

Clinical Significance of *Blastocystis* Sp. among Children with Leukemia

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ABSTRACT

Background: *Blastocystis* spp. distributes world widely and the genus *Blastocystis* include many subtypes that are isolated from human intestinal tract. It is considered the most common parasite detected in human being.

Objectives: To evaluate the incidence of *Blastocystis* spp. among leukemic children, to find out its association with the presence of symptoms (diarrhea and abdominal pain), and to assess the efficacy of different staining methods in detection of *Blastocystis* spp.

Type of the study: cross-sectional study.

Method: 103 children were enrolled in this study, 53 leukemic patients and 50 healthy controls. The study was performed during the period between January and June 2014, in Oncology department of Ibn Al-Atheer teaching hospital in Mosul city. Three consecutive samples were taken from each child and a thorough history was taken from them. The samples then treated with 3 stains, iodine, iron hematoxylin, and trichrome. The data was analyzed using minitab version 17, Fischer's exact test, Two-proportions test, and Correlation test. The results were considered statistically significant with P-value <0.05.

Results: *Blastocystis* spp. was detected in 18.87% of leukemic children and in 10% of healthy group. All specimens were stained by iodine, iron hematoxylin, and trichrome stains. Infection rates among patients were (9.4%,

11.3%, and 18.87% respectively) while among healthy children they were (4%, 6%, and 10% respectively). However, these differences were statistically nonsignificant. A higher infection rate was detected in the preschool children (33.3%) than school age group (6.9%). Furthermore, there was a significant association between *Blastocystis* spp. infection and intestinal symptoms (abdominal pain and diarrhea).

Conclusion: Our study reveals a higher rate of infection with *Blastocystis* spp. among leukemic children than normal controls and a significant association was seen between incidence of *Blastocystis* spp. infection and symptomatic children. Those results draw attention for the significance of *Blastocystis* spp. infection in immunosuppressed patients and lighten the way for further studies on its pathogenicity and diagnostic methods.

Key words: *Blastocystis*, Leukemia, Immunosuppression

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B *Lastocystis* spp. are unicellular, anaerobic enteric parasites that was considered as a harmless yeast in the past (1). It is distributed worldwide and found in almost all species of animals. It increases especially in developing countries in the tropics and sub tropics (2). *Blastocystis* spp. is one of the most common intestinal protozoa found in human intestinal tract (3). Nine subtypes (genotypes) of *Blastocystis* are described on the basis of small subunit ribosomal RNA gene analysis (4). As well, isolates from humans and animals can be divided into 12 species, several of which are found in humans and no subtype exclusive to them (5,6). Consequently, human isolate should be referred as *Blastocystis* spp. instead of *Blastocystis* hominis. All *Blastocystis* spp. that were isolated from humans and animals have been reported to be morphologically similar and cannot be differentiated microscopically (7). The morphological stages present in stool sample include vacuolar, granular, and amoeboid. The vacuolated form is most commonly found in feces and responsible for transmitting infection by fecal-oral route (8). The pathogenicity of *Blastocystis* spp. is still an issue of controversy. Initially, it was believed to be a commensal protozoan, but recent studies support its pathogenicity in

certain situations (9). The incidence of *Blastocystis* spp. increases in malignancy causing symptomatic or asymptomatic infections. The clinical manifestations among symptomatic individuals include diarrhea, vomiting, abdominal pain, flatulence (10). The disease progresses rapidly in patients with leukemia in particular (11,12). Leukemia is the most prevalent malignancy in children below 15 years. A large number of leukemic children become victims of infections that considered a lethal cause in leukemia (13). Diagnosis of *Blastocystis* spp. is based on microscopic detection using direct wet mount preparation method with lugol's iodine or trichrome stain which is more sensitive for identification of the protozoa (14). Monotherapy with metronidazole is the most commonly recommended drug with eradication rates vary from 0-100% (15). Studying the prevalence of *Blastocystis* spp. in patients with leukemia will help in assessment of its pathogenicity and correlation with diarrhea and abdominal pain among those immunosuppressed children.

