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Evaluation of the Level of some Immunological Parameters in Patients with Gastrointestinal Disorders Infected with the *Blastocystis Hominis* Parasite

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Abstract
Objectives: *The current research was conducted to investigate the Blastocystis hominis parasite in the stool of patients with gastrointestinal disorders and to detect some immunological effects.*
Methodology: *Descriptive case-control study design, the study included collecting 75 stool and blood samples from patients with gastrointestinal disorders and 75 stool and blood samples from healthy people. The ages of patients and healthy ranges from 10-70 years.*
Results: *The results of immunological examinations using the ELISA technique revealed a significant increase of $P<0.05$ in the level of IL-1 beta and IL-6 in the serum of patients with gastrointestinal disorders compared to control. There was a positive correlation between the IL-1 beta and IL- 6 in patients with gastrointestinal disorders infected with B. hominis parasite. The results of the immunological tests also showed a significant increase of $P<0.05$ in the level of IgG and IgM and a significant decrease in the level of IgA antibody in patients with gastrointestinal disorders with B. hominis compared to control.*
Conclusion: *Infection with the B. hominis parasite has an effect on stimulating the cellular immune response by increasing the level of IL-1 beta and IL-6 and stimulating the humoral immune response by increasing the level of IgG and IgM antibodies in patients with gastrointestinal disorders infected with B. hominis parasite compared to control.*

Key-words: *Blastocystis Hominis*, Interleukin-1 Beta, Interleukin-6, Immunoglobulin-A, Immunoglobulin-M, Immunoglobulin-G.

1. Introduction

The parasite *Blastocystis hominis* is widespread as it is one of the most common parasites that infect the intestines, with an estimated infection rate of about one billion people from all over the world(1).The innate immune system constitutes the first line of host defense and plays a critical role in preventing infection of bacterial pathogens The co-evolution of intestinal parasites with the

mammalian immune system has encouraged the development of complex interactions between parasites and hosts as *Blastocystis* is the most common parasite related to humans and represents a genetically diverse component of the gut microbiome[2]. The parasite *B.hominis* modulates the immune response against it, and this helps the parasite in the process of immune escape, and here it stimulates the body and secretes inflammatory cytokines such as (IL-8, IL-6, IL-1 beta, GM-CSF) in order to get rid of the foreign body[3]. *B.hominis* induce strong expression of the pro-inflammatory cytokines IL-1β, IL-6 and TNFα mediated by mitogen-activated protein kinases (MAPKs). The MAPK cascade is one of the most important evolutionarily conserved signaling pathways and plays an essential role in immunity[4]. IL-6, which is rapidly and transiently produced in response to infection and tissue injury, contributes to host defense by stimulating acute phase responses, hematopoiesis and immune reactions[2]. Microbial infection stimulates production of IL-1β, a key mediator of the inflammatory response that is essential for host response and resistance to pathogens, and exacerbates damage during chronic disease and acute tissue injury. It is therefore not surprising that there is a great deal of interest in how this protein is produced and exported from cells since IL-1β secretion occurs in a continuum, dependent on the strength of the stimulus and the requirements of extracellular IL-1β [5]. This study aims to evaluation the some immune parameters among gastrointestinal disorder patients infected with *Blastocystis hominis* in Al-Najaf province of Iraq.

2. Methodology

Study Sample

One hundred fifty six of respondents were chosen in this study included (78 case and 78 control) attended to Al-Furat Hospital and Al-Sajad Hospital in Al-Najaf city from October 2020 to March 2021. The patients who were diagnosed clinically by physicians, that they are suffering from gastrointestinal disorders and infected with *Blastocystis hominis*. Those respondents in age group (10-70) years.

Study Location

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

Inclusion Criteria

The cases were defined as any patients who are infected with *Blastocystis hominis* parasite and suffering from gastrointestinal disorders in the age group (10-70) years living in the location of the study. However, the control group was patients without any history of disease in the same age group at same study location.

Exclusion Criteria

The patients who have suffered from other than gastrointestinal disease such as bloody ulceration, cancer and others.

