

ABSTRACT: *Blastocystis* spp. was previously considered to be a member of normal intestinal flora; it is now receiving increased attention as a potential causative agent of human intestinal disease. The present study attempts to investigate the pathogenicity of *Blastocystis* spp. in clinically symptomatic patients. Eighty-six immunocompetent patients with intestinal *Blastocystis* spp. were evaluated clinically. Two consecutive stool samples were examined by wet mount, modified formol ethyl acetate concentration method and trichrome and Kinyoun acid-fast staining. Presence of more than 10 various morphological forms of *Blastocystis* spp. under 40× magnification was taken as a criterion for the presence of protozoan. Patients with other pathogens were excluded from the study. Metronidazole was administered for five to seven days. After treatment, stool sample of patients were re-examined for *Blastocystis* spp. There were 39 males and 47 females with a mean age of 39.8 years ± 18.8 (range 19–62 years). Abdominal pain was the most frequent symptom (77.9%) followed by diarrhea (65.1%). A second stool specimen was obtained from 61 (70.9%) patients after metronidazole therapy. Consecutive investigation revealed no intestinal protozoa in 56 (91.8%) patients. Symptoms persisted in five patients. Out of 56 patients who complained of diarrhea, 51 showed improvement in symptomatology with no protozoan found. If all other pathogens and factors causing intestinal pathologies are ruled out and only *Blastocystis* spp. is present in symptomatic patients, it is logical to consider them as pathogenic rather than commensals and treatment can be initiated in such cases. However, further studies for genetic association and prevalence are needed to strengthen this view.

KEYWORDS: *Blastocystis* spp., pathogen, abdominal pain, diarrhea

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Introduction

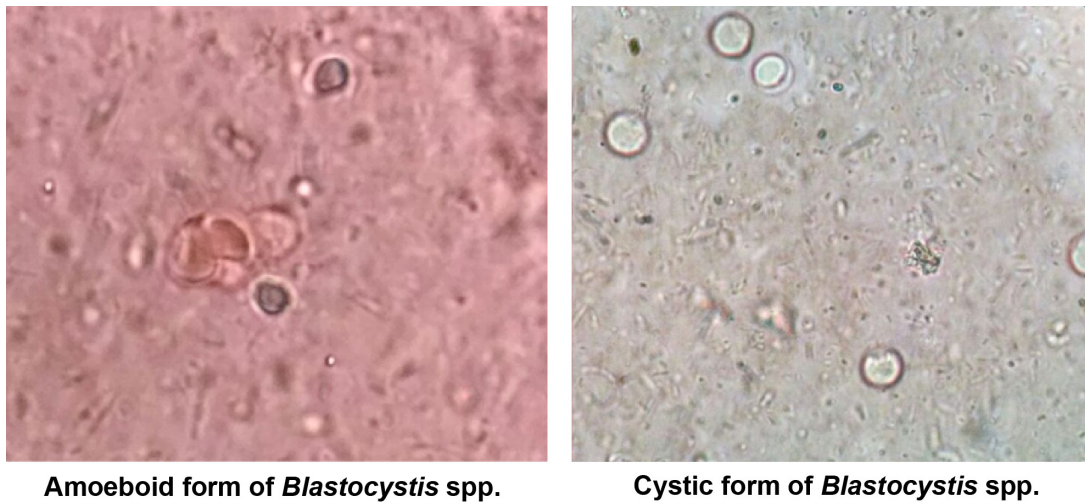
Blastocystis spp., a controversial unicellular protozoan, is among the most common parasites found in the human intestinal tract. It was first described in the medical literature by Alexeieff and was then well thought out as a harmless yeast. But *Blastocystis* spp. is now receiving significant attention as a potential causative agent of human intestinal disease.^{1–3} *Blastocystis* spp. in fecal samples of symptomatic and asymptomatic individuals was assessed as a potential cause of gastrointestinal complaints.¹ *Blastocystis* spp. has been accepted as a causative agent of travelers' diarrhea. It can also lead to persistent or recurrent diarrhea in patients with AIDS and other immunodeficiencies.^{4,5} It is also reported in nosocomial diarrhea cases.⁶

Infection with *Blastocystis* spp. has a worldwide distribution and occurs in all age groups. Watery diarrhea, abdominal pain, lack of appetite, and constipation are the common complaints that may be present in the patients with *Blastocystis* spp. infection.^{3,7} The incidence of *Blastocystis* spp. in various regions is reported to be between 2% and 65%.^{7,8} Detection rates of around 65% can be achieved in cases of acute gastroenteritis with trichrome stain as against 15%–20% with wet mount.⁹ At present, the first choice of chemotherapeutic agent is metronidazole.^{3,10}

In this study, 86 patients from whom *Blastocystis* spp. was detected in fecal samples were evaluated to clarify the clinical findings and pathogenicity of these protozoa and to analyze the effect of pathogen-specific treatment in terms of relief in intestinal symptoms and the presence of *Blastocystis* spp. cysts in posttreatment fecal samples. Bacteriological and parasitological examination of these fecal samples revealed no other microorganism to be responsible for the clinical symptoms.

