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Türkiye'deki Bir Üniversite Hastanesinde *Blastocystis hominis* Enfeksiyonunun Karakteristiği

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ÖZET: *Blastocystis* (*B.*) *hominis* enfeksiyonunun karakteristiğini belirlemek amacıyla 770 kişinin dışkı örneği hem basit hem de konsantrasyon yöntemiyle, hem lugol hem de trikrom boyası ile boyanarak Türkiye'de Hacettepe Üniversitesi Tıp Fakültesi Parazitoloji Laboratuvarı'nda incelenmiştir. 770 örnek arasından 94 (%12,2) tanesinde *B. hominis* saptanmıştır. Çalışma grubunda en sık rastlanan parazit *B. hominis* olmuştur. En sık *Dientamoeba fragilis* ile birlikte saptanmıştır. Gruplar arasında, *B. hominis*'in insidansı alerjik hastalarda kontrol grubuna göre daha yüksek bulunmuştur. İmmunsüpresif hastalar arasında *B. hominis* solid tümörü olan hastalarda belirgin olarak yüksek bulunmuştur. Dışkılarında sadece *B. hominis* saptanan 48 kişide en sık semptom karın ağrısı olarak saptanmıştır. Trikrom boyası ile birlikte konsantrasyon tekniği, basit yaymanın lugol ile boyanmasına göre daha duyarlı bulunmuştur. Solid tümörlü hastalarda *B. hominis* enfeksiyonunu ve *B. hominis* ve *D. fragilis* birlikteliğinin anlaşılması için daha fazla sayıda hasta ile araştırmalar planlanmalıdır.

Anahtar Sözcükler: *Blastocystis hominis*, *Dientamoeba fragilis*, Solid tümörler, Trikrom boyası

Characteristics of *Blastocystis hominis* Infection in A Turkish University Hospital

SUMMARY: In order to determinate characteristics of *Blastocystis* (*B.*) *hominis* infection; 770 individuals' stool specimens were examined both by simple and concentration techniques and stained with iodine solution and trichrome in the Parasitology Laboratory of Hacettepe University Faculty of Medicine, Turkey. Among the examined 770 specimens, *B. hominis* was detected in 94 (12.2%). *B. hominis* was the most common intestinal parasite among the study group. It was mostly detected with *Dientamoeba fragilis*. Among the groups the incidence of *B. hominis* in allergic patients was higher than controls. Among the immunosuppressed patients, *B. hominis* was detected significantly higher in patients who had solid tumours. Of the 48 individuals who had only *B. hominis* in their stool the most common symptom was abdominal pain. Concentration technique with trichrome stain was more sensitive than simple smear with lugol solution for the detection of *B. hominis*. Studies with more patients must be planed to understand the *B. hominis* infection in solid tumour patients and coexistence of *B. hominis* and *D. fragilis*.

Key Words: *Blastocystis hominis*, *Dientamoeba fragilis*, Solid tumours, Trichrome stain

INTRODUCTION

Blastocystis hominis, an enteric protozoon, is a common cause of gastrointestinal complaints throughout the world. It has a worldwide distribution, mainly in developing countries where the incidences were found to be between 30-50% (1-3). *B. hominis* is also found in the stool of healthy cases that don't have gastrointestinal complaints (4, 5). For that reason there are controversy about its clinical importance, pathogenicity, systematic position and epidemiology (2, 3, 6-9).

Today it is believed that *B. hominis* can be a pathogen under some conditions such as immunosuppression, malnutrition, transplantation or co-infections (9, 10-12) and can be successfully treated. It is accepted as the etiologic agent of gastrointestinal symptoms when it is found in high numbers in the stool and when there are no other parasites and illnesses that can cause gastrointestinal symptoms (13). Therefore when *B. hominis* is observed in the stool the frequency of the organism should be reported. (14-16). Although there are case reports that reported small numbers of *B. hominis* as a

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causative agent, it is believed that there is a correlation between the number of B. *hominis* in the stool and the symptoms (7, 17). In order to understand the clinical significance of B. *hominis,* it is important to estimate the characteristics of cases, probably infected with *B. hominis*. The objectives of this study were to estimate personal data (age, gender, illness, contact with animals, gastrointestinal symptoms, socioeconomic level) and the presence of symptoms/signs related with *B. hominis* infection. We also compared different techniques for the diagnosis of *B. hominis* and investigated the incidence of other intestinal parasites among the *B. hominis* observed cases.

