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RESEARCH ARTICLE

IMMUNOPATHOGENESIS OF *ENTAMOEBA HISTOLYTICA* AT NEWLY DIAGNOSED MALE PATIENTS, IN THE EASTERN REGION OF IRAQ.

Zaid Habeeb Abd-Alkareem¹, Jaber Hameed hussien¹, Qays Ahmed Al-Kafaji² and Mohammed Al-Aaraji¹.

1. Department of Clinical Laboratory Sciences, College of Pharmacy, AL-Mustansiriyah University. Baghdad, Iraq.
2. Kamal Al-Samarraee Teaching Hospital for Infertility and IVF.

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Abstract

This study aims to investigate the effect of *Entamoeba histolytica* infection on the cellular immunity of the patients, through measuring the following immune markers: IL-1α, IL-2 and IL-8 during acute amoebiasis, and to find out the correlation of serological parameter with the severity of infection. The study included 110 individuals (male only), 55 patients were diagnosed with *Entamoeba histolytica*, and 55 cases as a healthy control group. Immune markers IL-1α, IL-2 and IL-8 were assessed throughout the course of the infection. Results showed elevated levels of IL-1α and IL-2 in patients group compared with the healthy control group (8.216 ± 1.656) and (4.263 ± 1.128) respectively ($P < 0.001$). In addition, results showed a significant increase in IL-8 ($P < 0.001$) with a confidence interval of (3.512-4.881).

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Introduction:-

Entamoeba histolytica is an anaerobic parasitic protozoa, it infects a large part of gastrointestinal tract of human causing amebiasis, human is the initial reservoir of *Entamoeba histolytica*, and the infection is mainly transmitted via contaminated fresh water and food (Khan and Jahan, 2017). *Entamoeba histolytica* infection is distributed worldwide, it causes colitis, and extraintestinal amebiasis to 50 million with 100.000 deaths per year (Bercu et al, 2007). Patients with intestinal amebiasis may suffer from severe abdominal pain, and diarrhea. However, endoscopic assessment reveals the scattered colonic ulcerations and liver abscess in some cases. *Entamoeba histolytica* has a two-stage life cycle, which is represented by cysts and trophozoites (Peterson et al 2011). It is an intracellular protozoan, it attaches to the host tissue in a manner of contact-dependent, this process and *E histolytica* excretory-secretory products (ESP) is reported to play a key role in cellular invasion (Ahn et al 2018). Moreover, *E histolytica* is found to initiate a local, secondary immune reaction, an anti-amoebic secretory IgA (sIgA) is diagnosed in saliva, faeces, breast milk, and bile from infected patients (Carrero et al, 2007). The pathogenesis of *E histolytica* is still not fully understood; however, many studies revealed, that the pathogenicity of *E histolytica* is a multifactorial tactic (Espinosa-Cantellano M and Martínez-Palomo, 2011). Some manifestations of *E histolytica*'s pathogenesis are proposed, they are generally grouped according to the involved mechanisms in to the following: (a) interaction of *E histolytica* with the intestinal normal flora, (b) *E histolytica* lytic effect on the host cell via direct adhesion or by cytotoxic products (c) *E histolytica* phagocytic activity of target cell (Espinosa-Cantellano, 2000). The microscopic examination of rectal smears is the common diagnostic test, to detect the trophozoites of *E histolytica* that are found in the mucus and exudates of mucosal ulcers, laboratory test depends on detecting anti *E histolytica* is recently used (Abbas et al 1997). Cytokines are small secreted proteins released by immune cells have a specific effect on the

interactions and communications between cells, Interleukin-1-alpha, one form of interleukin-1, which is made mainly by white blood cell to fight infections. It also helps leukocytes pass through blood vessel walls to sites of infection and causes fever by affecting areas of the brain that control body temperature (Choi et al, 2006).

The IL-1 α precursor is constitutively present in epithelial layers of the entire gastrointestinal tract, lung, liver, kidney, endothelial cells, and astrocytes (Upon cell death by necrosis, as occurs in ischemic diseases such as myocardial infarction, stroke, acute renal failure and tumor necrosis, the IL-1 α precursor is released (Garcia-zepeda et al 2007). Interleukin 2 (IL-2) is a monomeric glycoprotein, it is produced by activated CD4+ T-cells, CD8+ T-cells and dendritic cells . It is characterized as a pro-inflammatory cytokine that is secreted by Th1 cells (Gaffen and Liu, 2004). IL-8 is a chemoattractant cytokine produced by a variety of tissue and blood cells; unlike many other cytokines, it has distinct target specificity for the neutrophil, with weak effect on other blood cells (Begum et al, 2015).

Patients and methods:-

This study included 110 cases, 55 were newly diagnosed with acute *Entamoeba histolytica* infection, and 55 cases were the healthy control. All studied cases were located at Wassit province, and male only were selected. Stool Samples were mixed with 0.9% sodium chloride solution (wet amount), then samples were subjected to direct microscopic examination; to detect the motile trophozoites of *Entamoeba histolytica*. Also, stool samples were stained with Lugol's iodine solution to identify trophozoites and cysts. Enzyme Linked Immunosorbent Assay (ELISA) technique was conducted to measure the levels of IL-1 α , IL-2 and IL-8 in serum.

Statistical analysis:-

The statistical analysis was applied using Anderson Darling test to assess the distribution of continuous variables. Discrete variables presented using there number and percentage used to present data, chi square test was used to analyse the discrete variable, and statistical difference between studied groups was rated using one way ANOVA and Tukey's multiple comparison. All experiments were carried out three times independently: N=3, and data was expressed as $\pm$ SE. Binary logistic regression analysis used to calculate the odd ratio (OR) and their 95% confidence intervals. SPSS 20.0.0 software package was used to make the statistical analysis; $p < 0.05$.

