DETECTION OF BLASTOCYSTIS HOMINIS IN CHILDREN WITH DIARRHEA AND THE EFFECT OF INFECTION ON NUTRITIONAL STATUS OF THEM IN TIKRIT CITY- IRAQ

DİYARELİ ÇOCUKLARDA BLASTOCYSTIS HOMINIS VARLIĞININ VE NÜTRİSYONEL DURUMLARINA ETKİSİNİN SAPTANMASI

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ABSTRACT

Objectives: Blastocystis hominis is a causative agent of blastocystosis. It is world-wide cause of illness and disease. Our aim was to show the distribution of B. hominis among patients with diarrhea aged 6-12 years old and its effect on nutritional status of the children.

Materials and Methods: A cross-sectional study was carried out on 1100 children attended to three clinical centers in Tikrit. The clinical examination was carried on for all children including anthropometric measurements and laboratory investigation including general stool examination, haematological and some biochemical parameters.

Results: The overall rate of B. hominis infection was 2.27%, the infection rate was highest among 10-12 years old. The haemoglobin concentration (g/dl) among infected and non-infected children was within normal range. The total WBC count in infected children was higher than non-infected ones. The infection lead to neutropenia, lymphocytosis, decrease monocytes but had no significant effect on eosinophil and basophils. The infection significantly decreased the level of serum zinc (mmol/l). Although the values of T-rosettes cells and B-lymphocytes increased but the percentage of Null cells decreased in infected children.

Conclusions: The rate of Blastocystis hominis in Kirkuk city was 2.27%. The infection did not lead to anemia, but increased total leucocytes count, lymphocytes; Null cells; decreased neutrophils, monocytes, B-lymphocytes, T-rosetts cells and serum zinc concentration.

Key Words: Blastocystis hominis, anthropometric measurements, haematological and biochemical parameters.

ÖZET

Amaç: Blastocystis hominis dünya çapında bir hastalık olan blastositozun etken ajanıdır. Bizim amacımız, bu çalışmada 6-12 yaş grubunda ishali olan çocuklardaki B. hominis dağılımını ve bu çocukların nütrisyonel durumlarına etkilerini incelemektir.

Materyal ve Metot: Tikrit'teki üç klinik merkeze getirilen 1100 çocuk üzerinde çapraz kesitsel bir çalışma yürütülmüştür. Tüm çocuklarda antropometrik incelemeleri içeren klinik incelemeler ve genel gayta inceleme, hamatolojik ve bazı biyokimyasal parametreleri içeren laboratuar araştırmaları yapılmıştır.

Sonuçlar: Kerkük şehrindeki B. hominis oranı %2.2 olup, enfeksiyon anemiye sebep olmamış, total lökosit sayısını, lenfositleri, Null hücrelerini arttırmış, nötrofil, monosit, B-lenfosit, T- rozet hücreleri ve serum çinko konsantrasyonunu azaltmıştır.

Anahtar Kelimeler: Blastocytes hominis, antropometrik ölçümler, hematolojik ve biyokimyasal parametreler.

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INTRODUCTION

Blastocystis hominis are anaerobic protozoan parasites found in human gastrointestinal tract. They are among the most common protozoan to be detected in faecal specimens[1]. Its prevalence varies between communities[2]. The parasite is transmitted by feco-oral route and via contaminated water. The parasites seem to be of poor host specificity; transmission occurs from human to human and between humans and animals[3].

Blastocystis spp. exhibit wide genetic diversity. There are about nine genotypes of *Blastocystis* and recent studies have shown that no single subtype specific to human exists and that all subtypes have been detected in human stool[3]. Therefore, human isolates of *Blastocystis* that were commonly referred to as *Blastocystis hominis* should be called *Blastocystis* spp[4]. *B. hominis* in stool samples of symptomatic and asymptomatic individuals was evaluated as a possible cause of gastrointestinal troubles[5].

