**Measurement of immunological marker IL-1 beta in blastocystosis patients with experience of intestinal disturbance**

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**Objectives**: To measure of IL-1 beta concentration level in sera of blastocystosis patients with experience of intestinal disturbance by using ELISA.

**Methadology:** Descriptive case-control study design, the present study 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 participitant (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 of examining 140 stool samples by light microscopy showed the presence of the parasite

Blastocystis hominis in 31 out of 70 stool samples from patients with intestinal disturbance, at a rate of 44.3%, while the control group consisted of 70 people all subjects were negative except 5 of them (7.1%), were positive for the human cystic parasite. The results of immune assay showed mean difference ± standard error difference of IL-1β was (-9.771 ± 10.819 Pg/ml) with a 95% confidence interval was (31.16511.623) in intestinal disorder patients with significant difference (P˂0.001).

**Conclusion:**

 The rate of infection with Blastocystis hominis parasite in this study was 44.3%. Serum levels of cytokine IL1β were significantly higher in the control group.

**Key words: Interleukin-11beta, Intestinal Disturbance, Blastocystosis**

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**Introduction**

 *Blastocystis hominis* was considered to be a member of normal intestinal flora in the past; recently it has been accepted as a controversial pathogen (Scanlan and Stensvold, 2013).

 Infection with *B.hominis* has a worldwide distribution and occurs in both children and adults and it is anaerobic unicellular protozoan frequently found in the human gastrointestinal tract, *B. hominis* is now getting acceptance as an agent of human intestinal diseases with potentially disabling symptoms diarrhea, nausea, flatulence, and abdominal pain (Alinaghizade *et al*., 2017).

 Any patient carrying Blastocystis infection is liable for development of Intestinal disorders about 73 times than non-infected individuals compared to healthy controls, Making it an organism of interest in intestinal disorders (Sayal *et al.*, 2020).

 Microbial infection stimulates production of IL1β, 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 IL1β (Vignali and Kuchroo, 2012).

The present study aimed to measure of IL-1 beta concentration level in sera of blastocystosis patients with experience of intestinal disturbance by using ELISA.

 **Methadology** blood and stool samples was collrcted from both patients with intestinal disorders (70 participitant) and control groups and their ages ranged between (6 - ≥ 60) years at Al-Sadr Medical city and AlHakim General Hospital and Al-Sajad Hospital through the period from the beginning of November 2021 to the end of February 2022.

**Samples Collection**

1. **Fecal Samples Collection**

 Fecal specimens were collected in suitable, clean and dry container, all samples were introduced for microscopic examination for detection *B. Hominis*.

1. **Serum Samples Collection**  three ml of venous blood from 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. **Microscopic Examination**

1. **Wet mounting**

 A wet mount was performed directly from fecal sample. The two type of wet mount were used for each of fecal sample (Saline and Iodine) were used for the initial microscopic examination of stool and to demonstrate the parasite (*B.hominis*).

 For direct saline and iodine mount, a drop of saline was put in the side of slide and a drop of iodine solution in the other side, a small of the stool sample (size of a match head) and mixed with a drop of saline and iodine with an applicator stick picked up and covered with cover slips *B.hominis* cyst can be examined with high power magnification.

1. **Formalin-ether precipitation method**

 This method was done when the stool was suspected a *B.hominis* was not seen in the wet mounts.

* Ten ml of 2%formalin was added to approximately 1g of stool and stirred using applicator stick until get a cloudy suspension.
* A gauze filter was fitted into a funnal and the funnal was placed on top of the centrifuge tube.
* The fecal suspension was passed through the filter into centrifuge tube until 7ml mark is reached.
* The filter was removed and discards with lumpy residue.
* A volume of 3ml ethyl acetate was added and mixed well for one minute.
* The fatty plug was loosed with an applicator stick, and supernatant was poured away by quickly inverting the tube.
* The tube was replaced in its rack and the fluid was allowed on the sides of the tube to drain down to the sediment, mixed well and transfer a drop to a slide for examination, also it was made iodine.

