three months between June 2019 to

September 2019 were studied. Patients were informed and appropriate consent was obtained before being recruited for the study.

Those patients who visited the hospital with illness like HIV, diabetes mellitus, tuberculosis, liver cirrhosis as well as positive Hepatitis B, taking steroid, on cytotoxic chemotherapy and immunosuppressant drugs etc., were considered as immunosuppressive patients and were included in the study. Inappropriate quality of stool sample was the exclusion criteria.

Total 120 immunosuppressive patients were included in this study. Each stool sample was collected in a clean, dry, leak proof, well-labeled wide mouth stool container with name and identification number and processed in the Parasitology section of the Microbiology department. Necessary data of patients were obtained from the Medical Record Department (MRD).

Macroscopic examination: Macroscopic analysis was done in terms of color and consistency and presence of adult worms as well as segments of tapeworm and larvae.

Microscopic examination: After macroscopic examination all samples were examined by direct microscopic method. Wet mount of the samples were performed as saline and iodine mounts to identify trophozoite and cysts of protozoan parasites as well as ova and larva. For the detection of intestinal coccidians *Cryptosporidium parvum, Cyclospora* and *Isospora belli* Modified Ziehl-Neelsen (MZN) stain was performed. Formal ether concentration technique was also done to detect eggs and larva of intestinal parasites [15].

STATISTICAL ANALYSIS

For the statistical analysis, Graph Pad Prism version 8 was used. Chi-square (χ^2) test was performed and a p-value ≤0.05 was considered as statistically significant.

RESULTS

Out of total 120 patients, 20 (16.7%) were found to be infected with parasite. Among all 120 immunosuppressive patients, 80 (66.7%) were males and 40 (33.3%) were females. Among the male population, 11 (9.2%) and among females, 9 (7.5%) were found to be positive for the infection. The age range of the population was 21-60 years, the mean age being 43.1±6.5 years. The age group of 41-50 years showed maximum parasitic infections. When two groups having highest (7 out of 46; 5.8% in age group of 41-50) and lowest prevalence (3 out of 27, 2.5% in age group 31-40) of parasitic infestation were compared; there was no statistical significant difference (p-value=0.73). No statistical significant difference was observed for socio-demographic variables like gender, age, education, employment, hygiene methods, (washing of hands before taking meals and after defecation), as well as with level of sanitation in form of defecation in open or having facility of toilet [Table/Fig-1].

Prevalence of parasitic infection was found to be high in HIV (24%), diabetes (14.75%), tuberculosis (17.65%) and patients having Hepatitis B infection (18.18%) while no parasitic infection was present in miscellaneous group [Table/Fig-2].

As shown in [Table/Fig-3], among the total 20 positive samples; 15 samples (75%) showed the presence of Protozoa in which 10 samples (50%) showed presence of *Entamoeba histolytica* [Table/Fig-4], followed by *Cryptosporidium parvum* [Table/Fig-5] in four samples (20%) and *Giardia lamblia* in one sample (5%). The presence of Helminths was found in five samples (25%), of which three samples (15%) showed the presence of *Ascaris lumbricoides* [Table/Fig-6] and Taenia species eggs [Table/Fig-7] were found in two samples (10%) [Table/Fig-3]. Prevalence ratio of protozoan to helminths parasites was 3:1.

Particulars	Frequency N (%)	Number of positive cases (%)	p-value
Gender			
Male	80 (66.7)	11 (9.2)	0.2253
Female	40 (33.3)	9 (7.5)	
Age (in Years)			
21-30	16 (13.3)	6 (5.0)	0.1123
31-40	27 (22.5)	3 (2.5)	
41-50	46 (38.4)	7 (5.8)	
51-60	31 (25.8)	4 (3.3)	
Education			
Illiterate	19 (15.8)	5 (4.2)	0.5765
Primary school	25 (20.8)	4 (3.3)	
High School	32 (26.7)	6 (5.0)	
Diploma	18 (15.0)	3 (2.5)	
Graduate	15 (12.5)	2 (1.7)	
Post graduate	11 (9.2)	0 (0)	
Job			
Government	15 (12.5)	3 (2.5)	0.6520
Farmer	46 (38.3)	7 (5.8)	
Student	27 (22.5)	3 (2.5)	
Housewife	23 (19.2)	6 (5.0)	
Other	9 (7.5)	1 (0.8)	
Habit of washing hand			
Before meal			
Yes	110 (91.7)	17 (14.2)	0.2373
No	10 (8.3)	3 (2.5)	
After defecation			
Yes	90 (75.0)	11 (9.2)	0.0237
No	30 (25.0)	9 (7.5)	
Use of toilet			
Yes	109 (90.8)	16 (13.3)	0.0659
No	11 (9.2)	4 (3.3)	

[Table/Fig-1]: Showing socio-demographic variables in frequency and percentage of intestinal parasites.

