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Immunological evaluation of IL-10 concentration level in sera of digestive disorders patients infected with *Blastocystis hominis* infection in Al-Najaf city

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Abstract---Objectives: To evaluating the concentration level of IL-10 in serum of patients suffering from digestive disorders *Blastocystis* parasitic infection and compared with control group by using ELISA. Methodology: Case-control study design was conducted on 70 patients (33 males and 37 females) at Al-Sadr medical city and Al-Hakim General Hospital and Al-Sajad Hospital. A control group of 70 participant (29 males and 41 females) who were without any history of disease were included. Their ages ranged between (6 - ≥ 60) years, during the period from the beginning of November 2021 to the end of February 2022. Results: The results showed (48.6%) of culture positive for *B.hominis*. The results of immunoassay showed mean difference ± standard error difference of IL-10 was (5.878 ± 10.462 Pg/ml) with a 95% confidence interval was (14.809-26.565) in intestinal disorder patients with significant difference (P<0.001). Conclusion: Interleukin-10 concentration levels have a significant effect on the results of Blastocystosis patients suffering from digestive disorders.

Keywords---interleukin-10, *Blastocystis hominis*, digestive disorders.

Introduction

B. hominis is cosidred as the most common parasites found in human stool, the several forms for *Blastocystis* observed within invitro (Jalallou *et al*, 2017). Culture comprise vacuolar, granular, amoeboid, cyst, avacuolar, multivacuolar forms. However, vacuolar, granular, amoeboid and cyst are the most commonly

observed forms, there are many studies have indicated potential linkage between *Blastocystis* and some clinical manifestations such as intestinal disorders. Intestinal disorders is a common clinical condition affecting up to 10% of the global population and characterised by abdominal pain, bloating and disturbance of bowel habit (Canvan, 2014).

Involuntary digestive disorders in Iraq, at this very moment methodical as yoke of the in the most suitable way customary cases caused by different enteropathogenes. As a result, the existent test unchanging to highlighting on this moocher which is wanton by researchers past the interpret of protozoa dawdle becoming to its upfront sphere as customary flora in intestine and the predicament of its sorting out in accustomed mandate of moderate dissection, in bells to mosey, in Iraq here was outline obtainable studies were conducted on this parasite based on cytokines level of its immunological effect that associated with intestinal disorders.

Interleukin-10 is a cytokine with anti-inflammatory properties, has a central role in infection by limiting the immune response to pathogens and thereby preventing damage to the host and also it is important in regulating inflammatory response as it reduces the production of chemotactic factors, such as IL-8 (IP *et al.*, 2017) The present study aimed to evaluating the concentration level of IL-10 in serum of patients suffering from digestive disorders *Blastocystis* parasitic infection and compared with control group by using ELISA.

Methodology

Patients and Control Group

Samples collection (blood and stool) was conducted for patients with intestinal disorders (70 participant) and for the control group (70 participant) during the period from the beginning of November 2021 to the end of February 2022 Al-Sadr medical city and Al-Hakim General Hospital and Al-Sajad Hospital and their ages ranged between (6 - ≥ 60) years.

Samples Collection

Fecal Samples Collection

Fecal specimens were collected in suitable , clean , dry container , all samples were cultured on modified jones medium for detection *B. Hominis*.

Serum Samples Collection

3 ml of venous blood from all study groups (intestinal patients and control group) were collected in a serum separating gel tube. The blood was left at room temperature for about 30 minutes for clotting and then placed in a centrifuge at 3000 rpm. for two minutes. The serum was collected in a sterile Abendrov tube and kept at low temperature (20–20) in the freezer.

Study Location

This case control study was conducted in Al-Najaf city of Iraq.

Excluded Criteria

Patients with diseases other than intestinal disorders, such as bloody ulcers, cancer, and others, were excluded.

Culture of Parasite (*Blastocystis hominis*) on Modified Jones' medium

Stock solution of this media was composed of the following and prepared according to Leelayoova et al (2002).