Blastocystis spp. have a global distribution, appearing more frequently in tropical and subtropical countries[6]. It ranges from 1.5% to 15% in developed countries, and from 30% to 50% in developing countries. It occurs in both children and adults; it can be transmitted by contaminated water, pets and raw vegetables[7]. It is considered as pathogenic in immunodepressed individuals, causing symptomatic or asymptomatic chronic carrier state[8].

In Iraq, it is detected that 8.6% out of 58 children with malignancy were infected with *Blastocystis hominis* and 6.9% had combined infection of *B. hominis* and *G. lamblia*[9]. In children attending Pediatric Teaching Hospital In Sulaimania City Iraq, a study was carried out on 307 stool samples collected from children aging from six months to 12 years old, using direct wet mount method[10]. The frequency of *B. hominis* was found to be 22.15%. The rate of infection in females (23.8%) was higher than in males (20.9%).

In Baghdad, a study was carried out on 200 adult patients of different ages and sexes, to show the pathogenicity of *B. hominis* and their coexistence with other enteropathogens, attending the medical city outpatient clinic with the complaint of diarrhea[11]. It was found that the number of cases with *B. hominis* was 82(41%); 58(29%) single infection 21(10.5%) mixed with other parasites; 3(1.5%) mixed with other bacteria.

A study was held on 40 primary school children in Baghdad to show the effect of parasitic infections on nutrition, growth and physiology of the host during the period from October 2008 to May 2009[12]. They found the mean serum zinc levels were lower in parasitically infected children (71.06) than the control (96.61) and heights and weights of the infected children were (100.59 cm/28.24 kg) lower than controls (109.78cm/31.89 kg). They also reported the rate of *G. lamblia, E. histolytica, Ent. coli, E. vermicularis and B. hominis* were 15%, 12.5%, 5%, 7.5% and 2.5%, respectively.

In Turkey, a study was conducted between January 2002 and June 2003 to evaluate the relationship between *B. hominis* and growth status in 89 infected children and compared them with that of 178 non infected children[13]. They found that the anthropometric measurements and body mass indices were significantly lower in the case group than in the control group. Also, a study evaluated the clinical symptoms associated with *B. hominis* infections among 61 cases of *B. hominis* (40 males and 21 females aged 5-69 years). It has been reported that, the main clinical symptoms associated with *B. hominis* cases were; abdominal pain (39.4%), pruritus (36.1%), diarrhea (6.6%) and constipation (3.3%) respectively[14].

In Yazd City in Iran, a cross sectional study was carried on one hundred and eighty children under six years to determine the prevalence of intestinal parasites in Day Care Centre. The rate of *B. hominis* was found to be 2.8%[15].

A study was carried out in two schools inTanzania on 657 children aged 7-15 years. On analysis of formalin-fixed stool samples, it was seen that 74.7% of the children harbored at least one intestinal protozoa. About half of the children (48.7%) were infected with at least one of three potentially pathogenic intestinal protozoa[16]. The potentially pathogenic *B. hominis* was diagnosed in 154(28%) of the children; i.e. 73(26.9%) girls and 81(29.0%) boys; with the rate of low 125(81.2%), moderate 25(16.2%) and heavy 4(2.6%) respectively. Odd ratio (OR) of being infected with *B. hominis* among school-aged children, assessed by logistic regression OR girls VS boys at (95% CI) was 0.94 (0.64-1.37).

Nutritional agents that play an important role in immune functions include zinc, selenium, vitamin E, vitamin B-6 (pyridoxine), and linoleic acid. Deficiencies of these compounds impair both circulating (humeral) as

well as cell-mediated immunity. The requirement for essential nutrients increases in geriatric individuals, because immune function and the bioavailability of these nutrients generally wane with aging[17]. As with any nutrient, however, excessive supplementation can lead to significant clinical problems, many of which are similar to the respective deficiency states of these ingredients.