Material and Methods

Eighty-six immunocompetent patients with intestinal *Blastocystis* spp. attending the tertiary care center in Karnataka, India, over a study period of two years were enrolled and evaluated clinically. The study was approved by the Institutional Ethics Committee of the Kasturba Medical College, Manipal. Patients with other pathogenic agents besides *Blastocystis* spp. were excluded from the study. Two consecutive stool samples were collected and examined macroscopically and microscopically by using a wet mount (direct and lugol iodine), modified formol ethyl acetate concentration method, and trichrome and Kinyoun acid-fast stain method. The presence of more than 10 various morphological forms of *Blastocystis* spp. under 40× magnification field was taken as a criterion for the presence of the protozoan (Fig. 1).



Amoeboid form of *Blastocystis* spp.

Cystic form of *Blastocystis* spp.

Figure 1. Morphological forms of *Blastocystis* spp. as observed in direct wet mount under 40× magnification.

For the bacteriological examination, stool samples were inoculated on to blood agar and MacConkey agar. Suspected colonies of pathogenic bacteria were further tested with sub-culture on Thiosulfate-citrate-bile salts-sucrose agar (TCBS) and deoxycholate agar. The patients with other pathogens were excluded from the study.

In addition, tests for occult blood and WBC counts of fecal sample were done. Metronidazole 250–750 mg was administered orally three times a day to the patients with *Blastocystis* infection for five to seven days. After treatment, patients were called for re-examination of stool to rule out the presence or absence of *Blastocystis* spp. Data were analyzed using SPSS software version 16.0.

Results

The result of this study shows no significant gender predilection for *Blastocystis* infection (Table 1). The highest incidence of infection was documented among age group 31–40 years, mean age being 39.8 years ± 18.8 (Table 2). Occult blood and fecal WBC were found in 82% and 86% cases, respectively (Table 3). Abdominal pain was the most frequent symptom (77.9%). Diarrhea followed it with a rate of 65.1%. Other symptoms and their frequency rates are given in Table 4. Intestinal symptoms (abdominal pain, diarrhea, flatulence, etc.) were noted in 75 (87.2%) of 86 *Blastocystis* detected patients. A second stool specimen could be obtained from 61 (70.9%) of 86 patients after metronidazole therapy. Among them, only five cases (8.2%) were found to be positive for the presence of amoeboid and/or cystic forms of *Blastocystis* spp. with the number of trophozoites ranging from 5 to 10 or >10.

Table 1. Gender distribution (n = 86).

SEX	NO. OF SUBJECTS (%)
Male	39 (45.4%)
Female	47 (54.6%)

Reevaluation of 61 out of 86 *Blastocystis* spp. positive patients was done for improvement in clinical symptoms after metronidazole therapy. Intestinal symptoms were relieved in 51 of 61 (91.8%) patients whose consecutive stool examinations revealed no intestinal parasites. Abdominal pain was relieved in 48 of 52 patients who returned for follow-up. Nausea and vomiting was relieved in all follow-up cases.

Discussion

The pathogenicity of *Blastocystis* spp. still remains a matter of controversy and debate in medical literature. The organism is considered as a potential pathogen by a few,^{11,12} whereas other authors concluded it to be merely an intestinal commensal.^{3,13,14} The investigators claiming the pathogenicity of this protozoa accepted more than five *Blastocystis* spp. cysts per 40× magnification field as pathogenicity criterion.¹⁵ El-Shazly et al showed that in 23 symptomatic patients, *Blastocystis* spp. was the only causative parasitic agent.¹ The most common presenting complaints were diarrhea (30.4%), abdominal pain (26.1%), flatulence (21.7%), and vomiting (13.1%). A large number of *Blastocystis* spp. cysts were found in symptomatic than in asymptomatic patients with significant statistical difference (8.2 cysts/100× field versus 3.8, respectively). The mean number of *Blastocystis* spp. cysts was significantly high in patients presenting with complaints of diarrhea and abdominal pain.¹ Our study showed that in patients complaining of diarrhea, fecal leukocyte counts and presence of occult blood in fecal

Table 2. Agewise distribution (n = 86).

AGE GROUP	NO. OF SUBJECTS (%)
18–30 years	18 (20.9%)
31–40 years	32 (37.2%)
41–50 years	17 (19.8%)
>50 years	19 (22.1%)

Table 3. Microscopic findings of fecal samples of patients.

MICROSCOPIC FINDINGS	TOTAL NO. OF CASES (n = 86)
Occult blood	71 (82%)
Leukocytes	74 (86%)
WBC + RBC	68 (79%)

samples were equally significant as number of *Blastocystis* spp. cysts as pathogenicity criterion (Table 3).

