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• BASIC RESEARCH •

Epidemiological survey of Blastocystis hominis in Huainan City, Anhui Province, China

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Abstract

AIM: To provide scientific evidence for prevention and controlling of blastocystosis, the infection of *Blastocystis homonis* and to study its clinical significance in Huainan City, Anhui Province, China.

METHODS: Blastocystis homonis in fresh stools taken from 100 infants, 100 pupils, 100 middle school students and 403 patients with diarrhea was smeared and detected with method of iodine staining and hematoxylin staining. After preliminary direct microscopy, the shape and size of Blastocystis homonis were observed with high power lens. The cellular immune function of the patients with blastocystosis was detected with biotin-streptavidin (BSA).

RESULTS: The positive rates of Blastocystis homonis in fresh stools taken from the infants, pupils, middle school students and the patients with diarrhea, were 1.0 % (1/100), 1.0 % (1/100), 0 % (0/100) and 5.96 % (24/403) respectively. Furthermore, the positive rates of Blastocystis homonis in the stool samples taken from the patients with mild diarrhea, intermediate diarrhea, severe diarrhea and obstinate diarrhea were 6.03 % (14/232), 2.25 % (2/89), 0 % (0/17) and 12.31 % (8/65) respectively. The positive rates of *Blastocystis homonis* in fresh stools of male and female patients with diarrhea were 7.52 % (17/226) and 3.95 % (7/177) respectively, and those of patients in urban and rural areas were 4.56 % (11/241) and 8.02 % (13/162) respectively. There was no significant difference between them ($P>0.05$). The positive rates of CD_3^+, CD_4^+, CD_8^+ in serum of Blastocystis homonis-positive and-negative individuals were 0.64 ± 0.06, 0.44 ± 0.06, 0.28 ± 0.04 and 0.60 ± 0.05, 0.40 ± 0.05 and 0.30 ± 0.05 respectively, and the ratio of CD_4^+/CD_8^+ of the two groups were 1.53 ± 0.34 and 1.27 ± 0.22. There was significant difference between the two groups ($P<0.05$, $P<0.01$).

CONCLUSION: The prevalence of *Blastocystis hominis* as an enteric pathogen in human seems not to be associated with gender and living environment, and that *Blastocystis hominis* is more common in stool samples of the patients with diarrhea, especially with chronic diarrhea or obstinate diarrhea. When patients with diarrhea infected by *Blastocystis hominis* , their cellular immune function decreases, which make it more difficult to be cured.

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INTRODUCTION

Blastocystis homonis (B.h) is increasingly recognized to be a cause of human enteric disease. Its presence has been reported in a wide variety of intestinal disorders resembling irritable bowel syndrome (IBS) such as bloating, flatulence, mild to moderate diarrhea, abdominal pain, and nausea[1-35].The geographic distribution of *Blastocystis homonis* appears to be global, with infections common in tropical, subtropical and developing countries[36-40]. In general, studies from developed countries report approximately a 1.5 % to 10 % overall prevalence of *Blastocystis homonis*[41-45]. However, few reports of the prevalence and the importance of the protozoan *Blastocystis homonis* as an intestinal pathogen in China have been found. In order to explore the epidemiological characteristics and clinical significance of blastocystosis in population of the city of Huainan, a prospective study was carried out from July to August in 2001.

MATERIALS AND METHODS

Population

The study was performed in the following groups of the population in Huainan: in a healthy population (n=300, normal group), including infants in day-care centers (n=100), pupils (n=100) and middle school students (n=100), and in outpatients with diarrhea (n=403, male 226 and female 177, aged from 6 to 52 years). In addition, we paid more attention to the patients with intractable diarrhea.

Methods

A questionnaire, administered by a nurse, was used to collect detailed information of each subject investigated. Information was collected by means of in-person, telephone and interview, including age, gender, history of present illness, anamnesis, symptomatology (i.e. fever, upper respiratory tract infection, nausea, diarrhea, abdominal cramps, bloating, steatorrhea), date of symptom onset, duration of symptoms, personal health habits, and living environmental condition and the date of stool sample collected.

Stool examination All individuals were asked to provide one stool sample in disposable stool boxes for analysis. Samples were sent to the Department of Etiology and Immunology, School of Medicine, Anhui University of Science & Technology in Huainan for *Blastocystis homonis*. Then the sample was smeared to semitransparent feces membrane on the surface of sheet slides. After these smears were left to dry naturally and fixed with methanol, iodine solution and hematoxylin staining were made, and examination under microscope was carried out. The shape and size of *Blastocystis homonis* were observed.

