

## ARE WE NEGLECTING BLASTOCYSTIS HOMINIS IN PATIENTS HAVING IRRITABLE BOWEL SYNDROME

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**ABSTRACT:** Blastocystis hominis has become unique and an interesting pathogenic parasite and has been reported to contribute to irritable bowel syndrome. The parasite is highly pleomorphic and its diagnostic yield increases when more than one laboratory technique is used. Blastocystis hominis responds to metronidazole and trimethoprim-sulfamethoxazole (TMP-SMX). The prevalence of Blastocystis hominis in patients having irritable bowel syndrome is reported to be 43% in the present study. Blastocystis hominis was seen in 25%, 30%, 18.5% and 43% samples using wet mount, trichrome staining, formol ether sedimentation technique and culture in modified Jones' medium respectively. Vacuolar forms were the predominant forms. 87.34% patients did not show Blastocystis hominis in stool samples after treatment whereas 12.65% showed the presence of parasite. Hence, it becomes mandatory to examine stool specimen of patients having irritable bowel syndrome for early diagnosis and treatment of these patients will reduce morbidity.

**KEYWORDS:** Blastocystis hominis, Blastocystosis, Irritable Bowel Syndrome (IBS).

**INTRODUCTION:** Blastocystis hominis, a unicellular, eukaryotic protozoan, once thought to be a mere commensal of the large intestine, has now become unique and an interesting pathogenic parasite.<sup>(1,2)</sup>

The infection due to Blastocystis hominis is known as blastocystosis and its prevalence has been reported to be 1.5–10% in developed as compared to 30-50% in developing countries.<sup>(3)</sup> It is acquired by faeco-oral route which leads to non-specific clinical features such as nausea, anorexia, abdominal pain, bloating, flatulence, acute and chronic diarrhoea.<sup>(1,2,4)</sup>

Blastocystis hominis has also been incriminated to play a role in functional bowel disorders such as irritable bowel syndrome.(IBS).<sup>(3,4,5-8)</sup> IBS is characterised by abdominal discomfort, abdominal pain and is associated with alteration in bowel habit in the absence of any detectable organic causes.<sup>(9)</sup> Yakoob et al.,<sup>(4)</sup> Eida et al.,<sup>(3)</sup> and Tungtrongchitr et al.,<sup>(10)</sup> have demonstrated Blastocystis hominis in stool specimens of patients having irritable bowel syndrome concluding that the parasite contributes to irritable bowel syndrome by inducing inflammation and irritability of the gastrointestinal tract. Various other studies have demonstrated significantly increased levels of specific IgG2 antibody against Blastocystis hominis in patients having irritable bowel syndrome as compared to asymptomatic controls.<sup>(6)</sup> Prevalence of Blastocystis hominis in patients having irritable bowel syndrome has been reported to be 1.5-10% in developed nations whereas up to 60% in the developing nations.<sup>(3)</sup>

Laboratory diagnosis of Blastocystis hominis relies upon direct demonstration of the parasite in stool samples.<sup>(1)</sup> Blastocystis hominis is highly pleomorphic and can be detected in stool samples in various forms like vacuolar, granular, amoeboid and cyst<sup>(1,2)</sup> and also its size varies from 2-200µm in diameter.<sup>(1)</sup> Vacuolated parasite is the most commonly encountered form<sup>(3,10)</sup> Other forms being

## ORIGINAL ARTICLE

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smaller in size may be missed on stool examination. Permanent staining and culture have been performed to improve its diagnostic yield.<sup>(3,4,11,12)</sup> Different culture media for cultivation of *Blastocystis hominis* have been used.<sup>(13,14)</sup> Of these, Jones' medium has been reported to have high sensitivity.<sup>(12,15)</sup>

Metronidazole, cotrimoxazole or nitazoxanide have been shown to successfully eradicate *Blastocystis hominis* from stool specimens and also relieve symptoms related to irritable bowel syndrome.<sup>(16)</sup>

With this background, a prospective study was conducted to determine the prevalence of *Blastocystis hominis* in patients clinically suspected of having irritable bowel syndrome which would help early detection of the parasite in stool and initiate treatment enabling symptomatic relief to them.