Specimen Collection

Blood samples were drawn from each patient and control groups by disposable syringe 5ml of blood were collected in sterile serum tube and left for one hour at room temperature. Then centrifuged at 3000 rpm for (15 minute) to separate the serum that was stored at -20 C^{o} until used. IL-1 beta, IL-6 (Abcam, USA), IgA, IgM, and IgG (Mybiosource, USA) ELISA kit were used to measure the serum levels of IL-1 beta, IL-6, IgA, IgM, and IgG and executed according to manufacturer.

Statistical Methods

The following Statistical analysis approach by using social sciences (SPSS) version 20 in order to analyzed and assess the data of the study, t test and LSD was applied to find out the significant difference between the data. Differences were recorded as significant whenever the probability (P) was less than 0.05.

3. Results and Discussion

A. The Level of IL-1 Beta and IL-6 in the Study Groups

The results of the statistical analysis in table (1) showed that there were significant $P<0.05$ differences between the mean value of IL-1 beta and IL-6 levels for patients with intestinal disorders

infected with *B. hominis* which amounted to (594.608 ±76.814pg/ml) and (787.75 ±72.206 pg/ml) respectively, compared to the control group, which reached (153.01±109.55pg/ml) and (203.54 ±157.350 pg/ml) respectively.

The reason for the increase in these cytokines is attributed to the fact that IL-6 is an anti-inflammatory cytokine initiating inflammation that inhibits some pro-inflammatory cytokines and activates and activates other cytokines that limit or reduce the inflammatory process[6], while IL-1 beta is an inflammatory cytokine associated with the generation of an inflammatory reaction, it plays a role in activating B and T lymphocytes, and, along with IL-6, stimulates the synthesis of acute phase proteins in hepatocytes[7], so we find that both are elevated in patients with intestinal disorders with *blastocystis* in order to get rid of the parasite. This indicates that infection with the blastocyst parasite has an effect on the immune system, and this contradicts what was mentioned in a study conducted at the University of Al-Qadisiyah / College of Medicine in which it was mentioned that Blastocystis live naturally in the human intestinal system without causing disease[8]. While there is a study conducted at Al-Furat University The results of the current study were not similar to the results of another study that reported that the level of IL-6 was slightly and insignificantly elevated in the serum of gastrointestinal disorders patients compared to the control group[9]. While the results of the current study are in agreement with the results of other studies that reported the presence of high levels of IL-6 in the serum of patients with gastrointestinal disorders compared to the control group[10,11]. Others reported that there was no significant difference in the ratio of IL-6 and IL-1beta levels between patients with gastrointestinal disorders and the control group[12,13]. One study reported that IL-6 deficiency is associated with decreased antibody response and susceptibility to infection[14].

The results of the current study are in agreement with the results of another study which indicated that there is an increase in the levels of IL-1beta in the serum of patients with gastrointestinal disorders compared to the control group[15]. The results of this study contradicted the results of another study that stated that there is no difference in the levels of IL-1 beta concentrations between males and females in patients with intestinal disorders and the control group[16].

Table 1 - Comparison of the Average Level of Interleukin-1 Beta and Interleukin-6 in the Study Groups

Type of cytokine	Study groups	Mean	SD	P-value
IL-1beta	Patients	594.608	76.814	0.000
	Control	153.01	109.55	
IL-6	Patients	787.75	72.206	0.000
	Control	203.54	157.350	

B. Correlation Analysis between IL-6 and IL-1 beta in Study Groups

The results in table (2) revealed a positive correlation at the probability level of P<0.001between the level of IL-1 beta and IL-6 in patients with intestinal disorders with *Blastocystis hominis*, where the correlation coefficient was 0.794.

The results of our study indicate that IL-1 beta works alongside IL-6 to stimulate the synthesis of acute phase proteins in hepatocytes. Thus, IL-6 as a pro-inflammatory cytokine and once a cytokine reduce or prevent inflammation, so there is a positive correlation between the two in order to increase susceptibility to inflammation and the body fights infection and inflammation. This association may be explained by the fact that *Blastocystis* induces strong expression of proinflammatory cytokines (IL-1beta, IL-6) in macrophages mediated by Mitogen-activated protein kinases (MAPKs) via the serine protease secreted by Bladder. *B.hominis* in a MAPKs-dependent manner, whereby MAPKs play a critical role in regulating cytokine expression in the immune response[17].