Zinc is ubiquitous in cellular metabolism. It is necessary for a wide range of biochemical, immunological and clinical infections[18]. Plasma zinc levels can be affected by a number of factors, such as low dietary intake, intestinal disease, infection and surgery[19,20]. Zinc deficiency is common in children from developing countries because of the lack of intake of animal products, high dietary phytate that limit zinc absorption and inadequate food intake. Zinc deficiency is commonly associated with acute and persistent diarrhea especially in children. There are also increased fecal losses of zinc during diarrhea[21].

The aim of the study was to detect the relationship among *Blastocystis hominis* existence and nutritional status of children with diarrhea aging 6-12 years.

MATERIALS AND METHODS

Study population: The total number of samples collected in Tikrit city composed of 1100 children aged from 6-12 years old (445 boys and 655 girls). Those were the children submitted to the clinical centers during the time of the study suffering from diarrhea. All the children were exposed to complete clinical examination in the three Medical Centers and private medical laboratories, including laboratory investigations. The study started on February 2010 and ended on the first of June 2012.

A special questionnaire form was arranged to collect information; direct interviewing with the eligible adults and with the children's parents was fulfilled.

Anthropometric measurements: The body weight of children were measured by electronic balance distributed by (WHO/UNICEF to primary health care's) with minimal clothing.

The height of the children measured to the nearest 0.5cm with a vertical measuring scale fixed to the metal bar connected to the beam balance.

The skin fold thickness was measured using Harpenden skin fold caliper with a constant pressure of 10gm/sq mm. It was used to serve as an index of subcutaneous fat.

The mid upper arm circumference was measured to the nearest 0.1 centimeter with a non-elastic fiber glass measuring tape, which was placed gently, but firmly round the middle of the left upper arm to avoid compression of the soft tissue.

Collection of the specimens: One-three stool samples were collected from each patient in a tightly covered wide mouth plastic disposable container. These were labeled with sample numbers, date and name of the patient. The sample was examined within 1 hour from collection to detect the parasitic infection. The samples were examined by direct wet mount method; formal-ether concentration and modified acid fast stain[22]. Blood samples were obtained as 10 ml from each patient; 9 ml of blood without EDTA and 1 ml of blood added with EDTA-was drawn intravenously from each subject. The former was used for T-and B-lymphocyte counts, biochemical tests, and zinc concentration. The blood with EDTA was used for hemoglobin estimation, total and differential WBC count.

Biochemical tests: The total serum protein and albumin were measured by using kits from Randox Laboratories Ltd., U.K. The value of serum globulin was estimated by subtracting the value of serum albumin from the total protein. The blood urea was estimated by using urea-kit S.180 (bioMerieux); the uric acid was estimated by using uric acid-enzymatic kit RAP 150 (bioMerieux). The serum creatinine was estimated by using creatinine-colorimetric method (RANDOX). The alkaline phosphatase was estimated by colorimetric method using kit of bioMerieux sa Marcyl Etoile-France.

The serum zinc level was estimated using atomic absorption spectrophotometer (Varian, Australia) after dilution of serum samples with deionized distilled water 1:4 to bring the metal concentration within the working range of atomic absorption spectrophotometer by direct aspiration of the samples.

Hematological parameters: The hemoglobin estimation was done by Sahli method, the test is done by dilution of 0.02 ml. of blood with 2 ml of 0.1 mol/hydrochloric acid converting the hemoglobin to acid hematin.

The white blood cell numbers were counted under light microscope (10X and 40X) using Neubauer counting

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chamber. It was estimated by diluting of whole blood in Turk's solution (glacial acetic acid, 1% with 1-2 drop methyl blue to give purple colour).

The differential leucocyte count was made by counting 200 leukocytes in blood smears, stained with Leishman's stain. These were differentiated into neutrophils, eosinophils, basophils, lymphocytes and monocytes and the percentage of each was calculated using oil lens microscope (100X).