**MATERIALS AND METHODS:** After obtaining Institutional Ethics Committee permission, a total of 200 patients in the age group of 18-55 years diagnosed to have irritable bowel syndrome in accordance with the Rome II criteria were recruited for the study. Written informed consent was taken from these patients. The study was carried out for one year at a tertiary care multi-speciality teaching institute.

**Rome II criteria used were as follows:**<sup>(3,9)</sup> At least 12 weeks, which need not be consecutive, in the preceding 12 months of abdominal discomfort or pain that has two of the following three features:

1. Abdominal pain relieved by defecation
2. Onset of abdominal discomfort with changes in stool frequency
3. Onset of abdominal discomfort associated with changes in form or appearance of stool

The patients who were on anti-parasitic treatment in the last one month and having any other organic diseases were excluded from the present study.

The patients were instructed to submit three stool specimens in a leak proof, dry, wide mouth plastic containers on non-consecutive days. The stool specimens were processed as per the standard procedure with respect to gross and microscopic examination.<sup>(17)</sup> Concentration technique of Formol ether sedimentation, saturated common salt solution were used. Trichrome staining and culture of stool specimen using modified Jones' medium were carried out.<sup>(17)</sup>

The patients having *Blastocystis hominis* in stool specimen were administered a course of metronidazole 400 mg three times a day for 10 days and asked to submit a repeat stool specimen one week after completion of treatment. To determine the efficacy of the treatment, the stool specimens were examined for the presence of parasite and the patients were interviewed for the symptomatic relief.

The results of all the tests were observed, interpreted, recorded and statistically analysed using Chi square test. P value <0.05 was considered as significant.

**RESULTS:** Out of the total 200 patients of irritable bowel syndrome, 105(52.5%) were males and 95(47.5%) were females.

Majority of patients (22%) showing *Blastocystis hominis* in their stool specimens belonged to the age group of 27-35 years.

## ORIGINAL ARTICLE

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Statistically significant difference in the prevalence of *Blastocystis hominis* between the genders was not noted.

Sensitivity of parasite detection increased when three samples of stool were examined.

Mixed parasitic infections were not detected.

*Blastocystis hominis* was seen in 25%, 30%, 18.5% and 43% samples using wet mount, trichrome staining, formol ether sedimentation technique and culture in modified Jones' medium respectively.

Vacuolar forms were the predominant forms seen in 39% specimens.

87.34% patients did not show *Blastocystis hominis* in stool samples after treatment whereas 12.65% showed the presence of parasite.

**DISCUSSION:** *Blastocystis hominis* was considered a commensal of the large intestine of humans for a number of years.<sup>(3,13)</sup> However, epidemiological, in vitro and animal studies strongly suggest pathogenic potential of the parasite<sup>(3,13,19)</sup> and also a link between *Blastocystis hominis* and irritable bowel syndrome.

(Table 1) Out of the total 200 patients recruited for the present study, 105 (52.5%) were males and 95 (47.5%) were females. Majority of patients, i.e. 43.5% who had irritable bowel syndrome were in the age group of 27–35 years. Yakoob et al in 2004 have also reported that irritable bowel syndrome was more common in the same age group.<sup>(4)</sup>

(Table 2) The prevalence of *Blastocystis hominis* was 43% (86/200) in the present study. Almost similar prevalence has been reported by various workers to be 46% and 44.6%.<sup>(3,4)</sup> Currently Rome III criteria are being used for clinical diagnosis of irritable bowel syndrome. Rome III criteria are as follows:

- Onset of symptoms at least 6 months before diagnosis
- Recurrent abdominal pain or discomfort, at least 3 days per month in the last 3 months, associated with two or more of the following:
  1. Improvement of pain with defecation; and/or
  2. Onset of abdominal discomfort associated with a change in frequency or stool; and/or
  3. Onset of abdominal discomfort associated with a change in form (appearance) of stool