Table 2 - Correlation Analysis between IL-6 and IL-1 Beta in Gastrointestinal Disorder Patients Infected with *B. Hominis*

Cytokines	**Correlation coefficient**	P-value
IL-6	0.794*	0.001
IL-1 beta		
*The correlation is significant at the P<0.001level (two-tailed).		

C. The Level of IgG, IgM, and IgA Antibodies in the Study Groups

The results of the statistical analysis in Table (3) showed that there was a significant increase of P<0.05 in the mean level of IgG antibody in patients with intestinal disorders infected with *B. hominis* which amounted to (109.30 ±17.471ng/ml), compared to the control group, which amounted to (71.34±20.775ng/ml), as the results showed in Table (3) a significant increase in the mean concentration of IgM antibody in patients with intestinal disorders infected with *B. hominis*, which was (228.75 ± 20.584 ng/ml), compared to the control group, which was (128.48 ± 55.007 ng/ml), and the results showed that there is a significant decrease in the mean concentration of IgA antibody in patients with intestinal disorders infected with *B. hominis*, which amounted to (2.34 ±0.874 ng/ml), compared to the control group, it reached (135.48 ± 59.723 ng/ml).

Immunity plays a major role in defense against extracellular pathogens by stimulating the production of different types of immunoglobulins such as IgG, IgA and IgM. in general, IgG constitutes 80% of our circulating immunoglobulins and is one of the most immunoglobulins that act against agents *Blastocystis* pathogenicity[18]. The humoral immune response is characterized by B cells producing antibodies that protect against infection and as the humoral response develops, B cells can secrete different types of antibodies[19]. IgM is generally the first type of antibody released in response to infection IgM antibodies were considered the first antibody to be released against infection and indicate the presence of a current infection and have a shorter life compared to IgG, which is characterized by a long period of presence in the body, which is later secreted and indicates the presence of a previous or old infection[20].

The results of our study indicated that there is a decrease in the level of IgA in people infected with *B.hominis* infection compared with the control group when the parasite *B.hominis* enters the human body, it begins to secrete Cystein Protease, which constitutes an adaptation mechanism for the parasite [21], which facilitates Colonization by protecting parasites in the intestinal environment against the host's immune system. Cystein Protease, in turn, binds with IgA and breaks down its degradation, because IgA is the most antibody present in the secretions, so it is called secretory. Thus, it prevents colonization or invasion of the microbe and also reduces the inflammation of the mucous membrane and tissues after colonization or invasion [22], thus the parasite works to modify the immune response against it and this helps the parasite in the process of immune escape. A study by [23] reported that patients with *B.hominis* had elevated levels of specific IgG antibodies in the blood. IgM is an antibody present on the surface of the B cell and is the first antibody produced to fight infection by humoral immunity [24]. The specific immune response to parasites leads to the production of antibodies. Infection with primary parasites is associated with the production of IgG and IgM [25]. Depending on the immune functions of IgG, IgA and IgM, previous studies evaluated these immunoglobulins in order to understand the immune response of the host infected with intestinal parasites, and one of those studies was a study that indicated a decrease in the level of IgA in people infected with *B.hominis* compared with the control group [26] and therefore the results of our current study are in agreement with the results of this study.

The results of the current study did not agree with the results of another study that reported that the level of IgM concentration in people with *B. hominis* was low compared to the control group [27].

Table 3 - Comparison of the Average Level of IgM, IgG and IgA in the Study Groups

Antibodies	Study groups				P-value
	Patients		Control		
	Mean	SD	Mean	SD	
IgG	109.30	17.471	71.34	20.775	0.000
IgM	228.75	20.584	128.48	55.007	0.000
IgA	2.34	0.874	135.48	59.723	0.000

4. Conclusion

Infection with the *B. hominis* parasite in patients with gastrointestinal disorders has an effect on stimulating the cellular immune response by increasing the level of IL-1 beta and IL-6 and stimulating the humoral immune response by increasing the level of IgG and IgM antibodies compared to control group.

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Ethics Approval

The research had the ethical approval which given by department of biology Science, College of Education for Girls, University of Kufa, An-Najaf Health Directorate\ Center of Training and Development of Staffs, also written consent was taken from all respondents.

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