- 9.46 g of Na₂HPO₄ in 1 L distilled water
- 9.08 g of KH₂PO₄ in 1 L distilled water
- 9.00 g of NaCl in 1 L distilled water

To prepare Jones' Medium

- The buffer was prepared by mixing a volume of 93.8 mL Na₂HPO₄ with 31.3 mL KH₂PO₄ and 562.5 mL NaCl and mixed well.
- Yeast extract 0.1% was added to buffered solution on the flask.
- The mixture was Autoclaved at 15 lb inch-2 for 15 min.
- Before use, 10% horse serum was added (heat inactivated at 56°C for 30 min) to Jones' Medium.
- For each faecal specimen transfer with a sterile swab 50 mg faeces (or so) to a centrifuge tube with screw cap containing Jones' Medium (3 ml will do). The culture was incubated at 37 °C for 48-72 h. (Note: The presence of bacteria in the sample will be enough for creating the anaerobic environment needed for *B. hominis* to grow). Subculturing was done by transfer about 50-100 uL of the sediment from each culture into 3 ml fresh Jones' medium and Subculture every 3-4 days.

ELISA Technique

Estimation concentration level of IL-10 done in lab according to Mybiosource/USA protocol.

Statistical Methods

Data were analyzed using SPSS program version 16 and Microsoft Office Excel 2007. Numeric variables were expressed as mean +SD while nominal variables were expressed as number and percentage. Independent sample t-test was used to study difference in mean between any two groups while chi-square was used to study association between any two variables. P-value was considered significant when it was less than or equal to 0.05.

Results and Discussion

Age distribution of intestinal disorders patients and control subjects

The results shown in Table (1) showed that the highest incidence of *Blastocystis hominis* for patients with intestinal disorders was in the age group (6-15) years, which amounted to 30%, while the age group (≥ 66) years had the lowest number with a percentage of 2.9 % in patients with intestinal disorders with *Blastocystis hominis*. While the results showed that the control group in which the age group (18-27 years) is the most numerous with a percentage of (27.1%), while the age group (58-67 years) is the least in number at a rate of (4.3%).

The results of the presented study almost agreed with a study conducted in the city of Samarra, in which it was mentioned that the most frequent infection with *Blastocystis* was in the age group (6-10) years (Mahmood and Khudher, 2018). While there is a recent study conducted in Saudi Arabia in which it was mentioned that the highest percentage of infections with the human *Blastocyst* parasite was in the age group (50-31) years, and this does not correspond to the results of our current study (Aldahhasi *et al.*, 2020). The results of the current study are almost similar to the results of other studies presented by (Abdalla *et al.*, 2014) and (Sayal *et al.*, 2020).

Table (1) Distribution of samples of intestinal disorders patients with *Blastocystis hominis* and control groups according to age

Age Groups For Cases					
		Frequency	Percent	Valid Percent	Cumulative Percent
	6 - 15	21	30.0	30.0	30.0
	16 - 25	8	11.4	11.4	41.4
	26 - 35	8	11.4	11.4	52.9
	36 - 45	16	22.9	22.9	75.7
	46 - 55	12	17.1	17.1	92.9
	56 - 65	3	4.3	4.3	97.1
	66+	2	2.9	2.9	100.0
	Total	70	100.0	100.0	
Age Groups For Control					
		Frequency	Percent	Valid Percent	Cumulative Percent
	8-17 y	9	12.9	12.9	12.9
	18-27 y	19	27.1	27.1	40.0
	28-37 y	14	20.0	20.0	60.0
	38-47 y	11	15.7	15.7	75.7
	48-57 y	9	12.9	12.9	88.6
	58-67 y	3	4.3	4.3	92.9
	68 and more	5	7.1	7.1	100.0
	Total	70	100.0	100.0	

Culture of *Blastocystis hominis*

All stool samples (140) were culture in modified Jone's media then incubated for 48 hours then subcultured and incubated for 72 hours then examined microscopically. The results of culture (48.6%) positive for *B.hominis* . *Blastocystis hominis* was the parasite to be significantly higher among patients 48.6% in comparison with control subjects 7.1% as shown in Table 2.