The time frame of Rome III criteria is less restrictive when compared to Rome II criteria (12 weeks of symptoms over 12 months) and is easier to understand and apply in research and clinical practice.<sup>(18)</sup> It has been observed by Sperber AD et al in 2007 that the prevalence rates for irritable bowel syndrome were 2.9 and 11.4%, respectively, when Rome II and Rome III criteria were used respectively. However, in the present study, Rome II criteria have been used as these are being followed in the tertiary care centre where the research has been carried out. We could have reported more prevalence of *Blastocystis hominis* if Rome III criteria were used for diagnosis of irritable bowel syndrome.

Comparatively higher female preponderance of infection with *Blastocystis hominis* is noted in this study (22.5%) however this is not statistically significant (P value=0.235). Major difference in the prevalence of *Blastocystis hominis* between genders has also not been reported in studies by Stenzel et al,<sup>(19)</sup> and Rapeeporn et al.,<sup>(20)</sup>

## ORIGINAL ARTICLE

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The prevalence of *Blastocystis hominis* was significantly higher, i.e.; 22% in the age group of 27–35 years than that in the other age groups. Similar observations have been made by Rapeeporn et al in 2005.<sup>(20)</sup>

*Blastocystis hominis* was detected 17.80%, 51.04% and 77.41% of patients who submitted one, two and three stool specimens respectively. Thus, there is an increase in sensitivity of detection of *Blastocystis hominis* when two or more stool specimens were examined instead of only one. Mixed infections, i.e. other protozoans and intestinal helminths were not detected in the present study.

Upon analysing clinical history of 86 patients in whom *Blastocystis hominis* was detected, it was found that 95.34% complained of diarrhoea, 91.86% complained of abdominal pain prior to defecation, 69.76% complained of indigestion, 65.11% complained of alternating diarrhoea and constipation, 11.62% complained of constipation whereas 43.02% complained of flatulence.

Considering such varied clinical presentation of patients having *Blastocystis hominis*, stool specimens need to be screened for the presence of parasite which will help in initiation of treatment and also in reducing their morbidity.

Various laboratory techniques are used to demonstrate *Blastocystis hominis* in stool specimens. There are reports mentioning differences in sensitivities of these techniques.<sup>(4,10,17,20)</sup> In the present study, *Blastocystis hominis* was seen in 25%, 30%, 18.5% and 43% patients using wet mount, trichrome staining, formol ether sedimentation technique and culture in modified Jones' medium respectively. (Table 3)

Though, not a routine practice, stool culture using modified Jones' medium has shown significantly higher yield than stool microscopy (P value=0.0001). Owing to differences in sensitivities of various techniques, a combination of techniques rather than a single one should be used to increase the diagnostic yield of *Blastocystis hominis*.

The vacuolar form of the parasite comprises a large central vacuole which occupies most of the cell space, limiting the cytoplasm and other intracellular components to a thin peripheral rim. This morphological form shows extensive variation in size ranging from 2µm to 200µm in diameter. The granular form structurally resembles the vacuolar form except for the presence of granules in central body and cytoplasm. The amoeboid form is irregular and also 10µm in diameter. The cystic form is spherical, 3-5 µm in diameter.<sup>(1,3,20,10)</sup> (Table 4) In the present study, vacuolar forms were seen in 39% specimens, amoeboid forms in 2% specimens and granular forms in 2% specimens. We could not detect any cystic forms of the parasite. This is in accordance to the findings of Eida et al.,<sup>(3)</sup> and Tungtrongchitr et al.,<sup>(10)</sup> Eida et al.,<sup>(3)</sup> have suggested that intensity of infection is based on the number of parasites present per high power field of the microscope. In the present study, there was no correlation between the number of parasites per high power field and symptoms. Also, we could not establish any correlation between morphologic form of the parasite and the disease process.

