



## Association between *Blastocystis hominis* and irritable bowel syndrome (IBS)

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### ABSTRACT

This study was designed to examine the association between *Blastocystis hominis* and irritable bowel syndrome (IBS). In this case-control study that conducted in Mashhad, Iran in 2014-2015, one hundred IBS cases and one hundred matched (age and sex) healthy people were participated. Direct stool examination, formalin-ether concentration technique and trichrome staining were done. The data were analyzed by SPSS<sub>20</sub> with Fisher's exact test and T-test. One hundred IBS patients (31 males and 69 females) had a mean age of 29.5 ( $\pm 7.4$ ) years. *B. hominis* were positive in 26% of IBS participants, and 9% in control group ( $P$ -value=0.002, Risk Estimate=3.5). *Giardia lamblia* were positive in 6% of IBS participants, and in none of control participants ( $P$ -value=0.01). Trichrome staining for detection of *B. hominis* was more sensitive than direct examination and formalin-ether concentration technique ( $P$ -value < 0/001). *B. hominis* was more frequent in patients with irritable bowel syndrome. For detection of *B. hominis*, trichrome staining was more sensitive than other methods. Authors suggest that in patients with IBS, stool should be examined with different methods for three times to obtain a more reliable diagnosis.

**Keywords:** *Blastocystis hominis*, Irritable bowel syndrome, Iran

### INTRODUCTION

*B. hominis* is an anaerobic unicellular eukaryote that inhabits the intestinal tract of humans and many animals; it was first described in 1912 [1]. Several forms of *B. hominis* are observed in fecal samples including vacuolar, multivacuolar, avacuolar, granular, amoeboid and cyst [2-3-4]. *B. hominis* prevalence in human often exceeds 5% in developed countries and can reach as high as 76% in developing countries [3-4]. However, prevalence data are largely dependent on the methods used for detection. The most common diagnostic technique used worldwide for identification of *B. hominis* is the permanent stain. The use of xenic cultures in which *B. hominis* grown *in vitro* have been shown to be more sensitive in detecting *B. hominis*, but it is not commonly used in the diagnostic laboratory [5-6-7]. However, most laboratories recognize only the vacuolar form as the diagnostic stage since it can be easily distinguished from other protozoa.

Role of *B. hominis* in human disease has been widely debated in the literature during the two last decades, numerous recent *in vivo* and *in vitro* studies strongly suggest that *B. hominis* is a pathogen [8]. Case reports have linked *B. hominis* infection with various gastrointestinal and extra intestinal symptoms including diarrhea, abdominal pain, depression, fatigue, vomiting, constipation, anorexia, urticaria, skin rash, headaches and flatulence, but this parasite may also play a significant role in several chronic gastrointestinal illnesses such as irritable bowel syndrome (IBS)

and inflammatory bowel disease [9-10-11]. According to many studies around the globe prevalence of IBS was reported to be 5.4–14.1% in USA and in Europe (1.1–10.5%) and in Asia (2.9–22.1%) [10]. Rome II criteria separated IBS into three subtypes: diarrhea-predominant, constipation-predominant and alternating. (10) Because of increasing the prevalence of IBS in Mashhad (north east of Iran) this study was done to examine the association between irritable bowel syndrome and *Blastocystis hominis* which could help us about understanding the cause and treatment of IBS.

## MATERIALS AND METHODS

This case-control study was conducted between Jan 2014 to Sep 2015 in Mashhad, Iran. Participants were 100 IBS patients as case group and 100 healthy people as control group. The case group had medical IBS symptoms at least in 3 months which confirmed with internal medicine specialist. Data of age, sex, occupation and gastrointestinal symptoms of participants was assessed by checklist based on Rome II criteria. Laboratory evaluations that included: direct stool examination, formalin-ether concentration technique and trichrome staining.

### Direct stool examination

Direct stool examination 2 mg of fecal sample place on a microscope slide. Then a drop of normal saline added on it and covered with a cover slip. The slide examine under a light microscope using the 10× objective and 40× objective as well.

### Formalin-ether concentration

For formalin-ether concentration technique using (1.0 g-1.5) g of faeces mixed with 10 ml of water and centrifuged for 2 minutes. Then the supernatant discarded and the sediment suspended in 10 ml of normal saline and adding 7 ml of 10% formaldehyde and 3 ml of ether and shaken it vigorously after that the tubes centrifuged for at 500g for 2 minutes. Four layers became visible and three upper layers discarded and the sediment suspended with a small amount of formalin for suspension. A drop of suspension transferred to a slide for examination under a cover slip. The preparation examined with the 10× objective.

Also a drop of mentioned suspension which produced for formalin –ether concentration method stained with trichrome staining. All these procedure were done for three times in Parasitology Laboratory of Imam Reza Hospital of Mashhad University of Medical Sciences, Iran.

The data were analyzed by SPSS software version 20 with Fisher's exact test and independent sample T-test. P-value less than 0.05 was considered as significant level.

Protocol of study was approved by the university's ethical committee of Mashhad University of Medical Sciences.

