Journal of the Egyptian Society of Parasitology, Vol. 55, No. 2, August 2025
J. Egypt. Soc. Parasitol. (JESP), 55(2), 2025: 243 - 250
Online: 2090-2549

CORRELATIONS AND IL6 LEVEL AMONG SOME BLASTOCYSTIS HOMINIS INFECTED ADOLESCENTS IN FAYOUM GOVERNORATE

By
FATEN A. M. MOHAMED* AND AYA A. H. EL-NAGGAR
Department of Medical Parasitology, Faculty of Medicine, Fayoum University, Egypt
(*Correspondence: fam02@fayoum.edu.eg).

Abstract

Blastocystis is a global protozoan affects the gastrointestinal tract of man and animals. It is frequently observed in human stool samples. The study evaluated *Blastocystis hominis* in 110 stool samples by Iodine smears, Trichrome staining, and Jones' culture, between IL6 and infection. Microscopic smears and Trichrome staining detected 21and 35 positive cases, with sensitivity of 50%, and 71.43% and specificity of 100% and 92.65% respectively. Culture method, used as diagnostic test identified more number of positive cases (42 cases). A significant association was found between infection and the presence of pets (p=0.03). Diarrhea (71.4%; p=0.01), flatulence (45.2%; p=0.01), vomiting (42.9%; p=0.002), and nausea (40.5%; p=0.001) were the patients' symptoms. The IL-6 concentration in stool and plasma positive samples was significantly elevated in patients compared to negative control (P <0.001). These results indicating that systemic inflammation is related to *Blastocystis* infection and highlighted the importance of the complementary use of microscopy, Trichrome stain, and culture techniques in the detection of *Blastocystis* infection.

Keywords: Fayoum University, *Blastocystis hominis*, Stained smears, Stained culture, IL-6.

Introduction

Blastocystis is the most prevalent eukaryote of phylum Stramenopila (Tan, 2008), inhabits the gastrointestinal tract of humans and various animals (Ibrahim *et al*, 2020). It inhibits the intestinal tract of humans and a wide range of animals with an estimated of about 1-2 billion people, with prevalence varies significantly, ranging from 0.5% to 30% in developed countries and from 30% up to 76% in developing regions, with some areas even reporting 100% infection rates (Fu *et al*, 2025). In blastocystosis at least 22 subtypes, nine of which can infect humans with and without gastrointestinal illness (Delshad *et al*, 2020)

Blastocystis sp. life cycle is not yet completely understood as infectious stage and various morphologic forms of this polymorphic organism that have been identified in the stool and/or culture constitute parasite distinct biologic stages in hosts' intestinal tract. Cyst form (3-5µm) is suggested to be an infectious stage, and the predominant form in hum an stool specimens is referred to as vac-uolar (or central body) form is of variable size (5-40µm), or much larger (CDC, 2019).

Blastocystis spp. was included in the water sanitation and health programmes (WHO, 2008). Blastocystosis can be transmitted by the fecal-oral zoonosis or from contaminated food or water and/or from human or animal feces (Liu *et al*, 2023). Signs and symptoms of blastocystis possibly include: watery diarrhea, constipation, nausea, fatigue, abdominal pain, bloating, excessive gas, loss of appetite, weight loss, ulcerative colitis and anal itching (Cleveland Clinic, 2023). Symptomatology was debated and may be related to the IL6 cytokine, which is the key for pro-inflammatory cytokines in parasitic infections (Aykur *et al*, 2023). The symptoms were more severe in the immunocompromised patients (abdellatif *et al*, 2025).

Diagnosis of *Blastocystis* infection is based mainly on direct microscopy of Trichrome stained smear and in vitro culture (Jha *et al*, 2921). *Blastocystis* spp. rapidly multiplies in culture medium supplemented with serum after 24-48hr of cultivation and considered culture as one of the most sensitivity methods (Elghareeb *et al*, 2015).

The PCR proved to be a valuable tool for diagnosing blastocystisosis, offering best sensitivity and ability to identify the different subtypes compared to all other traditional

methods (Elsayad *et al,* 2019). However, the selection of the most accurate diagnostic tests for *Blastocystis* with the least false results is essential (Zamani *et al,* 2021).