Methods: This study was conducted during January to June 2014 on two groups; fifty three children (28 male, 25 female) with leukemia attending Oncology

department of Ibn Al Atheer teaching hospital in Mousl City, and fifty apparently healthy children (25 male, 25 female) included patient relatives as a control group. All children were between ages of (1.5-12 years) and the two groups were matched by age and sex to each other. A special data was provided for each child in the study included (Age, Sex, Residence, presence or absence of diarrhea & abdominal pain).

Stool sample collection: Three consecutive stool specimens were collected from each child and stored in wide mouth tightly closed containers. These containers were labeled with the child name, age, date of collection. Each stool sample was stored in 10% formalin solution. The samples were centrifuged by ZnSO₄ and formal-ether (ethyl acetate) concentration technique. Sediments were examined by Wet mount with Lugol's iodine, Iron hematoxylin, and trichrome stain (16) (17).

Statistical analysis: Statistical interpretation of data was performed by using the computerized software program minitab version 17. Fischer's exact test and Two-proportions test were used to compare between the variables in the study, while Correlation test was used to detect a possible association between *Blastocystis* spp. infection and symptoms (diarrhea & abdominal pain). All P values > 0.05 were considered statistically insignificant.

Results: *Blastocystis* spp. was found in 10 (18.87%) of the 53 leukemic children and in 5 (10%) of the 50 healthy control group Fig.1. Table 1 shows the distribution of *Blastocystis* spp. in the study and control groups according to gender and area of residency. The infection rate was higher among male than female in both patients (25%, 21%) and controls (12%, 8% respectively). Also it was higher in rural than urban among leukemic children (20%, 17.4%) and healthy group (15.4%, 4.2%) respectively. Although, infection rates were higher among patients than controls, these differences were statistically insignificant. Fig. 2 All of the collected samples were stained by 3 types of stains iodine, iron hematoxylin and trichrome. The infection rates were (9.4%, 11.3%, and 18.87% respectively) in the patients group and (4%, 6%, and 10% respectively) in the control group. The highest infection rates were recorded when the samples stained with trichrome and higher infection rates were seen among the patients than the controls but the difference was nonsignificant and p value >0.05. In table 2 the leukemic children were divided according to the age in to two groups. The first group included children < 6 years (preschool) who showed an infection rate of (33.3%), while the second group included those ≥ 6 years (school age) with an infection rate of (6.9%). This difference was statistically significant with p value 0.004. Again the patients were divided into two groups (symptomatic & a symptomatic) according to the presence and absence of diarrhea and abdominal pain in order to study the correlation between *Blastocystis* spp. infection and clinical symptoms. 50% of the 1st group was positive for *Blastocystis* spp. while only 2.85% of the second group was positive.

The difference was statistically significant and a direct relationship was found between the appearance of symptoms (diarrhea & abdominal pain) and *Blastocystis* spp. infection Fig.3.

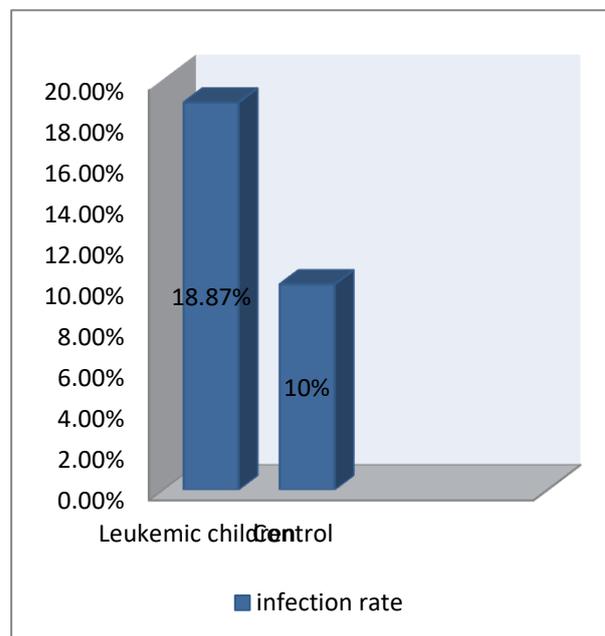


Fig.1: Difference between infection rate with *Blastocystis* spp. in leukemic children and control

