

Evaluation of IL-5 concentration level in Irritable Bowel Syndrome patients that suffering from *Blastocystis* infection in Al-Najaf Province

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Abstract

Objective: The current research aimed to evaluate the concentration level of IL-5 in irritable bowel syndrome patients infected with *Blastocystis* infection

Methods: Stool samples were collected from 156 of respondents (78 patients were diagnosed clinically by physician suffering from IBS and 78 control apparently healthy individuals without any history of disease) attended to three Hospitals in Al-Najaf Province of Iraq from August, to December 2019. Data was collected using self-administrated questionnaire. The whole questionnaire has five sections which included age, sex, family income, living area and occupation. Fecal specimens were collected and each sample divided into three parts, the first for microscopic examination and second for culture and third part were quickly frozen for Immunological evaluation of IL-3 by Sandwich ELISA, shipped on dry ice and kept at -20 °C prior to analysis.

Results: the *Blastocystis* was significantly predominant ($P < 0.001$) parasite associated with IBS which constituted (46.2%). The results of culture were higher than light microscopic examination (48.7%).

Individuals with *blastocystis* infection were found to have positive association with IBS (OR: 73.150 , 95% CI: 9.684-552.531).

Evaluation of immune status among IBS patients infected with *Blastocystis* through measuring IL-5 by SandwichELISA, were significantly elevated in the IBS *Blasto* group ($p < 0.05$) compared to control.

Key words: Irritable bowel syndrome, *Blastocystis*, Interleukin-5.

Background

Blastocystis is the most common anaerobic protozoa in the large intestine of human and many animals and considered as the most common parasites found in human stool, the several forms for *Blastocystis* observed within in-vitro culture comprise vacuolar, granular, amoeboid, cyst, avacuolar, multi-vacuolar forms. However, vacuolar, granular, amoeboid and cyst are the most commonly observed forms^[1]. There are many studies have indicated potential linkage between *Blastocystis* and some clinical manifestations such as irritable bowel^[2]. Irritable bowel syndrome (IBS) is a common clinical condition affecting up to 10% of the global population and characterised by abdominal pain, bloating and disturbance of bowel habit^[3]. The carriage of the enteric organism *Blastocystis* has been reported to be three times higher in patients with diarrhea predominant IBS (D-IBS) compared to healthy controls^[4]. Making it an organism of interest in IBS. Interleukin-5 was an interleukin produced by type-2 T helper cells and mast cells. The role of IL-5 appears to be largely restricted to eosinophil biology, fulfilling expectation, through binding to the interleukin-5 receptor, interleukin-5 and increases immunoglobulin secretion-primarily IgA and It is also considered as a key mediator in eosinophil activation^[5]. IL-5 first mobilizes existing bone marrow eosinophils and induces eosinophil production , these existing

and developing eosinophils then respond to eotaxin, resulting in their exit from the marrow and entry into the tissue, thus Very early eosinophil progenitors also respond to eotaxin, and when they reach the tissues, mature into eosinophils *in situ*, while in tissues, IL-5 also maintains eosinophil levels by inhibiting normal eosinophil apoptosis^[6].

Methods

Patients with IBS and controls

This case control study design was conducted in Iraq on 78 patients (40 males and 38 females) attended to three Hospitals in Al-Najaf Province of from August, to December 2019. The patients whom were diagnosed clinically by physician suffering from IBS and anonymous questionnaire form were done which including age, gender, water supply and residency area. Control group consist of 78 apparently healthy individuals (42 males and 36 females) without any history of disease were clinically considered as healthy control also included in this research.

Sample collection

Fecal specimens were collected in suitable , clean , dry container , all samples were divided into three parts, the first for microscopic examination (Wet mounting, Formalin-ether precipitation method and Trichrom staining) and second for culture (on Modified Jones Medium) and thrid part were quickly frozen for for Immunological evaluation of IL-5 by Sandwich ELISA , shipped on dry ice and kept at -20 °C prior to analysis.

Results and discussion

Isolation and identification of *Blastocystis* in Irritable Bowel Syndrome patients and control subjects

- **Microscopic examination**

Out of (156) fecal samples that which examined microscopically for *Blastocystis* by using light microscope under (40x), *Blastocystis* infection detected in 36 (46.2%) out of 78 IBS patients as shown in figure (1), this high percent of detection may due to *Blastocystis* consider normal flora in intestine and become opportunistic. All the control subjects (78) were negative for *Blastocystis* infection.

Microscopic examination techniques remain the main method for diagnosis of *Blastocystis* including wet preparation, concentration and stained smear by trichrome stain for the detection and identification of *Blastocystis* in stool, the role of *Blastocystis* as an etiological agent of IBS is inconclusive due to the controversial nature of *Blastocystis* as a human pathogen^[7].

This results of current research were nearly similar with results from another studies in which *Blastocystis* was isolated from 43(33%) of 127 samples in Iraq^[7] and 25(30,1%) of 83 samples from patients with IBS by using microscopic examination in Egypt^[8].