The present study aimed to evaluate *Blastocystis hominis* among some outpatients at Fayoum University Hospitals and to assess the link between the pro-inflammatory cytokine (IL6) and *Blastocystis hominis*.

Materials and Methods

Study design: A total of 110 samples were obtained from patients with enteric complaints and attending the out-patient clinics, El-Fayoum Teaching Hospitals, Egypt from May 2023 to July 2023. Study aim was explained and a written consent was obtained from their parents or guardians. Data were documented in a structure questionnaire. Patients received antiparasitic drugs or with systemic or immune disease weren't included.

Parasitological examination: Fresh stool samples were collected in clean labeled containers and transported to parasitology laboratory within 2 hours. All samples were examined with Lugol's iodine smear, Trichrome staining, and incubation in Jones's culture. All were examined under microscopy (40**X** &100X). those with the characteristic vacuolar forms were considered *Blastocystis* positive (Garcia *et al,* 2018). For stained and air-dried stool smears were immediately prepared and fixed in the Schaudinn's fixative. Smears were with Trichrome stained solution and examined for *Blastocystis* at 100X (Garcia, 2021).

The in-vitro Jones's culture (1946): About 50mg of all stool samples was directly inoculated in 5ml of Jones' medium supplemented with 10% horse serum and penicillin (100u/ml). Culture tubes were incubated anaerobically at 37°C. After 48-72hrs, all culture tubes were smeared and examined for *Blastocystis* forms and the results were recorded.

IL-6 cytokines assay: To exclude any confounding effects of other detected parasites on IL6 measurements, only pure positive *Blastocystis* cases and *Blastocystis* negative (control) cases were included. Patients with other detected parasites were excluded.

Morning stool samples (1gm) were collected emulsified and washed 3 times in (5ml) phosphate phosphate-buffered saline with protease inhibitors and centrifuged (5000xg for 10mins). Also, serum samples were obtained (by clotting for 2hrs then centrifugation at 2000xg for 10 minutes). The collected stool supernatants and serum samples were stored at -80°C for later usage. IL-6 levels were estimated by ELISA kits (Human IL6 Kit, ELK Biotechnology, USA, CAT, and No. ELK1156), and processed was carried out after the manufacturer's instructions. This kit employs the quantitative sandwich enzyme immunoassay technique. Each sample was added to plates containing antibodies against IL-6 and assayed in duplicate. Plates were read by ELISA reader at a wavelength of 450 nm. The assay included standard curves and internal controls for assay validation.

Ethical consideration: The study protocol was approved by the Scientific Research Ethics Committee, Faculty of Medicine, Fayoum University (NO: R 450/2023), which was in the agreement with the Helsinki Declaration (DoH) of the WMA (WHO, 2024).

Statistical analysis: Data were collected computerized and statistically analyzed by SPSS software (version 23, IBM Inc., USA).

To determine descriptive and the correlation between *Blastocystis* infections and participant variables, the Chi-square test was used. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of each method were calculated using culture as the standard diagnostic reference. Cohen's Kappa coefficient assessed agreement between the diagnostic tests. Differences in the IL6 between positive and negative patients were analyzed by Mann-Whitney U test. When the P-value < 0.05, it was considered significant difference (George and Mallery, 2003).

Results

Blastocystis hominis was detected among

23(54.7%) patient's age group (10 to 14 years) and in 19(45.2%) patient's age group (15 to 19 years, with (14±3.1), but without significant difference (P=0.69). Infection was in males (64.3%) than in female (35.7%), with (P=0.841), and not correlated with residence (P=0.69) or hand-washing (P=0.85). However, significant risk was between infection and pets (p=0.03, odd ratio= 0.23), diarrhea (71.4%; P=0.01; odd ratio=2.23), flatulence (45.2%;p=0.01; odd ratio= 2.68), vomiting (42.9%;p=0.002; odd ratio=3.88) and nausea (40.5%;p=0.001; odd ratio= 5.92) the most associated symptoms.