(Table 5) Stool samples of 86 (43%) patients in whom *Blastocystis hominis* was detected underwent treatment with oral administration of 400 mg metronidazole thrice daily for 10 days. 79 out of 86 patients submitted a repeat stool specimen for examination one week after completion of treatment whereas 7 patients were lost to follow up. Out of 79 patients, 69 (87.34%) did not show *Blastocystis hominis* using the above mentioned techniques, whereas it was detected in 10 stool specimens (12.65%). The patients who did not show *Blastocystis hominis* in stool specimens had symptomatic relief. Thus, parasitological cure and symptomatic relief was observed in 87.34% patients. Similar studies have been carried out by Nigro et al who have reported that 80% of patients demonstrated clearance of parasite from stool after completion of treatment.<sup>(21)</sup> 10 patients in whose

## ORIGINAL ARTICLE

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stool specimens, *Blastocystis hominis* was detected on repeat examination after treatment did not have any symptomatic relief.

The literature mentions that in at least 10% patients having irritable bowel syndrome, blastocystosis cannot be successfully treated. This could be due to decreased humoral and cellular immune functions in these patients.<sup>(10)</sup>

Thus, it is advisable not to neglect screening of stool specimens of all patients clinically diagnosed to have irritable bowel syndrome to look for the presence of *Blastocystis hominis* so that appropriate treatment can be initiated to provide parasitological cure as well as symptomatic relief to the patient.

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## ORIGINAL ARTICLE

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| Age (Years)  | Males      | (%)           | Females   | (%)           | Total      | (%)          |
|--------------|------------|---------------|-----------|---------------|------------|--------------|
| 18 - 26      | 13         | (6.5)         | 16        | (8)           | 29         | (14.5)       |
| 27 - 35      | 38         | (19)          | 49        | (24.5)        | 87         | (43.5)       |
| 36 - 44      | 35         | (17.5)        | 22        | (11)          | 57         | (28.5)       |
| 45 - 55      | 19         | (9.5)         | 8         | (4)           | 27         | (13.5)       |
| <b>Total</b> | <b>105</b> | <b>(52.5)</b> | <b>95</b> | <b>(47.5)</b> | <b>200</b> | <b>(100)</b> |

**Table 1: Age and Gender distribution of patients having irritable bowel syndrome (n= 200)**

| Age (Years)  | Number of patients positive for Blastocystis hominis |               |           |               | Total (%) |             |
|--------------|--|---------------|-----------|---------------|-----------|-------------|
|              | Males  | (%)           | Females   | (%)           |           |             |
| 18 - 26      | 6  | (3)           | 5         | (2.5)         | 11        | (5.5)       |
| 27 - 35      | 16   | (8)           | 28        | (14)          | 44        | (22)        |
| 36 - 44      | 14   | (7)           | 8         | (4)           | 22        | (11)        |
| 45 - 55      | 5  | (2.5)         | 4         | (2)           | 9         | (4.5)       |
| <b>Total</b> | <b>41</b>  | <b>(20.5)</b> | <b>45</b> | <b>(22.5)</b> | <b>86</b> | <b>(43)</b> |

**Table 2: Prevalence of Blastocystis hominis with respect to age and gender of patients having irritable bowel syndrome (n=200)**

## ORIGINAL ARTICLE

| Techniques                        | Number of samples positive for <i>Blastocystis hominis</i> (%) |        |
|-----------------------------------|--|--------|
| Wet mount                         | 50   | (25)   |
| Trichrome staining                | 60   | (30)   |
| Formol ether sedimentation        | 37   | (18.5) |
| Culture in modified Jones' medium | 86   | (43)   |