## RESULTS

IBS Participant were 31 males and 69 females with mean ( $\pm$ SD) age of 29.5 ( $\pm$ 7.4) years. Healthy control were 29 males and 70 females with mean ( $\pm$ SD) age of 29.3 ( $\pm$ 7.3) years that matched grouping with case group. Clinical symptoms of the IBS patients were reported respectively: diarrhea (64%), Constipation (16%), Constipation – diarrhea (19%).

*B. hominis* were positive in 26% of IBS participants, and 9% in control group (P-value=0.002, Risk Estimate=3.5), in other word risk of *B. hominis* in patients with IBS was 3.5 times more than control group.

*Giardia lamblia* was seen in 6% of IBS participants, and was not seen in any of control participants (P-value=0.01).

Table1: Prevalence of *B. hominis* based on laboratory test method

Laboratory Test Method	Formalin-ether		Direct examination		Trichrome staining	
	Frequency	Percent	Frequency	Percent	Frequency	Percent
IBS group	6	11.5%	6	23.1%	14	53.8%
Healthy group	1	11.1%	2	22.2%	6	66.6%
P-value	0.91		0.06		0.37	

In direct examination *B. hominis* was detected in 23.1% (6 of 26) of IBS group and in 22.2% (2 of 9) of healthy group (P-value =0.06). By trichrome staining 53.8% (14 of 26) of IBS group and 66.6% (6 of 9) of controls were positive for *B. hominis* (P-value =0.37). By formalin-ether concentration technique 11.5% (3 of 26) of IBS cases and 11.1% (1 of 9) of healthy group were positive for *B. hominis* (P-value = 0.91), (Table1).

Trichrome staining for detection of *B. hominis* was more sensitive than direct examination and formalin-ether concentration technique (P-value<0/001). Vacuole forms of *B.hominis* were found more frequently than other forms (77%) and there was a significant difference between the vacuole forms and other forms of parasites (P-value = 0/007) by trichrome staining.

#### DISCUSSION

Because of increasing the prevalence of IBS in Mashhad (northeast of Iran) we decided to evaluate the relation of this disease and parasitic infections in stool samples. This study was carried out to examine stool specimens of patients suffering from irritable bowel syndrome (IBS) for *B. hominis* with three laboratory methods, at Imam Reza hospital of Mashhad, Iran from Jan 2014 to Sep 2015.

In the present study, 69% of IBS patient who referred from internal specialist were female. There are many study which were in agreement with our research. Roshandel in 2006 reported 75% of 1023 IBS patients were female [10]. In another study by Yakoob in 2004 about irritable bowel syndrome in Pakistan, 46% of patients were male and 54% were female [11]. Also in another study in Shahrekord, Hosseini reported female to-male ratio among IBS patients was 1.17 to 1 [12]. So that we concluded there is a significant relationship between sex and IBS which is prevalent in female.

Mean age of patients in this study was 29.5 ( $\pm$ 7.4) years. Hosseini in Iran reported the mean age of IBS patients was 20 years [12]. But in another study by Roshandel in 2006 mean age was 25 years [10]. We concluded that young people are more susceptible to irritable bowel syndrome. In this research the most important clinical symptoms of patients were diarrhea (64%), in other studies by Hosseini and Yakoob the most common clinical manifestations of IBS patient was diarrhea (48% and 43% respectively) [12, 13]. Therefore in IBS the most common symptom is diarrhea.

Tungtrongchrit (in Southeast Asian) found 13.6% *B. hominis* and 1.7% *Giardia lamblia* in IBS patients [14]. In our study, stool specimens were positive for *B. hominis* in 26% of IBS patients, and 6% were positive for *Giardia lamblia*, while stool specimens in 9% of controls were positive for *B. hominis* and *Giardia lamblia* in none of them was diagnosed.

In this research, we detected risk of *B. hominis* in patients with IBS was 3.5 times more than healthy group. In some study related to socioeconomic situation and climate condition there were more prevalence of *B. hominis* in IBS patient compared with control group. The risk of *B. hominis* in patients with IBS by Cekin in 2012 and Yakoob (2010) is more than 3 times in comparison with healthy people [13,15]. We found that the risk of *B. hominis* in our study is higher than in other studies.

In this study, we used three methods for stool examination: direct smear, formalin-ether concentration technique and trichrome staining. Tengku Shahrul Anuar in Malaysia reported using trichrome staining increased the chances of finding *B. hominis* in stool samples which this item is in agreement with our results [16].

Although this research found vacuole form of *B. hominis* more than other forms. Many other studies like Tan KS and Zierdt are in agreement with our finding in this issue [17, 18].

#### CONCLUSION

According to this study in Iran, *B. hominis* was more frequent in patients with irritable bowel syndrome compared to healthy group. For detection of *B. hominis*, trichrome staining was more sensitive than direct examination and formalin-ether concentration techniques. Authors suggest that in patients with irritable bowel syndrome stool examination must be done with three different lab methods for three times in order of exact diagnosis and suitable treatment.

#### Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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