MATERIALS AND METHODS

Study and Population*:* Between May 2003 – February 2004, stool samples of 770 cases have been screened by means of *B. hominis* in the Parasitology Laboratory of Faculty of Medicine, Hacettepe University. Faculty of Medicine, Hacettepe University which is a reference center is located in Ankara the capital city of Turkey. Of the 770 cases, 475 had only gastrointestinal complaints, 82 had only allergic complaints, 23 had only chronic renal failure, 90 were immunocompromised (30 had solid tumours, 57 had hematologic malignancy, 3 had AIDS) and 100 healthy volunteers had no complaints and illness. Clinical and epidemiological data about age, gender, illness, intake of drugs, contact with animals, travelling, gastrointestinal symptoms, occupation, job, socioeconomic level from each subject were obtained using standardized questionnaires. On the basis of subjects' job, education, occupation socioeconomic level was classified into three groups; high, middle and low socioeconomic level. Diarrhea was defined as three or more unusual loose bowel movements per day. From each patient three consecutive stool specimen were evaluated, for routine ova and parasite examination. The characteristics of patients according to sex, age and socioeconomic level are shown in Table 1.

Collection, processing and examination of specimens*:* Fecal specimens were collected in clean plastic containers. After preparation of a smear from fresh stool samples, approximately 5 g of stool samples were added to the vials containing 10 ml MIF (thyomersole 0.02%, formaldehyde 2.5%, glycerol 1%) and centrifuged. The sediment was resuspended in saline and iodine solution, examined microscopically under x10 and x40 objectives. A second smear was prepared from the sediment with Mayer's albumin coated slides for trichrome staining and examined microscopically under x100 objective (18). Trichrome staining was also performed directly for fresh stool samples before concentration. Fecal specimens that had been obtained from immunosuppressed and diarrheic patients were also stained with Kinyoun's Modified Acid Fast Method and than examined for the presence of *Cryptosporidium* spp, *Cyclospora cayetenensis* and other *Coccidia* (7). Fecal specimens taken from diarrheic patients were cultured on selective media for bacterial enteropathogens.

When *B. hominis* was observed, 10 immersion objective areas was counted for quantification and regarded as rare, moderate or frequent. If the number of *B. hominis* was 2 and lower in 10 immersion objective it was accepted as rare, the number 3-9 accepted moderate and 10 or more frequent (18).

Statistical Analysis*:* Statistical analysis was done using the chi-square, cochran-a and Mc Nemal tests. A 'p' value of <0.05 was considered significant.

RESULTS

Of the 770 specimens examined, *B. hominis* was detected in 94 (12.2%). It was detected alone (without other parasites or pathogenic bacteria) in 48 patients. Table 2 shows the incidence of *B. hominis* and other parasites in the study group. *B. hominis* was the most common intestinal parasite among the study group. It was mostly found with *Dientamoeba fragilis* (Table 3). There was no significant difference in *B. hominis* detection among the groups but the incidence of B. *hominis* were higher in allergic patients than controls ($p= 0.667$). Among the immunosuppressed patients, *B. hominis was* detected significantly higher in patients who had solid tumours ($p= 0.031$) (Table 4). Among the study groups there was no correlation between gender and detection of *Blastocystis hominis* ($p=0.458$). Subjects ranged in age from 0 to 85 years (mean 28.8, the youngest case was 2 months old). *B. hominis was* detected significantly higher between the ages 0-19 compared to other age groups ($p= 0.039$). The detection of *B. hominis* was significantly lower in cases from high socioeconomic level ($p<0.05$). There was no correlation between *B. hominis* detection and contact with animals ($p= 0.534$). Of the 48 cases who had only *B. hominis* in their stool samples, the most common symptom was abdominal pain and was present in 20 cases (41.6%) (Table 5). Of the cases, 12 (25.3%) were asymptomatic. There was no significant difference between the study groups according to the number of *B. hominis* found in the stool samples ($p= 0.992$). However immunocompromised and allergic patients seems to have more *B. hominis* in their stool samples. For the detection of *B. hominis*, trichrome stained smears were more sensitive than lugol stained smears ($p<0.05$) (Table 6).