There have been many studies which use Jones medium as the medium of choice for xenic culture growth of *Blastocystis* (sayal *et al.*, 2020; Mohamed, 2016; Parkar *et al.*, 2007). The results of the current study were nearly similar to results the study of Amany and Mohamed, (2008), and sayal *et al.*, 2020 in which the isolation of *Blastocystis* was 41% and 48% respectively of Irritable Bowel Syndrome patients using culture method on modified jones medium.

The results of culture was in agreement with study of (Yousif and Hashem, 2021; Sayal *et al.*, 2020) where both they mentioned that the results of the culture of the *Blastocystis hominis* parasite were slightly higher than the results of the microscopic examination, and therefore this rise is non significant.

Table (2): Culture of *Blastocystis hominis* results on modified jones medium

Culture results of cases					
		Frequency	Percent	Valid Percent	Cumulative Percent
	Positive	34	48.6	48.6	48.6
	Negative	36	51.4	51.4	100.0
	Total	70	100.0	100.0	
Culture results of control					
		Frequency	Percent	Valid Percent	Cumulative Percent
	Positive	5	7.1	7.1	7.1
	Negative	65	92.9	92.9	100.0
	Total	70	100.0	100.0	

Distribution of cases and control according to the area of residence.

The results in Table (3) showed that there is an increase in the incidence of *Blastocystis hominis* in patients and control groups living in rural areas, which amounted to 52.9% and 70% compared to 47.1% and 30% in city areas respectively. The results of the current study are in agreement with the results of (Yousif and Hashem, 2021), and also in agreement with another study conducted in Egypt done by (Eassa and Masry, 2016), which both stated that the infection rate of the parasite *Blastocystis hominis* was higher in the rural than in the city. While the results of the current study did not agree with the results of the study conducted in Najaf governorate, which stated that the parasite infection rate was higher in the urban than in the rural (Sayal *et al.*, 2020).

Table (3) Percentage of Blastocystis infection by area of residence

Living results of cases					
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Urban	33	47.1	47.1	47.1
	Rural	37	52.9	52.9	100.0
	Total	70	100.0	100.0	
Living results of control					
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Urban	21	30.0	30.0	30.0
	Rural	49	70.0	70.0	100.0
	Total	70	100.0	100.0	

The results of the evaluation of immunoassay of IL-10 in study groups

The results of the statistical analysis shown in Table (4) shows mean difference \pm standard error difference of IL-10 was (5.878 ± 10.462 Pg/ml) with a 95% confidence interval was (14.809-26.565) in intestinal disorder patients with significant difference ($P < 0.001$). numerous studies have shown that chronic alterations of inflammatory cytokines are found in the peripheral blood and intestinal mucosa, which is consistent with the development of intestinal disorders symptoms at the molecular level, however, alterations of certain cytokines in different locations within the GI tract have not been systematically reported in intestinal disorders (Yang and Lan, 2015). IL-10, as a classical anti-inflammatory cytokine, decreases the inflammatory reaction through a number of mechanisms. It can diminish the production of inflammatory mediators including IL-1 β and IFN- γ in T cells and activate macrophages, it also can reduce the expression of major histocompatibisblity complex class II, co-stimulating, and adhesion molecules on the surface of antigen-presenting cells (Sabat *et al.*, 2010). Darkoh *et al.*, 2014 demonstrate the concentrations of anti-inflammatory cytokine IL-10 was significantly lower in the intestinal disorders groups, thus this not compatible with our results. In addition, IL-10 level was significantly lower in Intestinal disorders patients (0.11 ± 0.01) compared to control group (0.21 ± 0.02) ($P < 0.05$). Moreover, no significant differences were detected in IL-4, and IL-10 level among each group (Chen *et al.*, 2012). The results of the present study nearly compatible with results of (vara *et al.*, 2018) where reported that the serum level of IL-10 was significantly higher in patients with intestinal disorders compared with a healthy control group ($P = 0.02$).

