

CLINICAL, IMMUNOLOGICAL AND PARASITOLOGICAL PARALLELS IN PATIENTS WITH BLASTOCYSTOSIS

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ABSTRACT

On clinical and prophylactic indications, 7905 patients were parasitologically examined, and *Blastocystis hominis* was detected in 273 (3.4%) of them. Detailed investigations were carried out in 82 of the infected patients (19 males and 63 females), divided into two groups: individuals with clinical symptoms and asymptomatic carriers. A correlation was found between the number of parasites detected, the clinical presentation and the changes in the levels of total serum immunoglobulins (IgG, IgA, IgM) in the persons infected with *B. hominis*. Half of the patients investigated presented with gastrointestinal and allergic symptoms. Significant decrease of levels of serum immunoglobulin IgA, correlating with the number of parasites detected was found in 43 patients, irrespective of the presence or absence of clinical symptoms.

Key words: *Blastocystis hominis*; morphological forms; serum immunoglobulins; clinical presentation.

INTRODUCTION

There are few comprehensive studies on *Blastocystis hominis*, and its pathogenicity has long been discussed. The parasite is widely spread throughout the world and is a protozoon most often detected in human fecal material (1, 3, 9, 15, 18, 20). Several morphological forms are described in literature: vacuolar, granular, amoeboid and cyst forms (15, 19). Usually, the parasite is detected by light microscopy, and the most common form found is the vacuolar. There are a large number of publications with controversial data, in which the protozoon is defined as a commensal, a human pathogen, or an opportunistic protozoal microorganism. Most authors agree that *B. hominis* is capable of causing gastrointestinal disorders in both immunocompetent and immuno-compromized subjects (3, 4, 7, 14, 18, 21).

The aim of the study is to look for a correlation

between the clinical form of blastocystosis - asymptomatic and clinically manifested, and some clinical, parasitological and immunological indices.

MATERIALS AND METHODS:

2.1. Patients investigated.

In 2006, 7905 patients referred for prophylactic check-ups or on clinical indications were investigated. Some of the carriers of *B. hominis* (82 individuals) underwent detailed investigations. Special attention was paid to: determining the number of parasites found, the clinical and humoral immune status of patients. These patients were divided into two groups: group A - 41 persons presenting with clinical symptoms and positive for *B. hominis*; and group B - 41 asymptomatic patients without complaints and positive for *B. hominis*.

2.2. Materials investigated.

Fresh fecal samples were investigated, collected in chemically treated sterile glass vials. Venous blood samples were collected from the same patients, using a closed system, to investigate serum globulins.

2.3. Diagnostic methods.

Fecal smears – wet and stained with iodine solution, were microscopically investigated for intestinal protozoa. According to some authors, there is a correlation between the number of parasites detected and the severity of clinical presentation (14, 19). The parasitological finding was calculated as a mean sum of parasites detected in 100 microscopic fields (magnification 400), and the patients were grouped according to the number of parasites – mean numbers up to 5, and more than 5 parasites per a microscopic field, as proposed by Zierdt (19).

In cases with diarrhoeal symptoms, fecal mass or rectal secretion was investigated for pathogenic intestinal flora. Levels of IgG, IgA and IgM in blood sera were determined by the method of radial immunodiffusion

following the method of Mancini (17). The results were processed using variation and correlation analysis and SPSS for Windows V 13.0.

RESULTS

Of the 7905 subjects examined for intestinal parasites, 273 (3.4%) were positive for *B. hominis*. Further investigations were performed in 82 positive patients - 19 (23.2%) males and 63 (76.8%) females.

Age distribution was as follows: children and adolescents aged 2-19, n=23 (age 6.5 ± 4.3 years) and adults aged 20-78, n=59 (40.9 ± 11.3 years).

In 79 (96.3%) of the fecal samples investigated only *B. hominis* was found. In 3 samples (3.7%), other intestinal parasites besides *B. hominis*, such as - *Giardia lamblia* (2 cases) and *Enterobius vermicularis* (1 case) were detected.

The distribution of complaints by symptoms in group A patients is presented in **Table 1**.

Table 1: Main symptoms in patients with *B. hominis* and clinical complaints (group A)

Total number of patients	Number of parasites per field	No/percentage of patients with:					
		Abdominal pain	Meteorism	Unstable defecation	Persisting constipation	Clinically manifested allergy	Other complaints
41	>5	16 (39.0%)	13 (31.7%)	7 (17.0%)	4 (9.7%)	15 (36.6%)	2 (4.9%)
	<5 (2.4%)	1 (17.0%)	7 (24.9%)	2 (49.7%)	4 (12.4%)	1 (-)	

In the patients from group A, those with gastrointestinal disorders and clinically manifested allergy prevailed.

In the cases with gastrointestinal complaints, the leading symptom was abdominal pain. The clinical presentation also included meteorism, constipation or diarrhea. Patients with diarrhea were investigated using routine microbiological methods, and no pathogenic intestinal bacteria were detected.

Clinically, the allergy presented with allergic rash and/or swelling of the face. No other causes for the allergy

reaction were found.

A statistically significant difference was found between patients with more than 5 and less than 5 parasites, on the one hand, and abdominal pain and clinically manifested allergy, on the other hand. ($\chi^2=12.083$ P = 0.001).

Fig. 1 (A) and (B) presents the relative share of decreased values of serum immunoglobulins (IgG, IgA, IgM) in patients in groups A and B, which were lower than the normal range for the respective immunoglobulin.