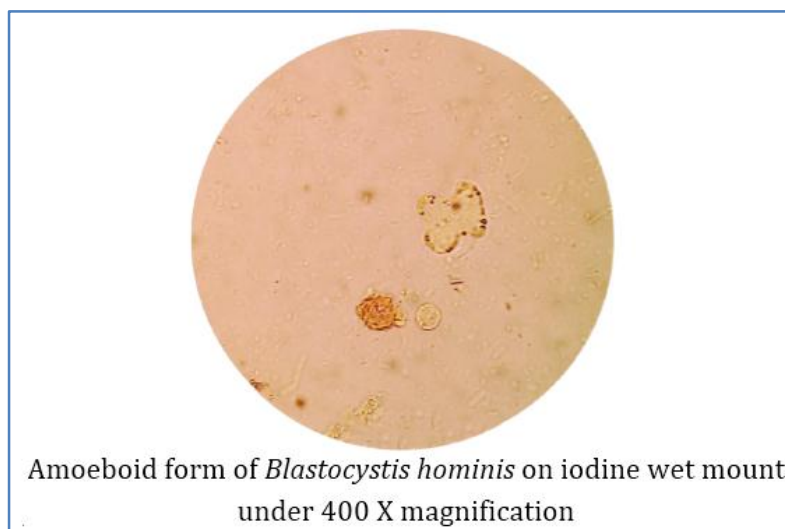
**Table 3: Distribution of *Blastocystis hominis* in relation to various laboratory techniques (n = 200)**

| Morphologic form | Total number (%) |             |
|------------------|------------------|-------------|
| Vacuolated form  | 78               | (39)        |
| Amoeboid form    | 4                | (2)         |
| Granular form    | 4                | (2)         |
| Cystic form      | 0                | (0)         |
| <b>Total</b>     | <b>86</b>        | <b>(43)</b> |

**Table 4: Distribution of different morphologic forms of *Blastocystis hominis* in patients having irritable bowel syndrome (n=200)**

| Total number of patients detected of having <i>Blastocystis hominis</i> | Number of patients who submitted a repeat stool specimen 7 days after treatment | Number of patients in whom <i>Blastocystis hominis</i> was detected after treatment (%) | Number of patients in whom <i>Blastocystis hominis</i> was not detected after treatment (%) |
|---|---|---|---|
| 86  | 79  | 10 (12.65%)   | 69 (87.34%)   |

**Table 5: Therapeutic response in patients detected of having *Blastocystis hominis* in stool specimen**



**FIGURE 1**

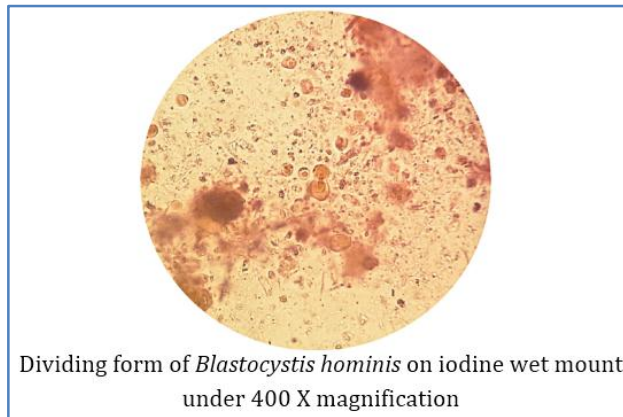


FIGURE 2

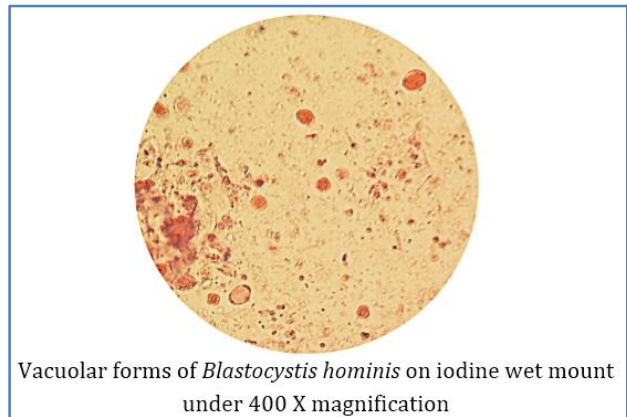


FIGURE 3

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