Table 1: The rate of infection with *Blastocystis* spp. according to the gender and place of studied children

Characteristic		patients			control		
		Examined No.	+ve	-ve	Examined No.	+ve	-ve
Gender	Male	28	7 25%	21 75%	25	3 12%	22 88%
	Female	25	3 12%	22 88%	25	2 8%	23 92%
Place	Urban	23	4 17.4%	19 82.6%	24	1 4.2%	23 95.8%
	Rural	30	6 20%	24 80%	26	4 15.4%	22 84.6%

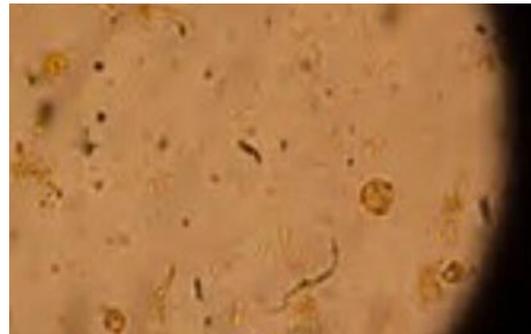
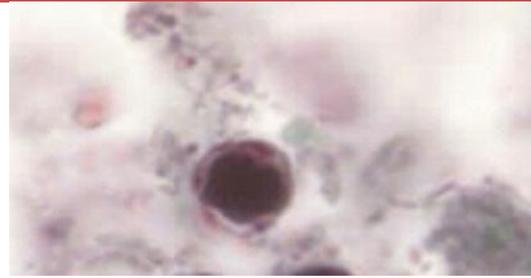
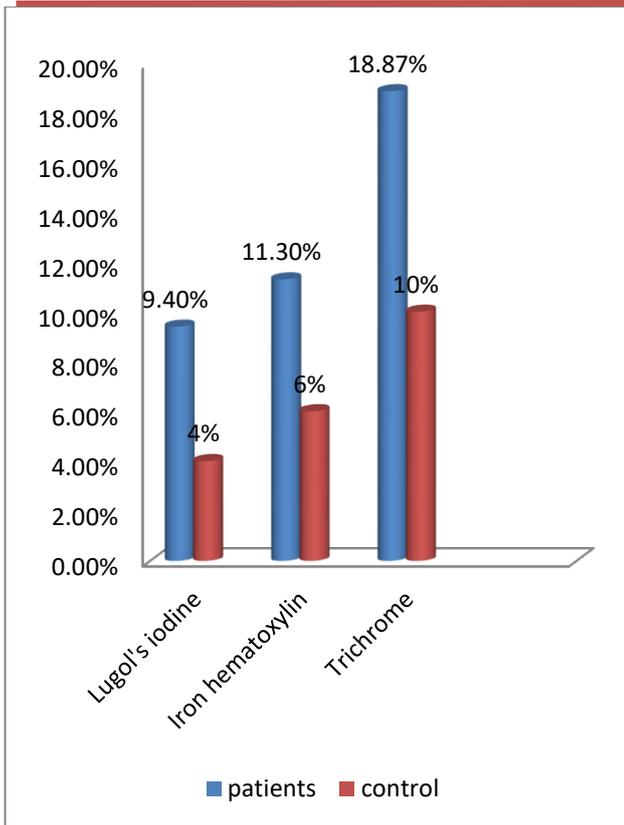
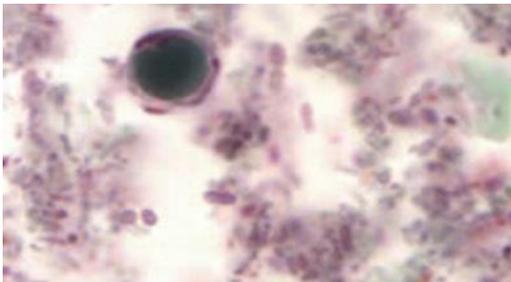