Statistical analysis: Both Chi-square and student's t-test were used according to the requirement of the test[23].

RESULTS

Table 1 shows the frequency of *B. hominis* among school children. The overall rate of infection among school children was 2.27%, the highest rate of infection was among 10-12 years old (3.43%) followed by 7-10 years and 6-7 years old respectively. No significant differences were found between infection and age groups ($\chi 2$=P>0.05).

Table 1. Distribution of Blastocystis hominis infection according to age in Tikrit

Age	No. examined	No. infected	Infection %
6-7	159	2	1.25
7-10	621	12	1.93
10-12	320	11	3.43
Total	1100	25	2.27
Chi-square $\chi 2$		P>0.05	

Table 2, shows the anthropometric measurements values (mean weight, height, skin fold thickness and mid upper arm circumference among children with *B. hominis* infection and non-infected controls of different age groups. Statistically, there was significant difference between infected and non-infected groups in all parameters.

Table 2. The anthropometric measurements values among B. hominis infected patients and none infected

Age	No. examined	Mean weight Kg	Mean Height cm	Mean SFT mm	Mean MUAC mm
Infected 6-7	2	18.2 ±0.6	110.2 ±0.5	8.3 ±0.5	17.0 ±0.4
Non-infected	157	18.7 ±0.3	111.7 ±0.3	8.9 ±0.3	17.8 ±0.5
Infected 7-10	12	26.2 ±1.9	129.8 ±1.4	9.3 ±1.4	18.4 ±0.7
Non-infected	609	26.6 ±0.5	129.8 ±0.8	9.8 ±0.8	20.2 ±1.2
Infected 10-12	11	42.2 ±0.3	145.6 ±0.6	11.3 ±1.4	22.5 ±0.9
Non-infected	309	42.7 ±0.7	145.9 ±0.4	11.9 ±0.8	22.9 ±0.7
Total Examined	1100	<0.05			

Mean values ± S.D.

Table 3 shows the hemoglobin concentration and total white blood cells values among patients of different age groups, infected and non infected with *B. hominis*. Although patients' haemoglobin values in infected children were lower than non-infected children, this value was within normal range. The white blood cell counts among different age groups of children was significantly higher among infected children than non-infected ones (P<0.01).

Table 3. The hemoglobin concentration and white blood cells count among B. *hominis* infected patients and controls

Age (year)	No. Examined	Hemoglobin g/dl Mean±S.D.	WBC count Cell/10^9/l Mcl Mean±S.D.
Infected 6-7	2	10±1.5	11500±1.9 **
Non infected	15	13.5±2.4	9000±2.6
Infected 7-10	12	11.6±1.6	9500±2.1 **
Non infected	14	12.5±2.3	8000±2.5
Infected 10-12	11	11.8±2.6	9600±1.0 **
Non infected	18	13.5±2.8	6800±2.6

** t-test sig. difference (P<0.01)

Table 4 shows the differential white cell counts among infected and non infected children with *B. hominis*. The neutrophyl and monocytes percentage in infected children were significantly lower than non infected ones, while lymphocyte percentage was higher in infected children than in non-infected ones. The eosinophil and basophil percentages did not vary significantly between infected and non-infected ones.

Table 4. Differential white blood cells count among infected patients and controls

Group	No. examined	Neutrophil %	Eosinophil %	Basophil %	Lymphocyte %	Monocyte %
Infected	32	49±1.1	1.8±2.6	0.6±3.1	40±0.5	3.8±0.4
Non infected	40	51±0.9	2±2.1	0.5±2.7	39±1.5	4±0.4
T test		<0.01	NS	NS	<0.01	<0.05

Table 5 shows significant the difference of zinc (µmol/L) concentration in serum of patients infected with *B. hominis* infection and control ones. Zinc concentration in serum among patients with *B. hominis* infection was significantly lower than non-infected ones (P<0.05).