The levels of serum immunoglobulins IgA and IgG

Fig. 1. Serum immunoglobulin in group A and B

Fig. 1 (A)

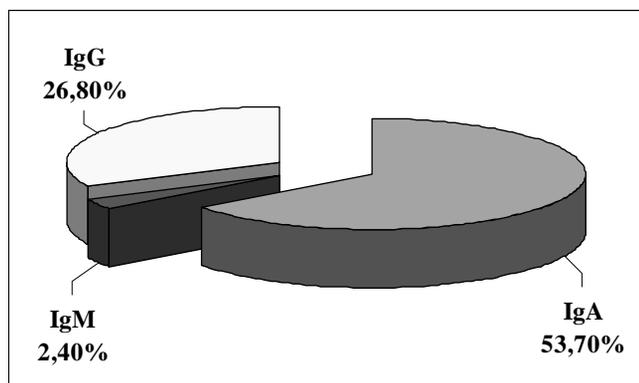
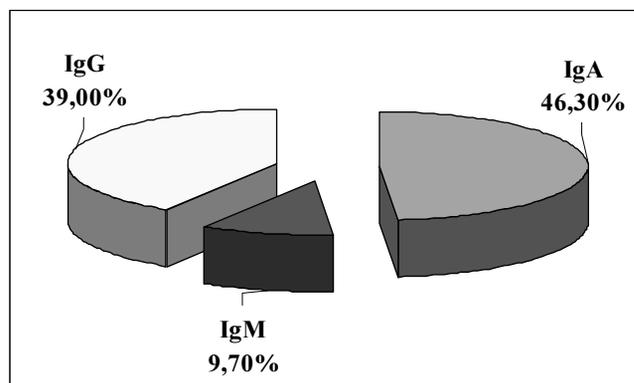


Fig. 1 (B)



in groups of patients A and B were lower than normal, and the decrease in levels of IgA in subjects from both groups was essential.

We found low levels of IgG in 11 patients in group A, that were below the lower limit and ranging from 820mg% to 481mg% (accepting as reference IgG values

1160±305mg%). The levels of IgA in 23 patients were between 134mg% and 84mg% (accepting as reference IgA values 200±60mg%). In group A patients, IgG levels were below reference values in 17 patients (between 527mg% and 820mg%), and IgA levels were decreased in 20 patients (from 62 mg% to 138mg%).

Comparison between the levels of serum immunoglobulins IgG and IgA in group A and B revealed a

low correlation ($r = -0.290$; $r = 0,079$), and lack of statistically significant differences between the two groups of investigated patients. ($p > 0.05$).

Table 2 demonstrates the changes in serum immunoglobulin levels against the background of the parasitological finding and the clinical symptoms.

Using the test of Mann-Whitney for comparison in

Table 2. Parasitological finding, clinical symptoms and changes in immunoglobulin levels in groups A and B (number of patients)

Groups of patients	Number of parasites	No/% of patients with: No/% of patients with reduced Ig levels				
		Gastrointestinal symptoms	Clinically manifested allergy	IgG	IgA	IgM
Group A n = 41	< 5	8 (19.5%)	1 (2.4%)	2 (4.8%)	3 (7.3%)	-
	> 5	23 (56.1%)	15 (36.6%)	9 (21.9%)	20 (48.8%)	1 (2.4%)
Group B n = 41	< 5	-	-	7 (17.0%)	6 (14.6%)	3 (7.3%)
	> 5	-	-	10 (24.4%)	14 (34.1%)	1 (2.4%)

irregular distribution of variables, we established significant differences between IgA values determined in patients with <5 parasites and with >5 parasites per microscopic field ($z = -2.51$; $p = 0.012$).

DISCUSSION

The study showed that half of the patients positive for *B. hominis* (group A) presented with specific clinical symptoms, of which abdominal pain and clinically presented allergy were most common. We assume that the absence of another etiological agent in these patients gives evidence of possible pathogenicity of this organism, that was pointed by other investigators, too (4, 9, 13, 20).

Some authors assume that there is a correlation between the number of parasites and the severity of clinical symptoms (14, 19). The studies of Shlim et al., (1995) however, do not confirm such correlation. In our study, a statistically significant difference was found regarding abdominal symptoms and clinically presented allergy between patients with less than 5 parasites and those with more than 5 parasites per microscopic field (magnification 400) in group A ($p < 0,05$). Among the patients with asymptomatic blastocystosis (group B), more than half of the patients had more than 5 parasites per microscopic field - 22 (53.6%) but they did not develop any clinical signs. We establish no correlation between the number of parasites and the clinical presentation of patients in group A and

group B. Despite the small number of individuals investigated and the controversial results for group A and B, in some of the cases the clinical symptoms were well expressed and correlated with the larger number of parasites detected. In Bulgaria there are only few studies on blastocystosis up till now and there is no knowledge on the features and especially on the virulence of the indigenous strains of *B. hominis*. Our data indicates the presence of pathogenic strains among human population in our country. Apart from the number of parasites, the pathogenicity of *B. hominis* seems to be attributable to a number of other factors such as strain characteristics, immune status of the host, number of times being infected, accompanying intestinal disorders of parasitic or other etiologies (4, 7, 14).

The investigations we carried on the humoral immune status in subjects with *B. hominis* showed a significant decrease in levels of serum IgA and IgG, and these changes correlate with the number of parasites detected in both groups.

IgA protease activity of *B. hominis* and other protozoa has been experimentally proved, which leads to an assumption that it is possible that the parasite is capable to surmount the barriers of intestinal mucosa by cleaving the secretory IgA (10, 11, 12). Persistent clinically presented forms of intestinal protozooses, associated with disturbances of humoral immunity have also been reported (8, 16). An assumption could be made that *B. hominis* may

possess similar characteristics.

The lowered levels of IgA in patients from group A and group B could be interpreted as either directly

attributable to the infection with *B. hominis*, or as a prerequisite for more frequent infections.

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