Detection of T lymphocyte subsets To investigate possible changes of cellular immune function in Blastocystis homonis-infected individuals, the level of CD_3^+, CD_4^+,CD_8^+ and CD_4^+/CD_8^+ in peripheral blood of Blastocystis homonis-positive individuals were tested with biotin-streptavidin (BSA) method. Firstly, peripheral venous blood of subjects was withdrawn, anticoagulated with heparin, and diluted with fluid free of Ca^{2+}, Mg^{2+}. Secondly, peripheral blood mononuclear cells were separated with lymphocytes separating medium, cleaned, and the number of cells was adjusted to (1-3)×10^9/L of which 10 μl was taken and smeared in an acid-proof varnish circle on the surface of the slides. When it dried naturally, McAb of anti-CD_3^+, anti-CD_4^+ and anti-CD_8^+ and sheep anti-guineapig IgG, SA- HRP were added into the circle. After development with DAB, the slides were observed under microscope. Only brown cytomembrane staining was regarded as positive, otherwise, as negative specimen. A total of 200 cells were counted, and the positive percentage of cells were analyzed respectively.

Statistical analysis

The positive rates were expressed as percentage, and the statistical analysis was carried out by using χ^2 and *t*-test. A probability value of less than 0.05 was considered statistically significant.

RESULTS

Stool examination

Of the 703 stool samples examined, 3.70 % (26/703) were found to be positive for *Blastocystis hominis*. Furthermore, the positive rate of *Blastocystis hominis* in 300 stools of healthy people was 0.67 % (2/300); and those of infants, pupils and middle school students were 1.00 % (1/100), 0 (0/100) and 1.00 % (1/100) respectively. In addition, The positive rates of *Blastocystis hominis* in the stools taken from the outpatients with mild diarrhea, intermediate diarrhea, severe diarrhea and obstinate diarrhea were 6.03 % (14/232), 2.25 % (2/89), 0 % (0/17) and 12.31 % (8/65) respectively. There was significant difference in the positive rates between each type of patients (P<0.05). The detailed results are showed in Table 1.

Table 1 The detective results of *B.h* in fresh feces (*n*, %)

Group	*n*	*B.h* positive	
		n	rate
[b]Normal	300	2	0.67
Infants	100	1	1.00
Pupils	100	1	1.00
Middle school students	100	0	0.00
[b]Diarrheic outpatients	403	24	5.96
[a]Mild	232	14	6.03
[a]Intermediate	89	2	2.25
[a]Severe	17	0	0.00
[a]Obstinate	65	8	12.31

[a]P<0.05, χ^2=7.9475; [b]P<0.01, χ^2=13.5181 *vs*: comparison with normal and abnormal and different diarrhea

Relationship between gender and infection of Blastocystis hominis

Of the 403 outpatients, the positive rates of Blastocystis hominis in male and female patients were 7.52 % (17/ 226) and 3.95 % (7/177) respectively. Statistics found no significant difference in positive rate between male and female.

Relationship between living place and infection of Blastocystis hominis

The positive rates of *Blastocystis hominis* in stools taken from patients with diarrhea living in urban and in rural areas were 7.52 % (17/226) and 3.95 % (7/177) respectively. There was no significant difference between the two groups (P>0.05).

Relationship between types of diarrhea and infection of Blastocystis hominis

The positive rate of *Blastocystis hominis* in stools of healthy people was 0.67 % (2/300), while that of diarrheic patients was 5.96 % (24/403). Among the patients with diarrhea, the positive rates of *Blastocystis hominis* in loose stools ,watery stools and mucopurulent bloody stools were 3.70 % (21/305), 4.23 % (3/81) and 0 % (0/17) respectively. There was no significant difference between each type of patients (P>0.05). Results are showed in Table 2.

Table 2 Relationship between types of diarrhea and infection of B.h (*n*, %)

Group	*n*	*B.h* positive	
		n	rate
Normal	300	2	0.67
Diarrhea	403	24	5.96
Loose stool	305	21	3.70
Watery stool	81	3	4.23
Mucopurulent bloody stool	17	0	0.00

P>0.05, χ^2=2.2767 *vs*: comparison with different diarrhea

Changes of cellular immune function in Blastocystis hominis-*infected individuals*

Compared with the negative group, the level of CD_3^+ ,CD_4^+ and CD_4^+/CD_8^+ of *Blastocystis hominis*-infected individuals decreased, but that of CD_8^+ did not change.