Immunocompromised status	Total number	Positive cases	Percentage (%)
HIV infection	25	6	24.00
Diabetes mellitus	61	9	14.75
Tuberculosis	17	3	17.65
Positive Hepatitis B	11	2	18.18
Miscellaneous group (Cirrhosis of liver (3), Chronic steroid therapy* (1), Cytotoxic chemotherapy with malignancy† (1), Immunosuppressant drugs‡ (1)	6 (3+1+1+1)	--	--
Total	120	20	16.67

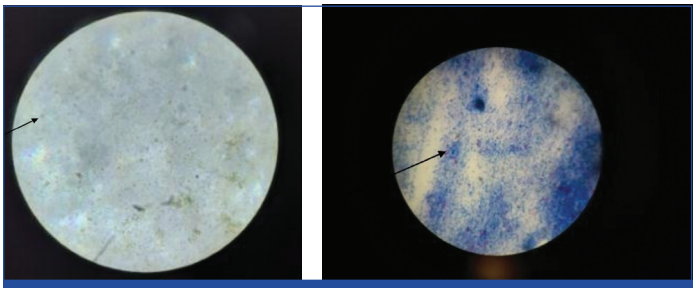
[Table/Fig-2]: Details regarding the immunocompromised states of the study population.
*Prednisolone therapy for more than three months, †Imatinib therapy for CML for last one year. ‡Azathioprine (AZA) for SLE,

DISCUSSION

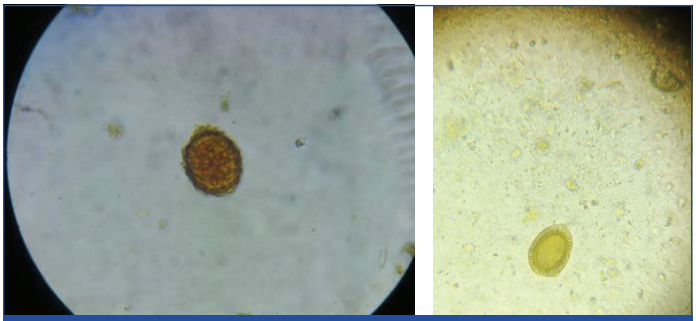
In this study the overall prevalence of parasitic infection in immunosuppressive patients was 16.7%. This study was primarily done to find out the risk factors involved in developing parasitic infection in immunosuppressive patients. Study done by Hailegebriel T showed that various socio-demographic factors like size of the family, hygienic practices like hand washing, nail cutting and wearing shoes had relation with intestinal parasitic infection [16]. In immunosuppressed patients, if same holds true, then there will be the need for additional interventional strategy. Moudgil V et al., in their study found co-prevalence of diabetes with parasitic infection of 8.6% [17]. In a study by Sabah AA and Temsah AG they showed parasitic infection amongst diabetic patients had predominance of *E. histolytica*, however their was no significant

Parasite	Number of samples with parasites	Percentage (%)
Protozoa		
Entamoeba histolytica	10	50.0
Giardia lamblia	1	5.0
Cryptosporidium parvum	4	20.0
Helminths		
Ascaris lumbricoides	3	15.0
Taenia species	2	10.0
Total	20	16.7 (20/120)

[Table/Fig-3]: Showing total number of parasite identified.



[Table/Fig-4]: Stool microscopy showing cyst of *Entamoeba histolytica* in saline wet mount (40X).
[Table/Fig-5]: Modified ZN stain showing *Cryptosporidium parvum* in stool sample under oil immersion field (100X power).