Patients were more likely to have other parasitic causing abdominal pain, as a total 54.8% had abdominal pain without blastocystosis significant association.

Blastocystis hominis was detected by iodine (19.1%), trichrome stained (31.8%) smears and 42/110 (38.18%) positive by culture media, with significant differences.

Microscopy detected 22/110(20%) with significant mixed infections, *G.* i*ntestinalis* (10%), *E. histolytica* (5.5%) and *H. nana* (4.5%), respectively. Samples 16(14.5%) were only *Blastocystis*. IL-6 concentration among positive cases was significantly elevated (58.3±5.34pg/ml) compared to negative ones (13.7±2.12), with (P<0.001). Elevated plasma IL-6 levels in *Blastocystis* samples was (76.13±9.56) compared to negative (11.6±3.57). A positive correlation (r=0.563) was between positive stool and IL6 concentrations among patients (P <0.001).

Details were given in tables (1, 2 & 3)

Table 1: Descriptive criteria of the participants and risk of *Blastocystis* infection:

		Basic Total n =110 %	Positive cases n=42%	P-value	Odd ratio	95% Confidence
Age	10-14	57(51.8%)	23(54.7%)	0.69	0.82	0.38-1.78
	15-19	53(48.2%)	19(45.2%)			
Sex	Male	69(62.7%)	27(64.3%)	0.841	0.89	0.40- 1.99
	Female	41(37.3%)	15(35.7%)			
Residence	Rural	51(46.4%)	18(42.9%)	0.69	0.79	0.36-1.72
	Urban	59(53.6%)	24(57.1%)			
Hand washing	Regular	55(50%)	20(46.6%)	0.85	1.17	0.54-2.52
	Irregular	55(50%)	22(52.4%)			
Pet animal	Yes	100(90.9%)	35(83.3)	0.03*	0.23	0.06-0.95
	No	10(9.1%)	7(16.7%)			
Diarrhea	Yes	63 (57.2)	30(71.4%)	0. 01*	2.23	0.99-5
	No	47(42.7	12(28.6%)			
Abdominal pain	Yes	50 (45.4%)	23(54.8%)	0.16	1.83	0.84-4
	No	60 (54.5)	19(45.2%)			
Nausea	Yes	24(21.8)	17(40.5%)	0.001*	5.92	2.18-16.03
	No	86(78.2%)	25(59.5%)			
Vomiting	Yes	29(26.4%)	18(42.9%)	0.002*	3.88	1.59-9.45
	No	81(73.6%)	24(57%)			
Flatulence	Yes	35 (31.8)	19(45.2%)	0.01*	2.68	1.17-6.14
	No	75(68.2%)	23(54.8%)			

* P< 0.05 significant

Table 2: Microscopy diagnosis by Iodine smears versus Trichrome stained culture as a diagnostic reference test:

Culture	Iodine stained smear			P value	Trichrome stained culture			P value
	Negative	Positive	Total		Negative	Positive	Total	
Positive	0	21	21	0.000*	5	30	35	0.000*
Negative	68	21	89		63	12	75	
Total	68	42	110		68	42	110	
Sensitivity%	50% (95%CI: 34.19-65.81)				71.43% (95%CI: 55.42-84.28)			
Specificity%	100% (95%CI: 94.72-100)				92.65% (95%CI: 83.67-97.57)			
Positive predictive value	100% (95%CI: 83.89-100)				87.55% (95%CI: 74.76-94.35)			
Negative predictive test	73.42% (95%CI: 67.12-78.89)				81.74% (95%CI: 73.43-87.89)			
Accuracy%	79% (95%CI: 70.20-86.18)				83.74% (95%CI: 75.49-90.08)			
Kappa	0.55 (Moderate)				0.66 (Good)			

* P< 0.05 significant

Table 3: Parasites detected among 110 stool samples

Parasites	Total No,	Positive %	P value
Only *Blastocystis* positive	16	14.5%	0.000*
Pure *Blastocystis* negative	72	65.5%	
Giardia intestinalis	11	10%	
Entamoeba histolytica	6	5.5%	
Hymenolepis nana	5	4.5%	
Total	110	100%	