Table 5. Serum zinc levels among children infected and non-infected children

Age (year)	Number of samples	Zn (µmol/L Mean value ± S.D.	t-value
Infected 6-10	6	8.0 ± 0.6	P<0.05
Non infected	6	10.0 ± 0.6	
Infected 10-12	6	12.9 ± 0.7	P<0.05
Non infected	6	15.1 ± 0.5	
Total	24		

Table 6 shows values of total T-rosettes forming cells, B-lymphocytes and Null cells percentages among *infected and* non-infected children. In infected children, the values of total T-rosettes forming cells and B-lymphocytes were lower while the Null cell was higher than non-infected cells. Statistically, there were significant differences in all values between infected and non-infected children.

Table 6. Values of T-rosettes forming cells (%), B-lymphocytes (%) and Null cell (%) among B. hominis infected children and controls

Group	No. examined	Mean values ±S.D.		
		T-rosettes Cells	(%) B-lymphocytes	(%) Null cell
		53 ± 4.2	19 ± 6.8	29 ± 4.8
Infected	12	59 ± 5.1	22 ± 8.6	20 ± 5.6
Non infected	10	P<0.01	P<0.01	
t-test				

Table 7 shows some biochemical parameters (mean serum alkaline phosphatase (ALP), total proteins, albumin and globulin) in infected and non-infected patients. The mean value of alkaline phosphatase, total protein, albumin and globulin did not vary significantly between infected and non infected groups (P>0.05).

Table 7. Some biochemical parameters in infected and non infected patients (gr/dl)

Group No.	No. examined	ALP	Total protein	Albumin	Globulin
Infected	25	95.3 ± 3.40	5.4 ± 0.60	3.9 ± 0.73	1.6 ± 0.80
Non infected	40	94.3 ± 4.13	5.5 ± 0.76	4.0 ± 0.86	1.5 ± 0.83
t-test		P>0.05	P>0.05	P>0.05	P>0.05

DISCUSSION

The overall rate of *Blstocystis hominis* among 1100 children aged from 6-12 years old was 2.27%. This finding is almost identical to those reported (2.5%) in Baghdad[12]; 2% in Kuwait[24], 2.8% in Iran[15], but lower than those reported as 3.16% in Kirkuk[25]; (22.15%) in Sulaimania[10]. The difference in the rate of infection in various studies may be related to sample size, place and period of the study, age of children, hygienic conditions, acidity of stool samples and techniques used for identification of the parasite. In Kirkuk province 4.17% was reported among Internal Iraqi displaced people[26]. Regarding the acidity of stool samples, it was reported that stool samples belonging to irritable bowel syndrome patients were 90.47%; in a high rate (57.89%), pH ranged between 7.1-8.5, in the stool samples in 36.84% and 5.26% with pH ranged between 6-7 and 4-6, respectively[27].

Although statistically no significant differences were found between infection and age groups of children, the rate of infection was highest in 10-12 years old children. This could be due to the fact that older children go outside more and may be exposed to infection more than younger ones. This finding is similar to that reported in some studies[10,28].

Parasitic infections are detrimental to children's nutrition status that can be easily measured by some anthropometric values all over the world in addition to the harm of inadequate or imbalanced dietary intakes. Children may also suffer from acute diarrhea and vomiting decreasing iron absorption, especially when radically infected with parasites. Moreover, pathogenic parasites which are carried by over half of the parasitic infected children's population may generate more severe physiological disorders as reported[24].

The lower weight, height, skin fold thickness and mid upper arm circumference found in infected children indicates the parasite lead to growth retardation among the studied groups. Similar findings were reported among children aged 6-12 years old in Hawija district, Kirkuk Governorate[29]. This is also confirmed in a study evaluating the relationship between *B. hominis* and growth status in children; they showed that the anthropometric measurements and body mass indices were significantly lower in the case group than in the control group[13].