Figure (1): *Blastocystis* staining by trichrome stain under light microscope
(The arrows→ refer to cyst of parasite 40X).

● Culture of *Blastocystis*

Out of (156) stool samples of IBS patients and controls were culture in modified Jone's media then incubated for 48 hours then subcultured and incubated for 72 hours then examined microscopically by light microscope

as shown in figure (1). The results shown 38 out of 78 IBS patients were positive for *Blastocystis* infection accounted for (48.7), and 40 patients were negative for infection accounted for (51.3). While all the control subjects (78) were negative for *Blastocystis* infection except one of them was positive for infection with *Blastocystis* this is may be because this person have latent infection or because an old infection.

The results of culture were higher than light microscopic examination (48.7%), as shown in table (1). This is because the culture method is more sensitive and accurate method than the microscopic examination and considered gold standard for detection of this parasite although this method take time between (2-3 days).

Table (1): Detection methods of *Blastocystis*

Method		IBS (No. = 78)	Control (No. = 78)	P-value
		(No. , %)	(No. , %)	
Light Microscope	Positive	(36 , 46.2)	(0, 0.00)	0.001
	Negative	(42 , 53.8)	(78 , 100.00)	
Culture	Positive	(38 , 48.7)	(1 , 1.3)	0.001
	Negative	(40 , 51.3)	(77 , 98.7)	

There have been many studies which use Jones medium as the medium of choice for xenic culture growth of *Blastocystis*. The result of current research was nearly similar to results the study in which the isolation of *Blastocystis* were 44.09 %^[7] and 41% of IBS patients using culture method on modified jones medium^[8].

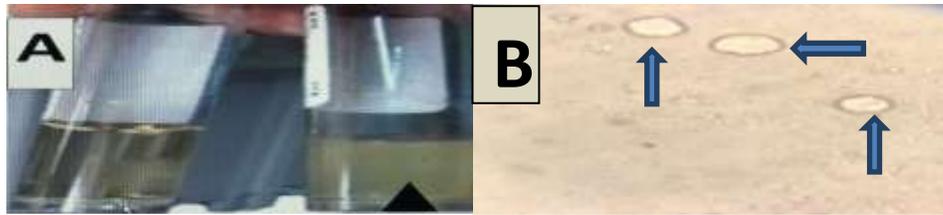


Figure (2): *Blastocystis*, (A) growth on modified Jones medium (The arrow→ shows turbidity) ;(B) Stained prepared slide with iodine (The arrows show the cyst of *Blastocystis* (40X)).

Risk of *Blastocystis* in relation to IBS

The Odd ratio statistic (OR) was used for assessment of the amount of risk submitted by *Blastocystis* in association with IBS, Adjusted OR was 73.150 with a 95% confidence interval was (9.684-552.531). This can be interpreted as that any patient carrying *Blastocystis* infection is liable for development of IBS about 73 times than non-infected individuals, this mean there are positive association between the infected patients with *blastocystis* and IBS as shown in table (2).

Table (2): Risk of *Blastocystis* in relation to IBS

Risk Estimate			
	Value	95% Confidence Interval	
		Lower	Upper
Odds Ratio for Culture (Positive/Negative)	73.150	9.684	552.531
For cohort Coding = Cases	2.850	2.205	3.683
For cohort Coding = Control	.039	.006	.271
N of Valid Cases	156		

Table (3) IL-5 concentration in studies groups of IBS infected with *blasto* infection and control.

Group Statistics					
Clinical group	Cytokine type	Mean \pm Std. Deviation	t. Test value	Df	P- value
IBS infected with <i>blasto</i> infection	Con_IL5	56.0474 \pm 18.66323	25.432	154	0.001
Control		2.0832 \pm 1.69587			

This table show the highest concentration in research groups appear in IL-5 in IBS infected with *blasto* infection compared to controls with significant difference($P < 0.001$).

The pathogenesis of *Blastocystis* parasite mainly involved adhesion of parasite to the intestinal epithelium, apoptosis and degradation of tight junction proteins of intestinal epithelial cells resulting in increases intestinal permeability, degradation of IgA, and induction of proinflammatory cytokine response thus *Blastocystis* parasite associated with IBS [2].

Conclusions

1-Any patient carrying *Blastocystis* infection was most liable for development of IBS than non-infected individual.

2- There were positive association between the infected patients with *blastocystis* and Irritable Bowel Syndrome.

3- Patients with IBS infected with parasite showed an increase in the interleukin-5 levels demonstrate that *Blastocystis* does have an effect in the immune system.

Recomendations

1-It was important to shed light on the re-evaluation of the role of cytokines especially IL-5 in the immune response against Blastocystosis according to different subtype of *blastocystis* species.

2-Genetic studies of the association between IL-5 levels and different kinds of cytokines are great importance.

References

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