Table 3 Tlymphocyte subsets of patients with *B.h* in faeces ($\bar{x}\pm s$, number fraction)

B.h	*n*	CD_3^+	CD_4^+	CD_8^+	CD_4^+/CD_8^+
Positive	26	0.64±0.06	0.44±0.06[a]	0.28±4.44	1.53±0.34[b]
Negative	30	0.60±0.05	0.40±0.05[a]	0.30±5.12	1.27±0.22[b]

[a]P<0.05, [b]P<0.01, *vs* negative

DISCUSSION

Results from this study showed that *Blastocystis hominis* as an intestinal pathogen in humans was found in Huainan area by stool examination, and the prevalence was not related to gender

and living circumstances, and that statistically significant association was observed between the presence of diarrhea and infection with *Blastocystis hominis*.

In this study, *Blastocystis hominis* was found in 26 (3.70 %) of the 703 stool specimens examined. The positive rates of male was similar to that of female, and there is no significant difference in the positive rates between the diarrhea patients living in urban areas and those in rural areas (P>0.05), which showed the prevalence of the organism was not related to gender and living environment of the individuals examined.

The results of this study supported the idea that *Blastocystis hominis* was associated with diarrhea. The positive rates of *Blastocystis hominis* in stools of the healthy people was 0.67 % (2/300), while that of the diarrheic patients was 5.96 % (24/403), and the difference between them was significant (P<0.05). To be exact, the positive rates of *Blastocystis hominis* was high in stools of the patients with mild diarrhea, intermediate diarrhea and obstinate diarrhea, but there was no *Blastocystis hominis* found in stools of patients with severe diarrhea. In accordance with other reports[46-49], vacuolar *Blastocystis hominis* were found in stools of patients with diarrhea with iodine solution and hematoxylin staining. This finding suggested that vacuolar *Blastocystis hominis* might be the main type of *Blastocystis hominis* causing diarrhea. Although the reasons why the organism had been found in both symptomatic and asymptomatic individuals have been largely unknown[50-56], one possibility was that it was due to infection time, infection dose, poly-infection with bacteria and the ability of host immunity that might decide whether the symptom turned up or not, because only over 24 h could the cysts of *Blastocystis hominis* develop into a large number of vacuolar forms[57-58].

In addition, this experiment demonstrated that the hematoxylin staining offered a very convenient and easy method to differentiate the various stages of *Blastocystis hominis*. As a matter of fact, there is high affinity between hematoxylin and *Blastocystis hominis*. By hematoxylin staining, the walls, nucleus, chromatoid bodies and other structures of *Blastocystis hominis* can be observed clearly, and vacuolar, granular, metamorphotic *Blastocystis hominis* can be easily differentiated from small amebae which do not cause any disease[59-61].

Our study provided evidence for the changes of cellular immune function in *Blastocystis hominis*-infected individuals. In this paper, the level of CD_3^+,CD_4^+,and CD_4^+/CD_8^+ decreased in *Blastocystis hominis*-infected individuals , but that of CD_8^+ was normal. Compared with the *Blastocystis hominis* negative group, the difference was significant (P<0.05).Recent advances in *Blastocystis hominis* found that in subjects suffering from immunodepression *Blastocystis hominis* showed a significant association with gastrointestinal symptoms[62-71]. All of these showed that the infection of *Blastocystis hominis* was related to the hosts' cellular immune function.

The level of CD_4^+/CD_8^+ is key to immunoregulation. When decreased, it suggested that T helper lymphocytes took part in the course of diarrhea caused by *Blastocystis hominis*. Indeed, both the ability of humoral immunity and that of cellular immunity decreased in the patients with low level of CD_4^+/CD_8^+, which made it difficult to cure diarrhea[72-75]. Because of low ability of immunological kill mediated by CD_8^+ cell, the cellular immunity of human bodies played an important role in the course of diarrhea.

In conclusion, Blastocystis hominis should be kept in mind of parasitologists and physicians when dealing with patients with diarrhea. Blastocystis hominis has long been described as a non-pathogenic protozoan parasite until recently, when claims have been made that it can result in pathogenic conditions[76-78]. Many labs do not know that it is now considered harmful to human bodies, or do not know how to test for it. Moreover, because of absence of specific symptoms, the disease was easily confused with other intestinal diseases and was easily misdiagnosed. The authors suggested that stool examination should be carried out on patients with diarrhea in order to decide whether or not the patients were infected by *Blastocystis hominis*, and the stool samples should be collected more than once from patients showing clinical signs and symptoms.

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