

ORIGINAL RESEARCH

To find the Incidence of Chronic *E. Histolytica*/ *E. Dispar* Infection and its Co-Infection in Patients with Inflammatory Bowel Disease on the Basis of Gender Differentiation With Routine Microscopy and Serum IgG ELISA in a Tertiary Care Centre in Gwalior District: A Cross- Sectional Observational Study

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ABSTRACT

Background: *Entamoeba histolytica*/*Entamoeba dispar* are two morphologically indistinguishable species and can mimic symptoms of inflammatory bowel disease. This infection can be acquired through the consumption of contaminated food or water and has a high chance of flaring up due to already damaged intestinal mucosa. Our study aims to find the incidence of this parasite in patients of IBD so as to differentiate between these two closely symptomatically resembling diseases ultimately benefitting the general public by timely diagnosis and also a gender differentiation if present.

Methods: 100 patients of IBD were enrolled in the study and routine microscopy of stool, aspirates of pus in case of liver abscess were performed. Approximately 4 ml of blood was taken under aseptic precautions and serum was separated and subjected to *E. histolytica*/*E. dispar* IgG ELISA.

Results: 37 patients (37%) were positive for cysts in their stool and out of these, 18 patients (48.64%) also came out to be positive for *E. histolytica*/*E. dispar* serum IgG and remaining 19 patients (51.35%) were negative. Among these 18 patients, 13 were males (72.22%) out of which only 2 males had liver abscess with cysts in routine microscopy of pus aspirates and 5 were females (27.78%) without liver abscess.

Conclusion: It was observed that the incidence of *E. histolytica*/*E. dispar* was significantly higher among males than in females with IBD which can be due to the fact that the male population usually belongs to the working class and daily wage workers in a district like Gwalior where the rural population is dominant as compared to the urban population who are devoid of proper infrastructure for waste disposal and sanitation measures and a general lack of awareness about the spread of the disease.

Keywords: Inflammatory bowel disease; *Entamoeba histolytica*; *Entamoeba dispar*; cyst; serum IgG ELISA

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INTRODUCTION

Entamoeba histolytica/*E. dispar* are anaerobic parasites which are morphologically indistinguishable and part of the genus *Entamoeba*. It is found that it mostly infects humans and other primates causing Amoebiasis. It is

the third leading cause of death after malaria and schistosomiasis. The diagnosis of amoebiasis is based on microscopy, culture, isoenzyme analysis, and serology-based techniques. Mainly there are 4 clinical forms of invasive intestinal amoebiasis which are

dysentery or bloody diarrhoea, fulminating colitis, amoebic appendicitis and ameboma of the colon. After the onset of clinical disease, the symptoms vary from profuse diarrhoea (10 per day with blood tinged mucous), fever, electrolyte imbalance and weight loss [1,2] In severe conditions the infection may reach the liver through the bloodstream via the portal system and cause hepatic disease characterized by liver abscesses, hepatomegaly and tenderness. This condition is known as Amoebic Liver Disease. The presence of *Entamoeba histolytica* in patients of Inflammatory Bowel Disease (IBD) comprising of two major forms, namely, ulcerative colitis and crohn's disease, has also been reported in many regions throughout the world with an incidence of 9.31% in India [3] and it has become a serious concern in the field of diagnostic microbiology. The diagnosis of *Entamoeba histolytica* can be made on the basis of different techniques, for example, routine microscopy with wet mount preparations of stool culture, antigen detection with the help of immunoassays used for the detection of Gal/GalNAc lectin, PCR targeting 3 SSU (small subunit rRNA gene) for differentiation between *Entamoeba* spp. Antibody detection by Indirect Hemagglutination Assays(IHA) and ELISA for IgM and IgG antibodies. [4,5]

METHODS

This study was conducted in the department of Microbiology in association with the department of Medicine and the department of Community Medicine, Jayarogya Group of Hospitals, Gajra Raja Medical College, Gwalior from January 2022 till June 2022. A sample size of 100 patients was taken, who consented to participate in the study and among these, 63 were males and 37 were females. All of these patients were confirmed cases of inflammatory bowel disease with chief complaints of diarrhoea, loss of appetite, blood in stool and ulcers through colonoscopy and barium studies. The presence of *Entamoeba histolytica/Entamoeba dispar* was made on the basis of routine microscopy and serum IgG ELISA was performed only on those patients who were positive for the presence of bi-/quadri-nucleated cysts in stool. Approximately 10 – 12gm of stool in a sterile wide mouth screw-capped container and 2-3 mL of liver pus aspirates were taken

on three different occasions and wet mounts were made and observed in a microscope under 40x magnification. Approximately 4 mL of blood under aseptic precautions was taken and serum was separated by centrifugation for the detection of IgG by NovaLisa *E.histolytica/E.dispar* IgG ELISA kit (ENTG-078). The procedure of ELISA was performed as per the manufacturer's instructions and the calculation of results was on the basis of the cut-off value which is the mean cut-off control determinations calculated to be 0.43 as per the kit guidelines and the final absorbance value of the samples was calculated in terms of NTU units (NovaTech Units). The formula for which is, Sample (mean) absorbance value x 10 and the value is divided by cut-off. A value of more than 11 NTU is considered as positive, 9 – 11 NTU as equivocal and a value less than 9 NTU is considered as negative as per the manufacturer's instructions.