[Table/Fig-6]: Stool microscopy showing ova of *Ascaris lumbricoides* in saline wet mount (40X).
[Table/Fig-7]: Stool microscopy showing *Taenia* species ovum in saline wet mount (40X).

difference between patients having diabetes and controls [18]. They assumed that *E. histolytica* can be a factor for poor sanitation and environment pollution. Predominance of *E. histolytica*, was found in present study also however no significant relation was found with socio-demographic risk factors. It can therefore be presumed that immunosuppression was responsible for parasitic infections than socio-demographic factors; however a larger population based study may be needed for significant conclusion. Immunosuppression due to HIV may also have opportunistic infection by enteric parasite. In this study of 120 patients having immunosuppression, 24 were HIV positive of which 6 had parasitic infection (25%). Swathirajan CR et al., studied 829 HIV positive individuals of which 23.4% had enteric parasitic infections [19]. Study done by Ghoshal U et al., on detection of opportunistic parasitic pathogens in immunocompromised patients, of 10,233 stool samples received in 11 years by Institute parasitology laboratory, 64 were found to excrete oocysts of Cystoisospora, of which 37/64 (57.81%) were HIV positive [20]. The prevalence of parasitic infection varies between different countries, places or even regions. Hence, present study shows relatively lower prevalence than other studies. This could be because of different method adopted for finding out this data.

In this study, *Entamoeba histolytica* was the most common parasite observed followed by *Cryptosporidium parvum, Ascaris lumbricoides*, Taenia species and *Giardia lamblia* was least observed parasite. Study carried out in immunocompetent and immunocopromised patients have different parasitic infestations; like opportunistic enteric parasitic infections like cystoisosporiasis is greater in HIV positive than in negative individuals [19]. In another study by Kaniyarakkal V et al., [21] on 200 seropositive HIV patients, *Cystoisospora* and *Cryptosporidium* spp were most commonly observed parasite followed by *Microsporidium* spores and *Chilomastix mesnili. Cystoisospora* spp, common opportunistic parasitic infestation in HIV positive individuals can cause diarrhoea and may be related to poor sanitation and lack of hygiene. *Cryptosporidium* and *Microsporidium*, are associated with severe immunosuppression that may require high index of suspicion and need different staining methods for diagnosis.

Study carried out by Saraswathi R et al., from patients of different speciality units of rural teaching hospital, 55 of 726 stool samples (7.6%) were positive for intestinal parasites [22]. In present study, of 120 stool samples of immunocompromised patients, 20 (16.7%) had parasitic infection. Present study and other studies have reported higher prevalence of intestinal parasites in immunocompromised patients than reported by studies in immunocompetent and general population [18,19,22,23]. Gender related distribution of parasitic infection is reported higher in males in comparison with females in general population, for the reason that males are more exposed to environment [14,22]. There is lack of specific gender related intestinal parasite prevalence data and also of their relation to socio-demograhic risk factors in immunocopromised individuals. Present study did not show significant gender wise difference and it also did not show any significant connection between the prevalence of parasite infections and socio-demographic variables of immunocopromised patients. Study by Shahdoust S et al., showed no significant association between the prevalence of parasitic infestation and the variables of socio-demographic data in patients who were referred to hospital of Tonekabon Mazandaran province, Iran [14]. Studies done in general population as well as in immunocompromised patients recommend for improvement on local risk factors like poor sanitation, contact with soil, contact with domestic animal and others, which requires community education in health and hygiene [5,23,24].

In present study of 120 immunosuppressed patients, 61 had DM of which 9 (14.75%) had intestinal parasitic infection. In a case control study by Mohtashamipour M et al., on 118 DM patients and same number of control group healthy individuals, parasitic infection prevalence was 26.3% in diabetic patients which was significantly higher than in control group [5].

Immunosuppression is an important risk factor for development of parasitic infections. Though some of the evidences generated in general population can be broadly applied to immunosuppressed patients, there seems to be a distinctive and separate pathophysiological mechanism, prevalence, profile and type of parasite involvement in immunosuppressed patients [16,17,19,20]. For better management of such patients, diseases causing immunosuppression and parasitic involvement, like diabetes should be linked with blood sugar and HbA1c control; HIV/AIDS to be related to CD4 count and ART treatment and post transplant as well as patients having malignancy to type and duration of immunomodulators/chemotherapeutic/drugs [4,5,10,11,18].

Limitation(s)

One of the limitations of the study was a small sample size. Larger sample with more representation of different groups of immunosuppressed patients like Diabetes/HIV/AIDS/Post transplant group can give better insight.

CONCLUSION(S)

The prevalence of parasite infection among immunosuppressive patients was 16.7% in this study. *Entamoeba histolytica* was the most common observed parasite followed by *Cryptosporidium parvum*. There was no significant relation between socio-demographic risk

factor and parasitic infections in immunosuppressed patients. Mostly of the time parasitic infection can be diagnosed by stool microscopy. Risk factors and profile of parasitic infections can vary in immunosuppressed patients, which require further research so that treatment and prevention can help to decrease the morbidity in immunosuppressed patients.

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