Table (4): Comparison of the average level of interleukin-1-beta, interleukin-4 and interleukin-10 in the study groups

Independent Samples Test		
	Levene's Test for Equality of Variances	t-test for Equality of Means

		F	Sig.	t	df	Sig. (2tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
IL-10	Equal variances assumed	5.051	.026	.562	138	.575	5.878	10.462	- 14.809	26.565
	Equal variances not assumed			.562	128.959	.575	5.878	10.462	- 14.822	26.577

Correlation between standards in cases and controls

The results of Table (5) show that there is statistical correlation between age and culture at level 0.01. On other hand, there is statistical correlation culture and IL-10, between living and IL- 10 at significant level 0.05.

Table (5): Correlation between standards in cases

Correlations					
		Age	Living	Culture	IL-10
Age	Sig.	1			
Living	Sig.	.738	1		
Culture	Sig.	.000**	.151	1	
IL-10	Sig.	.000**	.011*	.032*	1
*. Correlation is significant at the 0.05 level (2-tailed).					
**. Correlation is significant at the 0.01 level (2-tailed).					

The results of Table (6) show that there is no statistical correlation between age, living, culture and IL- 10 at significant level 0.05 and 0.001.

Table (6): Correlation between standards in controls

Correlations					
		Age	Living	Culture	IL-10
Age	Sig.	1			
Living	Sig.	.485	1		
Culture	Sig.	.719	.619	1	
IL-10	Sig.	.238	.980	.166	1
*. Correlation is significant at the 0.05 level (2-tailed).					
**. Correlation is significant at the 0.01 level (2-tailed).					

Conclusions

1. The ratio of infection with blastocystosis was high in this study.
2. Interleukin-10 concentration levels have a significant effect on the results of Blastocystosis patients suffering from digestive disorders.

Recommendations

It was recommended to study the immunological relationship between IL-10 and IL-1 beta with other parasite in intestinal disorders patients.

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Ethical Standards

The current study obtained ethical approval by the Department of Medical Laboratories in the College of Health and Medical Technologies / Kufa, Najaf Health Department / Training and Development Center, and written consent was taken from all participants in the research (patient group and control group).

References

1. Abdulla, N. B.; Alsadig, A. A.; Abosalif, K. O. and Saad, M.B. (2014). Gastrointestinal parasites among patients with irritable bowel Syndrome (IBS) in Khartoum state, Sud. Med. Lab. J., 2(2): 18-26.
2. ALDAHASI, W. T., TOULAH, F. H., & WAKID, M. H. (2020). Evaluation of common microscopic techniques for detection of *Blastocystis hominis*. Journal of the Egyptian Society of Parasitology, 50(1), 33-40.
3. Amany, M. E. and Mohamed M. E. (2008). Identification of *Blastocystis hominis* in Patients with Irritable Bowel Syndrome using Microscopy and Culture Compared to PCR. *Parasitologists United Journal (PUJ)*, 1, (2): 87-92.
4. Canavan, C., West, J., and Card, T. (2014). The epidemiology of irritable bowel syndrome. *Clinical epidemiology*, 6, 71.
5. Chen, J., Zhang, Y., & Deng, Z. (2012). Imbalanced shift of cytokine expression between T helper 1 and T helper 2 (Th1/Th2) in intestinal mucosa of patients with post-infectious irritable bowel syndrome. *BMC gastroenterology*, 12(1), 1-6.
6. Darkoh, C., Comer, L., Zewdie, G., Harold, S., Snyder, N., & DuPont, H. L. (2014). Chemotactic chemokines are important in the pathogenesis of irritable bowel syndrome. *PloS one*, 9(3), e93144.
7. Eassa, S. M., Ali, H. S., El Masry, S. A., & Abd El-Fattah, A. H. (2016). *Blastocystis hominis* among immunocompromised and immunocompetent children in Alexandria, Egypt. *Ann Clin Lab Res*, 4(2), 92-98.
8. Ip, W. E., Hoshi, N., Shouval, D. S., Snapper, S., & Medzhitov, R. (2017). Anti-inflammatory effect of IL-10 mediated by metabolic reprogramming of macrophages. *Science*, 356(6337), 513-519.