Mixed infections were not included * P< 0.05 significant

Discussion

Generally speaking, Zhou *et al.* (2003) in USA studied the interleukin-6 role in *Giardia lamblia* in infected mice, and concluded that IL-6 was indicated in early control of acute *G. lamblia* infections. Smith and Maizels (2013) in United Kingdom reported that in vivo, IL-6 limited Th2 response, modified the Treg-cell phenotype, and promoted host susceptibility after helminthic infection. Wilairatana *et al.* (2022) in Thailand reported increased significantly levels of IL-6 in patients with severe malaria compared with normal control and that IL-6 is a good marker in severe malaria. Apart from parasites, Coomes and Haghbayan (2020) in Canada reported that IL-6 inhibition might be a novel target for therapeutics for managing dysregulated host responses in Covid-19 patients, but high-quality studies in this field were required. AbdelKader *et al.* (2024) in Egypt reported that IL-6 was a key diagnostic indicator in Covid-19 development and progression. Karamati *et al.* (2021) in Iran reported that the lack of significant relationship between protease activity of *Blastocystis* sp. subtypes and the expression levels of pro-inflammatory biomarkers suggests that either *Blastocystis* spp.- or host-related factors besides proteases participate in stimulation of pro-inflammatory biomarkers. Said *et al.* (2021) in Sultanate of Oman found that IL-6 was associated with different diseases and viral infections, but in healthy blood donors varied between 0 and 43.5pg/ml. (95% confidence interval [CI]: 4.631, 5.740). They added that by one year age increased, IL-6 values increased by 0.0pg/ml (95% CI: 0.02, 0.09; p <.01).

In the present study, the prevalence of *B. hominis* infection by culture was 38.18% among 110 adolescents without age's significant association. This more or less agreed with Khorshidvand *et al.* (2021), who reported that, the global range (~ up to 60%). In contrast, El-Nadi *et al.* (2017) in Sohag elementary school reported 1% Blastocystis spp. Hamdy et al (2020) in Beni-Suef reported (53.6%) *Blastocystis* spp. among school children. Mohamed and Khalil (2023) in Fayo um reported (19.1%). Abroad, Dagci *et al.* (2014) in Turkey reported (16.9%) among patients aged 10-19 years.

Sheishaa *et al.* (2023) in Cairo reported that blastocystis is a prevailing gut parasite, with or without symptoms, but only GIT symptoms showed significant correlation when blastocystis was found in their stools Mokhtar and Youssef (2018) in Ismailia reported STs among blastocystosis infected animals showed broad genetic diversity among *Blastocystis* spp. isolated from animals but, they detected 4/412 STs among both man and poultry fecal samples, which showed anthroponotic transmission and possibility of fecal cross-contamination from other potential reservoir animals. Sebaa *et al.* (2021) in Algeria reported that *Blastocystis* spp. caused symptoms.

In the present study, microscopy showed *Blastocystis hominis* (20%) as well as *Giardia intestinalis*, *Entamoeba histolytica* and *Hymenolepis nana*. This agreed with El-Nadi *et al.* (2017) Hamdy *et al.* (2020), Abd Ellah *et al.* (2024) and Rodríguez *et al.* (2025), who reported other intestinal protozoa in the diarrheic schoolchildren. Also, Sheishaa *et al.* (2023) reported severe diarrheic schoolchildren due to *Blastocystis* (21.6%) and other protozoa (44.6%) without any helminthes.

In the present study, the sensitivity of Lug-

ol's iodine and Trichrome stain were low in detecting *B. hominis* as compared to the gold standard Jones' medium. This agreed with Hegazy *et al.* (2021), who reported the culture medium was the best in diagnosing human *B. hominis*. But, Boutahar *et al*. (2021) in Morocco reported that PCR was the gold standard in diagnosing *Bla- stocystis* spp. followed by the culture in Jones' medium and the least one was the microscopical examinations. Forsell *et al.* (2016) in Tanzania reported that at least three stool samples must be examined on three successive or alternating days**.** Mohammad *et al.* (2018) in Iran reported that trichrome stain was considered as a good standard method (kappa =0.66) with a higher negative predictive value (81.74%) and (83.7%) diagnostic accuracy than Iodine smear.