DISCUSSION

Blastocystis hominis is often the most frequent protozoon reported in human Fecal samples both from symptomatic patients and from healthy cases. In our study it is also the most frequently detected parasite in the Fecal samples. It has a worldwide distribution, mainly in developing countries where the incidences are 30-50% (1, 2, 3). In general reports from Turkey, the incidences of *B. hominis* in patients having gastrointestinal complaints were between 1.05-15% and 0.09-35.86% in healthy volunteers (19, 20, 21, 22). In our study the incidence of B. *hominis* in the patient groups ranged between 11.2-17.1% and the incidence in healthy volunteers was 13%.

Table 1. Characteristics of study group according to gender, age and socioeconomic level

	n (%)	Gender	Age	Socioeconomic Level		
		Female/Male	Mean (Range)	Low n (%)	Middle n (%)	High n (%)
Patients with gastrointestinal complaints	475(61.8)	251/224	20.1(0-80)	89(19)	315(66)	71(15)
Patients with allergy complaints	82(10.5)	50/32	20(2-72)	20(24.4)	52(63.4)	10(12.2)
Immunocompromised patients	90(11.7)	42/48	45.8(4-85)	19(21.1)	61(67.7)	10(11.2)
Patients with Chronic renal failure	23(3.0)	10/13	23.0(4-69)	4(17.3)	17(74.0)	2(8.7)
Healthy cases	100(13.0)	40/60	23.3(0-72)	21(21)	66(66)	13(13)
Total	770(100)	393/377	28.8(0-85)	153(19.8)	511(66.4)	106(13.8)

Table 2. The incidence of *B. hominis* and other parasites in the study group.

	Patients with gastro intestinal complains n=475	Patients with allergy complains n=82	Immunoco mpromised patients n=90	Patients with Chronic renal failure n=23	Healthy cases n=100	Total n=770
	Positive n (%)	Positive n (%)	Positive n (%)	Positive n (%)	Positive n (%)	Positive n (%)
B. hominis	53 (11.2)	14 (17.1)	11(12.2)	3 (13)	13 (13)	94 (12.2)
Entamoeba histolytica	2 (0.4)	1 (1.2)	0	0	1 (1)	4 (0.5)
Entamoeba coli	6 (1.2)	2 (2.4)	2 (2.2)	0	0	10 (1.2)
Dientamoeba fragilis	15 (3.1)	3 (3.6)	0	0	3 (3)	21 (2.7)
Giardia intestinalis	20 (4.2)	3 (3.6)	1 (1.1)	3 (13)	3 (3)	30 (3.8)
Chilomastix mesnili	0	0	0	1 (4.3)	0	1 (0.1)
Isospora belli	0	0	0	1 (4.3)	0	1 (0.1)

Table 3. The coexistence of *B.hominis* and other intestinal parasites.

	Number of patients
B.hominis* and *D.fragilis	21
B.hominis* and *G.intestinalis	13
B.hominis* and *E.coli	7
B.hominis* and *D.fragilis G.intestinalis	2
B.hominis* and *D.fragilis E.histolytica	2
B.hominis* and *E.coli E.histolytica	1

Table 4. The incidence of *B.hominis* in patient groups.

Immonocompromising Disorder	*Blastocystis hominis*	
	Positive n (%)	Negative n (%)
Solid Tumours (N=30)	7 (23.3)	23 (76.7)
Hematologic Malignancies (N=57)	3 (5.2)	54 (94.8)
AIDS (N=3)	1	2
Chronic Renal Failure(N=23)	3 (13)	20 (87)

Table 5. Symptoms of patients who had only *B. hominis* in their stool and patients who had no parasite in their stool.