 **ELISA Technique**

 Estimation concentration level of IL-1 beta 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**

1. **Mean age and gender distribution of intestinal disorders patients and control subjects**

 Table (4-1) shows that the mean age of intestinal disorders patients was 31.36+18.276 years and the mean age of control subjects was also

34.50+17.200 years.

Table 1: Mean age of patients and control subjects

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Age  | Groups |  N  | Mean  | Std. Deviation  | Std. Error Mean  |
| Case  | 70  | 31.36  | 18.276  | 2.184  |
| Control |  70  | 34.50  | 17.200  | 2.056  |

1. **Sex-** **distribution of patients with intestinal disorders and the control groups**

 The sex distribution of patients with intestinal disorders and the control group is shown in Table (2), where the proportion of male and female patients with intestinal disorders was 47.1% and 52.9%, respectively, while the proportion of males and females from the control group was 41.4% and 58.6%, respectively. There are several factors that make women more susceptible to intestinal disorders than men, including life stresses, raising children, and hormonal changes, especially during the menstrual period. During menstruation, this may be caused by increased levels of prostaglandins during the menstrual cycle.

Prostaglandins are hormone-like substances that contribute to pain and inflammation, and this increases uterine muscle contractions (Fajrin *et al*., 2020).

 Another hormone that can affect the intestines is testosterone, because the levels of this hormone are high in men and they have a wide stomach wall, which means less risk of bloating (Kim and Kim, 2018).

 In addition to hormones, the shape of the intestines differs in men and women. On average, the female colon is 10 cm longer than the male’s, and has many curves. In addition, he needs to compete to find space for himself in the pelvis with the genitals of women, and of course this makes them more susceptible to digestive diseases. (Mohammed *et al.,* 2010).

The distribution of patients and the control group according to gender in this study is dis agreement with the distribution of other studies (Yousif and Hashem, 2021; Sayal *et al*., 2020; Mohammed, 2016; and Abdalla *et al.,* 2014) Although there were small, non-significant differences.

 A study was conducted at Sebha

University/College of Science in Libya in which it was mentioned that the prevalence of intestinal parasites between male and female patients was not statistically significant (P-value > 0.05) (Abidu *et al*., 2000).

 Also, a study conducted in the city of Samarra / Salah al-Din Governorate reported that there were no statistically significant differences between males and females (Mahmood and Khudher, 2018).

 While a study conducted at the University of Kirkuk showed statistically significant differences between males and females (Salman, 2015).

 The results of the current study contradicted results from other studies in which it was mentioned that the percentage of males was higher than females, and this may happen due to the nature of male work in many areas, while women work mainly at home (Aldahhsi *et al*., 2020; Hamdy *et al*., 2019 and El-Safadi *et al*., 2016).

**Table (2) Distribution of samples of intestinal disorders patients and control according to sex.**

|  |  |  |
| --- | --- | --- |
| Group  | Male  | Female  |
| Frequency  | percent  | Frequency  | percent  |
| Control  |  |  |  |  |
| (n =70)  | 29  | 41.4  | 41  | 58.6  |
| Cases  |  |  |  |  |
| (n = 70)  | 33  | 47.1  | 37  | 52.9  |

**3. Diagnosis of Blastocystis hominis parasite in feces of patients with intestinal disorders and a control group**

 The results of examining 140 stool samples by light microscopy showed the presence of the parasite *Blastocystis hominis* in 31 out of 70 stool samples from patients with intestinal disorders, at a rate of 44.3%, while the control group consisted of 70 people all subjects were negative except 5 of them, were positive for the human cystic parasite as shown in Table (3).

 A number of studies used the method of microscopic examination in diagnosing the parasite *Blastocystis hominis* in Iraq, including the study that was recently conducted in Al-Najaf, where the parasite was diagnosed in 13 stool samples out of 75 samples from patients with intestinal disorders, at a rate of 17.% (Yousif and Hashem, 2021). Likewise also study conducted in Najaf

Governorate, in which the parasite was diagnosed in 36 stool samples out of 78 samples of patients with gastro-intestinal disorders, with a percentage of 46% (Sayal *et al.,* 2020).