The lower hemoglobin concentration among infected children reflect that *Blastocystis hominis* infection af-

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fect the general health of those children. Similar findings were reported in Sinniya district, Salahaddin province[30].

The white blood cells' main function is to fight infection, defend the body by phagocytosis against invasion by foreign organisms, and to produce or at least transport and distribute antibodies in the immune response. In this study there was increase in total white blood cell counts in all ages, while the highest increase in white cell counts was among 6-7 years old. The increase in white blood cells during *B. hominis* infection is also reported somewhere else[31]. On the other hand, it was found that the workers infected with *B. hominis* had a lower leukocyte count than those who were not, mainly caused by a reduced neutrophil count[32]. They also found the hemoglobin and hematocryte were also reduced in *B. hominis* workers. In the current study, although the neutrophyl and monocyte percentages decreased, the lymphocyte percentage increased in infected patients; no significant difference was seen in eosinophyl and basophil percentages. A study was carried out in Kindergarten children in Gaza to investigate possible changes in blood parameters that are associated with gastroenteritis infection, they found the most common enteric pathogens to be *Entamoeba histolytica* and *Giardia lamblia*[33]. They showed that the WBC was higher in cases compared to the control group. However, the mean score result of Hb concentration was slightly lower in cases compared to control; the other blood parameters (RBC, HCT, MCV, MCHC and PLT) had almost the same mean score results.

All children from 6-12 years old infected with *B. hominis* had lower serum zinc concentration than non-infected ones in our study as in another study which found low zinc levels in serum of children aged 6 years infected with diarrhea[34]. The low zinc level is also reported in *B. hominis* infection among primary school children in Baghdad[12]. The same results were shown among children suffering from persistent diarrhea in Guatemala[35], while no changes in zinc level in *B. hominis* infected patients were found in another study[36]. In Turkey[37], it was demonstrated that *G. lamblia* infection is associated with lower serum zinc level. It has been reported that a decrease in serum zinc could be due to dietary factors; i.e. intake of food low in antioxidants, so the body will use endogenous cytoplasmic superoxide dismutase to scavenge the over production of reactive oxygen species as a result of parasitic infection which leads to adecrease serum zinc levels[38].

The results of serum alkaline phosphatase estimation in children infected with *B. hominis* in different age groups among patients and control were without any significant differences at $P>0.05$. Among children infected with *B. homini,* no elevated report was documented. It has been reported that ALP and WBC elevated in amoebic liver abscess[39].

Because albumin is synthesized by the liver, decreased serum albumin may result from liver disease. It can also result from kidney disease, which allows albumin to escape into the urine. Decreased albumin may also be explained by malnutrition or a low protein diet. This study did not report the ability of *B. hominis* to produce any other disease than acute diarrhea, so no elevation in albumin was recorded[40].

Blood serum contains large amounts of proteins. Two major groups of proteins in blood serum are albumin and globulins. A total serum protein test measures the total amount of protein in blood serum as well as the amount of albumin and globulin. Because the total protein represents the sum of albumin and globulins, it is important to know which protein fraction is high or low than what the total protein is[41]. The results of total protein and serum immunoglobulin estimation in children infected with *B. hominis* and suffering from diarrhea among different age groups among infected and non-infected ones were without any significant differences.

CONCLUSION

The rate of *B. hominis* among children between 6-12 years old in Tikrit was 2.27%. The highest rate of *B. hominis* was recorded among 10-12 years old school children. It had no significant effect on haemoglobin concentration or eosinophil and basophil counts; but total WBC and lymphocytes increased; it lead to neutropenia and decreased the monocyte counts. The infection had no significant effect on total protein, albumin, globulin and alkaline phosphatase, but significantly lead to decrease serum zinc concentration and total Null cells and increased T-rosetts cells and B-lymphocytes.

Further studies are recommended to carry on to show the frequency of *B. hominis* in different parts of the country using different diagnostic technique such as cultivation, serological and polymerase chain reaction techniques.

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