9. Jalallou, N., Iravani, S., Rezaeian, M., Alinaghizade, A., and Mirjalali, H. (2017). Subtypes distribution and frequency of Blastocystis sp. Isolated from diarrheic and Non-diarrheic patients. *Iranian journal of parasitology*, 12(1), 63.
10. Mahmood, O. I., & Khudher, T. A. A. (2018). Diagnostic and Epidemiological study of Blastocystis hominis in Samarra city, Iraq. *Tikrit journal of pure science*, 21(6), 28-31.
11. Mohamed, S. K., (2016). Prevalence of Blastocystis hominis and other Enteropathogenes and Their Relationship with Irritable Bowel Syndrome in Al-Muthanna Province.
12. Parkar, U.; Traub, R. J.; Kumar, S.; Mungthin, M.; Vitali, S.; Leelayoova, S.; Morris, K. and Thompson, R. C. (2007). Direct characterization of Blastocystis from faeces by PCR and evidence of zoonotic potential. *Parasitology*, 134, 359-67.
13. Ramli, R. M., Sinrang, A. W., & Aminuddin. (2021). Levels of alpha-1 acid glycoprotein (AGP) in stunting and non stunting tolls age 36-60 months . *International Journal of Health & Medical Sciences*, 4(1), 145-149. <https://doi.org/10.31295/ijhms.v4n1.1666>
14. Ratnawati, I. G. A. A., Suandayani, N. K. T., & Sutapa, G. N. (2019). The linearity of x-ray devices radiation output and its relationship with patient thickness. *International Journal of Physical Sciences and Engineering*, 3(3), 1-6. <https://doi.org/10.29332/ijpse.v3n3.351>
15. Sabat, R., Grütz, G., Warszawska, K., Kirsch, S., Witte, E., Wolk, K., & Geginat, J. (2010). Biology of interleukin-10. *Cytokine & growth factor reviews*, 21(5), 331-344.
16. Sayal, R. A., Hameed, S. A., & Faisal, M. M. (2020). Evaluation of IL-5 concentration level in Irritable Bowel Syndrome patients that suffering from Blastocystis infection in Al-Najaf Province. *European Journal of Molecular & Clinical Medicine*, 7(2), 3807-3817.
17. Suryasa, I. W., Rodríguez-Gámez, M., & Koldoris, T. (2021). Health and treatment of diabetes mellitus. *International Journal of Health Sciences*, 5(1), i-v. <https://doi.org/10.53730/ijhs.v5n1.2864>
18. Vara, E. J., Brokstad, K. A., Hausken, T., & Lied, G. A. (2018). Altered levels of cytokines in patients with irritable bowel syndrome are not correlated with fatigue. *International Journal of General Medicine*, 11, 285.
19. Yang, B., Zhou, X., & Lan, C. (2015). Changes of cytokine levels in a mouse model of post-infectious irritable bowel syndrome. *BMC gastroenterology*, 15(1), 1-7.
20. Yousif, J. J., and Hashem, Z. A. K. (2021). Evaluation of the Level of some Immunological Parameters in Patients with Gastrointestinal Disorders Infected with the Blastocystis Hominis Parasite. *REVISTA GEINTEC-GESTAO INOVACAO E TECNOLOGIAS*, 11(4), 2322-2330.