	Patients who had only *B. hominis* in their stool n (%)	Patients who had no parasite in their stool n (%)
Abdominal pain	20 (41.6)	288 (56.5)
Diarrhea	2 (4.1)	37 (7.2)
Nausea	2 (4.1)	39 (7.6)
Constipation	4 (8.3)	43 (8.4)
Allergy	8 (16.6)	67 (13.1)

Table 6. Detection rate of *B.hominis* with different techniques.

	Blastocystis hominis	
	Positive n (%)	Negative n (%)
Lugol Stain	39 (5.9)	731 (94.1)
Trichrome Stain (Direct)	78 (10.1)	692 (89.9)
Trichrome Stain (Concentrated)	94 (12.2)	676 (87.8)

Among our study groups there was no significant difference in *B. hominis* carriage, but the highest incidence was in patients with allergic skin disease (17.1%). Some reports also have suggested that *B. hominis* may be the causative agent of a variety of allergic diseases such as chronic urticaria and atopic dermatitis (23, 24).

Infection with *B. hominis* appears to be common in immuno-compromised patients (11, 25-37). Incidence of *B. hominis* carriage in immunocompromised patients was reported between 0.5-15% in the literature (26, 28-30, 33, 35, 37). We found the incidence of *B. hominis* 12.2% in immuno-compromised patients. Among the immunocompromised patients the highest detection rate of *B. hominis* was found in patients with solid tumours (23.3%). In a case report a patient who had solid tumour, the number of *B. hominis* was increased due to intestinal obstruction and after the surgery it disappeared (38). In our study none of the patients having solid tumour had intestinal obstruction.

Symptoms commonly attributed to infection with *B. hominis* are non-specific and include Diarrhea, abdominal pain, cramps or discomfort, nausea, constipation (7, 8, 14, 39-42). We found no correlation between *B. hominis* carriage and gastrointestinal complaints. The most common symptom was abdominal pain among the patients who had *B. hominis* alone in their stool. Other symptoms included allergic skin disease, constipation, Diarrhea and nausea. Although some studies have reported slightly increased female to male incidences, there does not appear to be any major difference in the incidence of *B. hominis* between genders (2, 17, 40). In this present study we also found no correlation between gender and detection of *B. hominis.*

Young adults have the highest rates of infection but some studies reported higher incidences in adults than in children (2, 17, 41). We found *B. hominis* incidence highest in 10-19 age group, significantly.

Lower socioeconomic groups or those with lower standards of hygiene may show higher *B. hominis* incidences (43, 44). This finding is similar to our findings as we also found significantly lower *B. hominis* incidences in cases from high socioeconomic level.

Zoonotic transmission of *B. hominis* has been suggested by some studies. However, too few animal isolates of *B. hominis* were genetically proven to be identical to those human isolates (45, 46). In our study we found no association between animal contact and *B. hominis* carriage.

In this study we used both direct wet mount and concentration techniques to detect *B. hominis* carriage. Some authors suggested that concentration techniques were unsuitable to detect *B. hominis* from Fecal specimens because the parasite could be easily disrupted (47). In contrast several other studies have reported that concentration techniques are useful (8, 48). In this study of the *B. hominis* positive specimens 82% were detected by simple trichrome stain, 100% were detected by trichrome stain from concentration technique. Our results showed that concentration techniques were suitable for the diagnosis of *B. hominis* infection.

Our findings about the characteristics of the patients that have *B. hominis* in their stool are similar to the literature except the high incidence in solid tumour patients among the immunocompromised group and co-existence of *B. hominis* and *D. fragilis* in high numbers. In conclusion, *B. hominis* infection should be ruled out in the patients having allergic complaints of unknown origin and that can not be treated with standard allergic drugs. Studies with high number of patients must be planed to understand the B. *hominis* infection in solid tumour patients and coexistence of *B. hominis* and *D. fragilis*.

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