In the present study, iodine smear showed vacuolar forms surrounded by a thin cytoplasm rim contained up to six nuclei with average size (~8-10μm), and trichrome stain showed better *Blastocystis* with blue-green cytoplasm and central vacuole. This agreed with Garcia (2021). Also, Sheishaa *et al.* (2023) reported that Lugol's iodine and trichrome stained smears were simple, easy, rapid, and economic tests for diagnosing blastocystisosis in low-resource settings. Culture technique enhances detection of different pathogenic blastocystisosis were granular and amoeboid forms (Tan and Suresh, 2006; Suresh *et al,* 2009).

The present study found a high rate of infection among male adolescents who lived in urban areas without a significant relation. Also, infection was not correlated with hand-washing habit. This agreed with Mokhtar and Youssef (2018); Hamdy *et al*. (2020); Mohamed and Khalil (2023), but this disagreed with El-Badry *et al*. (2018), Asghari *et al*. (2021) and Sheishaa *et al*. (2023). However, Ali and Abass (2022) found a significant difference between ages and *B. hominis*.

In the present study, pets caused elevated significant risk in the *Blastocystis* infection. This agreed with Ahmed *et al*. (2022), who reported that nearly all Egyptians in rural and some urban ones keep pets indoors or even stray ones. But, Abd Ellah *et al.* (2024)in Upper Egypt didn't found significant association between *Blastocystis* infection and ages, residence or even with animals contact.

In the present study, *Blastocystis* infected patients suffered from diarrhea, flatulence, vomiting, nausea, and abdominal pain as common presenting symptoms. This agreed with Abdulsalam *et al*.(2013),who reported gastrointestinal disturbance among *B. hominis* patients. But, this disagreed with Ahmed *et al*. (2022), who reported *Blastocystis* infection as a silent infection in most ipatients, and Abd Ellah *et al*. (2024) who didn't find significant association between *Blastocystis* and gastrointestinal symptoms.

Generally speaking, the pathogenesis of *Blastocystis* and symptomatology are not clearly defined, possibly due to complex interaction between its genotypes, immune responses, gut microbiota of the host and the presence of other intestinal parasites, or microorganisms causing same digestive symptoms (Guard, 2024). Rayan *et al.* (2007) in Ismailia found that diarrhea was 38.5% in *D. fragilis* patients compared to 50% in *G. lamblia* ones and abdominal pain was 41% & 33.3% respectively. Farghaly *et al.* (2014) in Sharkia G. reported that *B. hominis* caused recurrent symptoms, and asymptomatic ones were due to genetic subtypes.

In the present study, the IL6 level was significantly elevated in stool and blood test of *Blastocystis* positive (r=0.23) was more correct. A significant positive relation was between stool and blood IL6 levels in infected patients, reflected local intestinal immune responses. This agreed with Azizian *et al.* (2016), who reported that *Blastocystis* is an inflammatory process and that can be a biomarker to assess blastocystisosis severity. Also, Norouzi *et al*. (2021) and Ismail *et al*. (2022) accepted this. But, Jimenez-Gonzalez *et al*. (2012), Amsal *et al*. (2024) didn't accept that elevated IL-6 assessed severity.

Conclusion

Blastocystis hominis remains the predominant gastrointestinal protozoan detected in many patients. The associated gastrointestinal symptoms as diarrhea, flatulence, vomiting, and nausea, may or may not be predictors among *B. hominis* infected patients. Microscopically diagnosed stained smears were valuable. However, the culture medium is more dependable diagnosis usually with the best detection rate. The elevated IL6 in stool and blood among *Blastocystis* infected patients can be considered as a biomarker for infection severity and immune status.

More studies on *Blastocystis hominis* role in health and disease manifestations are ongoing and will be published in due time elsewhere.

Authors' Declaration: They declared that neither have any conflicts of interest nor received any funds.

Authors' Contribution: The authors equally shared in the data collections, writing and revising the manuscript as well as approved its publication.

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