RESULTS

After the completion of our study, it was observed that out of the 100 patients of IBD, 63 patients (63%) were found negative, hence excluded and 37 patients (37%) were found to have bi-/quadri-nucleated cysts in routine microscopy as shown in Figure 1, comprising of 23 males (62.16%) and 14 females (37.84%), hence were subjected to *E. histolytica/ E. dispar* serum IgG ELISA. Among these patients, 18 patients (48.64%) were positive and the remaining 19 patients (51.35%) were negative as shown in Table 1, but the presence of cysts may still indicate that the patients may have acquired the infection recently. Among these 18 patients, 13 were males (72.22%) and out of which, the formation of liver abscesses and the presence of cysts in pus aspirates was observed in 2 individuals only and remaining 5 were females (27.78%), all without liver abscess. This indicates that the incidence of infection with *E. histolytica/ E. dispar* is significantly higher among the male population as compared to females as shown in Table 2 and Figure 2. We were unable to find trophozoites as they are primarily invasive and can be predominantly observed in intestinal tissue biopsy material or in freshly passed stools but the chances of presence of cysts remains higher as trophozoites are immediately killed once the stool is passed.

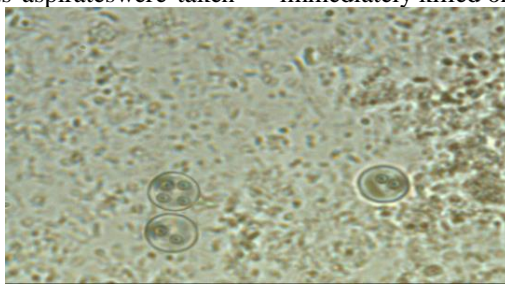


Figure 1: Binucleated and Quadrinucleated cysts showing chromatid body in one of the binucleated cysts present in stool under 40x magnification

Table: 1 Data showing total distribution of patients with the presence of cysts as well as gender differentiation

Total number of Patients with IBD= 100			
Patients with cysts = 37		Patients without cysts = 63	
Males		Females	
Number	%	Number	%
23	62.16%	14	37.84%

Table : 2 Data showing a higher incidence of *E. histolytica/ E. dispar* infection in males as compared to females out of 18 patients with positivity for cysts and *E. histolytica/ E. dispar* serum IgG

Gender	Cases	Percentage
Male	13	72.22%
Female	05	27.78%

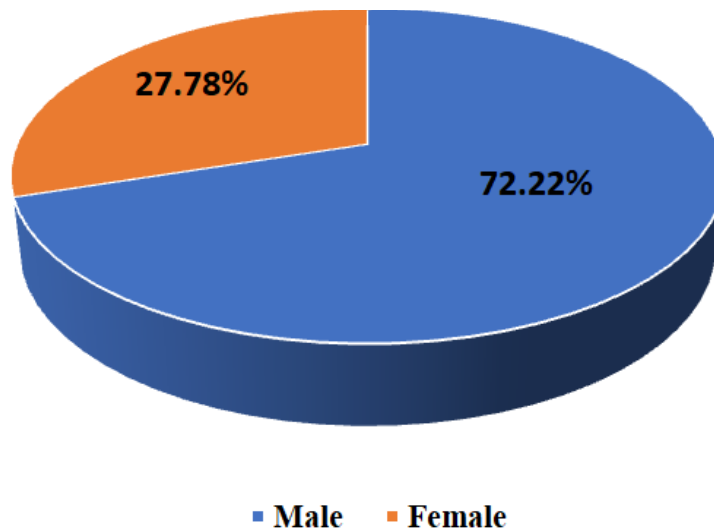


Figure: 2 Pie Chart showing the incidence of *E. histolytica/ E. dispar* infection among males and females

DISCUSSION

Infections of *E. histolytica/ E. dispar* can most commonly be found in patients with inflammatory bowel disease and ileocecal tuberculosis, in which *E. histolytica* is known to cause amoebic colitis which can be easily confused with IBD and can only be diagnosed by routine microscopy and/or serological techniques and culture or by molecular diagnostic techniques like polymerase chain reaction and iso-enzyme analysis targeting specific genes and enzymes. [5] In our study, out of 100 patients of IBD, 37 patients (23 males and 14 females) were found positive for *E. histolytica/ E. dispar* cysts in routine microscopy and were selected for further diagnostic confirmation through *E. histolytica/ E. dispar* serum IgG ELISA and 18 patients (48.64%) were found to be positive which indicates that the incidence of the parasite is high in these patients and the symptoms closely resemble to that of IBD or a co-infection with the amoeba and the remaining 19 patients (51.35%) who were negative for serum IgG may have a recent infection or the presence of dead cysts which is

higher as compared to previously published studies which may be due to the fact that India is still a developing country without the access of proper sanitation measures among the population belonging to rural districts. [6,7] and also could be due to the fact that IBD and amoebiasis are not recognized as public health problems in our country. Another finding came to surface that out of the 18 patients positive for the presence of cysts and serum IgG both, 13 were males and out of these male patients, liver abscess was found in 2 male patients with the presence of cysts in the pus aspirates which may suggest a disseminated disease and may have cysts and/or trophozoites in other organs as well. An overall higher incidence among the male population was also found in our study as compared to females. Through our study, it was suggested that there is a high need of proper diagnosis of *E. histolytica/ E. dispar* infection which mimics or exists as a co-infection in conditions like IBD, which may help to differentiate between the two closely symptomatically resembling diseases and our study also suggests to

increase the awareness of the disease and the need for proper sanitation measures, proper cooking of food and filtration of drinking water as the most commonly affected population belongs to male gender which may be daily wage workers.

CONFLICT OF INTEREST: NONE

FUNDING: NONE

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