Fig.2: Comparison between efficacy of different staining methods for diagnosis of *Blastocystis* spp. in leukemic children



Blastocystis spp. cyst stained by Trichrome stain (large central vacuole stained gray-green color and the peripheral nuclei stained red-purple color)



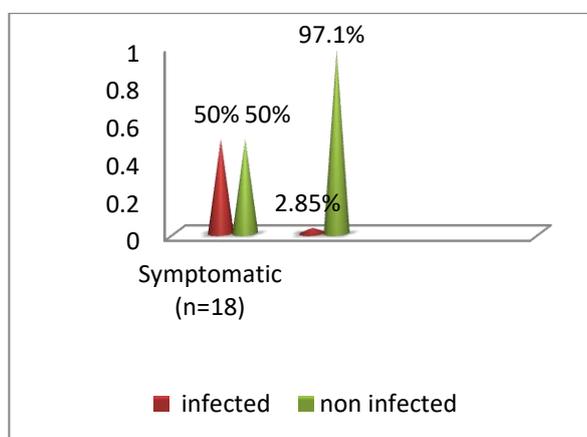
Blastocystis spp. cyst stained by Lugol's iodine stain (large central vacuole stained yellow-brown color and the peripheral nuclei stained dark brown-black color)

Blastocystis spp. cyst stained by Iron hematoxylin stain (large central vacuole stained gray color and the peripheral nuclei stained black color)

Table 2: The relationship between age group of leukemic children and *Blastocystis* infection

Age group	Examined no.	Positive	Negative
preschool age (< 6 years)	24	8 33.3%	16 66.67%
School age (≥ 6 years)	29	2 6.9%	27 93.1%
Total	53	10	43

Figure-3- Association of *Blastocystis* spp. infection with diarrhea in patients group.



Discussion: *Blastocystis* spp. is a ubiquitous parasite with a wide world distribution. Its prevalence differ from country to country and in variable communities within the same country (18,19). However, the truth about it's being a commensal or a pathogenic parasite in certain conditions is still a questionable matter (21).many reports about the incidence of *Blastocystis* spp. in immunocompromised individuals are available (20). In our study, 18,87% of the patients were positive for *Blastocystis* spp. compared to 10% of the controls and the difference was statistically insignificant. A similar study done by Aksoy *et al.* who found no statistically significant difference in the incidence of *Blastocystis* spp. in patients with malignant neoplasms (26%) compared to a healthy group which was 4% (16). Essa *et al.* results were against that and he attributed his findings to the lower exposure of leukemic children to pathogens due to lower activity and more care by their food and hygiene (13). Furthermore, there was no significant difference in the incidence of *Blastocystis* spp. among male and female groups and among rural and urban groups is in agreement with many similar result studies (3,13,22). In the present study, we use different staining methods including iodine, iron hematoxylin, and trichrome stains that shows an infection rates of (9.4%, 11.3%, and 18.87% respectively) in immunosuppressed group while (4%, 6%, and 10% respectively) in the healthy controls. We noticed that trichrome staining method detected more positive cases than the other two methods and this is in agreement with many other studies that showed trichrome as the most sensitive stain for diagnosis of *Blastocystis* spp. (23, 24). We noticed that the *Blastocystis* spp. infection rates were higher in the preschool than the school age group. That may be attributed to the higher activity of children under 6 years old and less awareness of hygienic aspects (16). In the other hand, we found that infection by *Blastocystis* spp. was significantly correlated with the presence of diarrhea and abdominal pain, and that was similar to El Shazly *et al* and kaya *et al* studies (25, 26). Other authors suggested that such symptomatic individuals had no correlation with being positive for *Blastocystis* spp. (27, 28). This disparity is controversy, but it may be attributed to the wide variety of the isolated subtypes and host defense factors like age and immune status.

Conclusions: Our findings reported that *Blastocystis* spp. infection was seen more frequently among leukemic children and there was a significant association with the presence of diarrhea and abdominal pain. Those findings are expected to produce anawareness about the right diagnostic method for *Blastocystis* spp. and open doors for further studies on its prevalence and pathogenicity.

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