Baldo et al reported that among 172 children of the residential institutions and street communities in Philippines, the prevalence for *Blastocystis* spp. infection was 40.7%. This high prevalence of *Blastocystis* spp. was attributed to be because of poor water quality and sanitation in the shelters.¹⁶ In another study conducted in Jordan, fecal specimens were collected from 180 patients who presented with acute or persistent diarrhea and other intestinal symptoms. Pathogens and potential enteropathogens were identified from 140 (77.8%) of the patients, 54 of whom were infected with *Blastocystis* spp. and in 32 cases of 54 it was the sole pathogen isolated.¹⁷

Taşova et al have investigated the clinical significance and frequency of *Blastocystis* spp. in patients suffering from hematological malignancy who presented with intestinal symptoms during chemotherapy-induced neutropenia. The study concluded that *Blastocystis* spp. is not rare (13%) and should be considered in patients with hematological malignancy who have gastrointestinal complaints while being treated with chemotherapy.¹⁸

Doğan reported the presence of intestinal symptoms like abdominal pain, distention, lack of appetite, and diarrhea in 88 patients with *Blastocystis* spp. detected in stool examination.⁸

In a study assessing the intestinal parasitic infections in children in an orphanage in Thailand, *Blastocystis* spp. was found at the highest prevalence (45.2%). During the investigation, stools of all infected cases were noted for six characteristics, namely, formed, soft, loose, mucous, loose-watery, and watery, and the symptoms disappeared after chemotherapy.¹⁹

In our study, the common symptoms in patients with *Blastocystis* spp. were abdominal pain and diarrhea. *Blastocystis*

spp. was re-detected in consecutive stool samples of only five of the total 61 postchemotherapy follow-up patients. Intestinal symptoms improved in 51 (91.8%) of 56 follow-up patients after metronidazole therapy. This was consistent with a study done by Kaya et al, wherein parasite was detected in only two of 41 patients in consecutive stool samples after chemotherapy and improvement was observed in 92.3% cases.²⁰

Re-detection of *Blastocystis* cysts in five of 61 patients in consecutive stool examinations after chemotherapy can be attributed to using medicines out of order. One recent study conducted on metronidazole resistance and subtype dependent variation in drug susceptibilities of *Blastocystis* revealed that subtype 7 and subtype 4 are resistant to metronidazole and also exhibit cross-resistance to tinidazole, indicating that new unknown mechanisms of activation and/or resistance may be involved.²¹ In summary, a variety of drug treatment options are available for *Blastocystis* infections. Metronidazole seems to be the most effective drug for the treatment of *Blastocystis* infection, despite some evidence for treatment failure. In such cases, Trimethoprim/sulfamethoxazole (TMP-SMX) and nitazoxanide may be considered as second choice drugs. Treatment should be instituted if diarrhea is persistent and no other causative pathogen is identified in fecal specimens.

Conclusions

Intestinal symptoms improved in 91.8% of follow-up patients after metronidazole therapy. So we can put forward this protozoan as possible pathogen, in accordance with the favorable response to metronidazole therapy in these patients, but treatment directed at eradication of *Blastocystis* spp. is generally not indicated. Therapy should be limited to patients with persistent symptoms subsequent to a complete workup for alternative etiologies.²² Noninfectious causes of intestinal symptoms should also be carefully ruled out. However, in the absence of an alternative explanation, a presumptive treatment with metronidazole may be offered keeping in mind that the resolution of symptoms may be secondary to elimination of unidentified pathogens rather than to the treatment of *Blastocystis* spp.³

Table 4. Clinical presentation and response to treatment.

SYMPTOMS	NO. OF PATIENTS (n = 86)	FREQUENCY OF FOLLOW UP (n = 61)	FREQUENCY OF RESPONSE AFTER FOLLOW UP
Intestinal symptoms	75 (87.2%)	56	51 (91.8%)
Abdominal pain	67 (77.9%)	52	48 (92.5%)
Diarrhea	56 (65.1%)	51	46 (91%)
Nausea/vomiting	15 (17.4%)	15	15 (100%)
Constipation	3 (3.5%)	1	0 (0%)
Weight loss	2 (2.3%)	2	0 (0%)
Flatulence	16 (18.6%)	14	12 (87.5%)
Fever and other non-specific complaints	6 (7%)	2	0 (0%)



Author Contributions

Conceived and designed the experiments: VK. Analyzed the data: KT. Wrote the first draft of the manuscript: VK. Contributed to the writing of the manuscript: KT. Agree with manuscript results and conclusions: CS. Jointly developed the structure and arguments for the paper: VK, CS and KT. Made critical revisions and approved final version: CM. All authors reviewed and approved of the final manuscript.

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