 The study conducted in Al-Muthanna

Governorate in which the parasite was diagnosed in 43 out of 127 stool samples from patients with intestinal disorders, at a rate of 33% (Mohammed, 2016).

**Table (3) Microscopic diagnosis of *Blastocystis hominis* parasite in patients with intestinal disorders and the control group**

|  |  |  |  |
| --- | --- | --- | --- |
| Parasite  | Control (n = 70)  | cases  (n = 70)  |   |
|  |  |  |  |  |
|  | No.  | %  | No.  | %  | Pvalue  |
| *Blastoc ystis*  | Positi ve  | 05  | 7.1  | 31  | 44.3  | <0.001  |
| *homini**s*  | Negat ive  | 65  | 92.9  | 39  | 55.7  |  |



**Figure (1): *Blastocystis homins* under microscopic examination (40 X)**

1. **The results of the evaluation of immunoassay of IL-1 β in study groups**

 The results of the statistical analysis shown in Table (4) shows mean difference ± standard error difference of IL-1β was (-9.771 ± 10.819 Pg/ml) with a 95% confidence interval was (31.16511.623) in intestinal disorder patients with significant difference (P˂0.001).

 Darkoh *et al.,* 2014 demonstrate the concentrations of pro-inflammatory cytokine IL-1β was higher in intestinal disorders patients than the healthy volunteers.

 But Conversely, of our study serum levels of cytokine IL-1β were significantly higher in the control group (p=0.03).

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **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-1beta  | Equal variances assumed  | 2.245  | .136  | -.903-  | 137  | .368  | -9.771-  | 10.819  | -31.165-  | 11.623  |
| Equal variances not assumed  |   |   | -.904-  | 134.6 11  | .368  | -9.771-  | 10.808  | -31.146-  | 11.603  |

 |

1. **Correlation between standards in cases and controls**

 The results of Table (5) show that there is no statistical correlation between gender, light microscopic diagnosis and IL- 1 beta at significant level 0.05 and at level 0.001.

**Table (5): Correlation between standards in cases**

|  |
| --- |
| **Correlations**  |
|   | **Gender**  | **Diagnosis By L\_M**  | **IL-1beta**  |
| **Gender**  | **Sig.**  | 1  |   |   |
| **Diagnosis By L\_M**  | **Sig.**  | .106  | 1  |   |
| **IL-1beta**  | **Sig.**  | .147  | .145  | 1  |
| \*. Correlation is significant at the 0.05 level (2tailed).  |
| \*\*. Correlation is significant at the 0.01 level (2tailed).  |

 The results of Table (6) show that there is only statistical correlation between light microscopical diagnosis and IL-1 beta at significant level at 0.001.

**Table (6): Correlation between standards in controls**

|  |
| --- |
| **Correlations**  |
|  | **Gender**  | **Diagnosis By\_L.M.**  | **IL-1beta**  |
| **Gender**  | **Sig.**  | 1  |   |   |
| **Diagnosis By L. M.**  | **Sig.**  | .320  | 1  |   |
|  |
| **IL-1beta**  | **Sig.**  | .269  | .004\*\*  | 1  |
| \*. Correlation is significant at the 0.05 level (2tailed).  |
| \*\*. Correlation is significant at the 0.01 level (2tailed).  |

**Conclusions**

1. The rate of infection with Blastocystis hominis parasite in this study was 44.3%.
2. The proportion of female with intestinal disorders was higher than male.
3. Serum levels of cytokine IL-1β were significantly higher in the control group.

**Recommendations**

1. Molecular diagnostic study for detection of Blastocystis in stool of patients with intestinal disorders.
2. Immunolological re evaluation the role of cytokine IL-1 beta in the immune response against Blastocystosis according to different subtype of blastocystis species that infect human.

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 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).

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