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

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Methods: Blastocystis hominis 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 hominis 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 hominis 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 hominis 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 CD5+, CD8+, and CD4+ 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 CD4+/CD8+ 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.
Detection of T lymphocyte subsets To investigate possible changes of cellular immune function in Blastocystis hominis-infected individuals, the level of CD3+, CD4+, CD8+ and CD4+/CD8+ in peripheral blood of Blastocystis hominis-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 Ca2+, Mg2+. Secondly, peripheral blood mononuclear cells were separated with lymphocytes separating medium, cleaned, and the number of cells was adjusted to (1-3)x10^6/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-CD3+, anti-CD4+ and anti-CD8+ 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 was analyzed respectively.

Statistical analysis
The positive rates were expressed as percentage, and the statistical analysis was carried out by using χ² 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 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   | n   | rate |
|---------------------|-----|-----|------|
| Normal              | 300 | 2   | 0.67 |
| Infants             | 100 | 1   | 1.00 |
| Pupils              | 100 | 1   | 1.00 |
| Middle school students | 100 | 0   | 0.00 |
| Diarrheic outpatients | 403 | 24  | 5.96 |
| Mild                | 232 | 14  | 6.03 |
| Intermediate        | 89  | 2   | 2.25 |
| Severe              | 17  | 0   | 0.00 |
| Obstinate           | 65  | 8   | 12.31 |

P<0.05, χ²=7.9475; P<0.01, χ²=13.5181 vs comparison with normal and abnormal and different diarrhea

Stool examination, and the prevalence was not related to gender

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 % (21/570), 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   | 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, χ²=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 CD3+, CD4+ and CD4+/CD8+ of Blastocystis hominis-infected individuals decreased, but that of CD8+ did not change.

Table 3 T lymphocyte subsets of patients with B.h in faeces (x±s, number fraction)

| B.h | n   | CD3+ | CD4+ | CD8+ | CD4+/CD8+ |
|-----|-----|------|------|------|-----------|
| Positive | 26 | 0.64±0.06 | 0.44±0.06 | 0.28±4.44 | 1.53±0.34 |
| Negative | 30 | 0.60±0.05 | 0.40±0.05 | 0.30±5.12 | 1.27±0.22 |

P<0.05, ᵇP<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.

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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[56-59], 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[56-59], 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[57-59].

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, metamorphic 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 CD4+ CD8+ and CD4+/CD8+ decreased in Blastocystis hominis-infected individuals , but that of CD8+ 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 CD4+/CD8+ 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 CD4+/CD8+, which made it difficult to cure diarrhea[72-75]. Because of low ability of immunological kill mediated by CD4+ 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 as a non-pathogenic protozoan parasite until recently, when claims have been made that it can result in pathogenic conditions[76-79]. 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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