Results:-

Interleukin-1 alpha (IL-1 α)

A significant increase was seen in IL-1 α levels in patients compared to the healthy control, (6.143 $\pm$ 1.476) for the infected patients and (2.383 $\pm$ 0.71) for the healthy control group respectively, $p < 0.05$, data represented as Mean $\pm$ SD, as in table 1 and figure 1.

Table 1:- IL- α levels during *Entameobia histolytica* infection. Data represented as Mean $\pm$ SD. C.I, t-test shows a significant difference in IL-1 α levels in patients and healthy control groups.

IL-1 α	control	Patients	95% Confidence Interval	
			Lower Bound	Upper Bound
Valid numbers	55	55	(3.140-4.379)	
Mean $\pm$ SD	2.383 $\pm$ 0.711	6.143 $\pm$ 1.476		

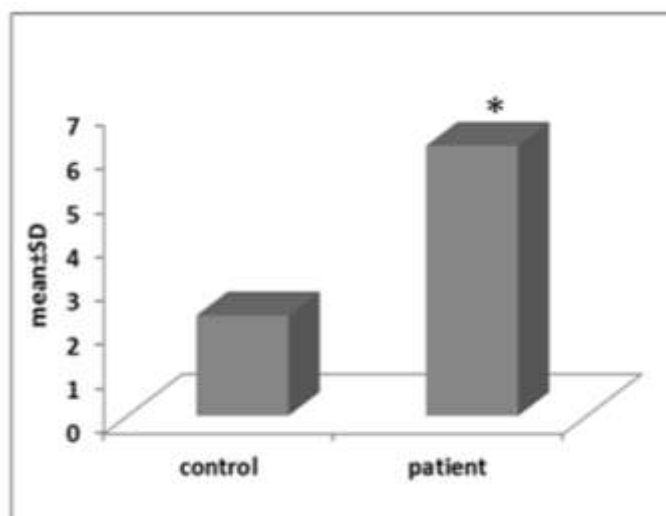


Figure 1:- IL- α levels during *Entameobia histolytica* infection. Mean $\pm$ SD for Interleukin 1 α in patients infected with *Entameobia histolytica* (right bar) and the healthy control group (left bar).

Interleukin-2 (IL-2)

Results showed elevated levels of IL-2 in patients comparing with the healthy control group (8.216 ± 1.656) and (4.263 ± 1.128) respectively with a significant differentiation between two groups $P < 0.005$, also the t -test showed 10.826 and confidence interval (3.225-4.679). As shown in table (2) and figure (2).

Table 2:- The histogram represents IL-2 levels in patients infected with *Entameobia histolytica* and the healthy control group. Data represented as Mean $\pm$ SD, t -test, $p < 0.005$.

IL-2	control	Patients	95% Confidence Interval	
			Lower Bound	Upper Bound
Valid numbers	55	55	(3.225-4.679)	
Mean $\pm$ Std. Deviation	4.263 $\pm$ 1.128	8.216 $\pm$ 1.656		

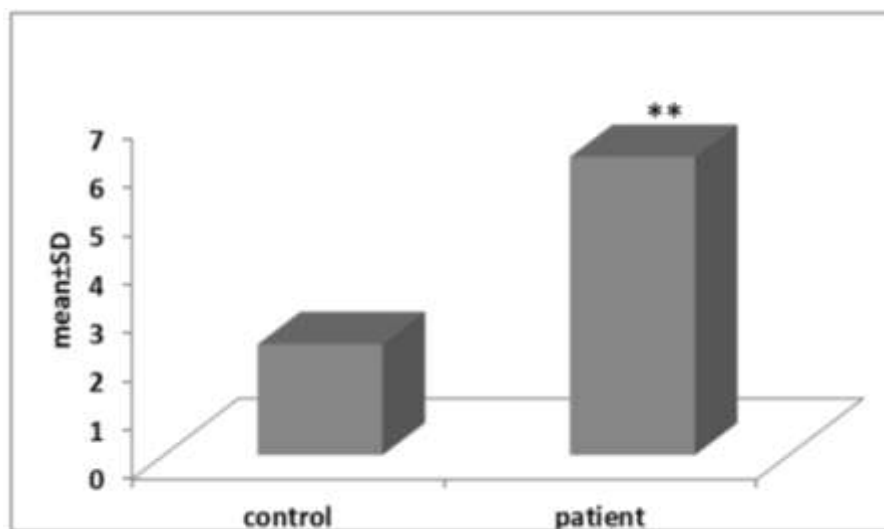


Figure 2:- The histogram represents IL-2 levels in patients newly infected with *Entameobia histolytica* and control group, $p < 0.005$.

Interleukin 8

The study results involve significant elevated in IL8 in patients groups than control groups $P < 0.001$ with confidence interval (3.512-4.881), t-test (12.211) and Mean $\pm$ SD (5.439 $\pm$ 1.545), 9.636 $\pm$ 1.367 respectively. Table 3 and figure 3.

Table 3:- The table represents IL-8 levels in patients newly infected with *Entamoeba histolytica* compared with the healthy control group. Data represented as Mean $\pm$ SD, t-test, $p < 0.005$.

IL-8	control	Patients	95% Confidence Interval		t	Sig. (2-tailed)
			Lower Bound	Upper Bound		
Valid numbers	55	55	(3.512-4.881)		12.211	$P < 0.001$
Mean $\pm$ SD	5.439 $\pm$ 1.545	9.636 $\pm$ 1.367				

*significant at $P \leq 0.05$

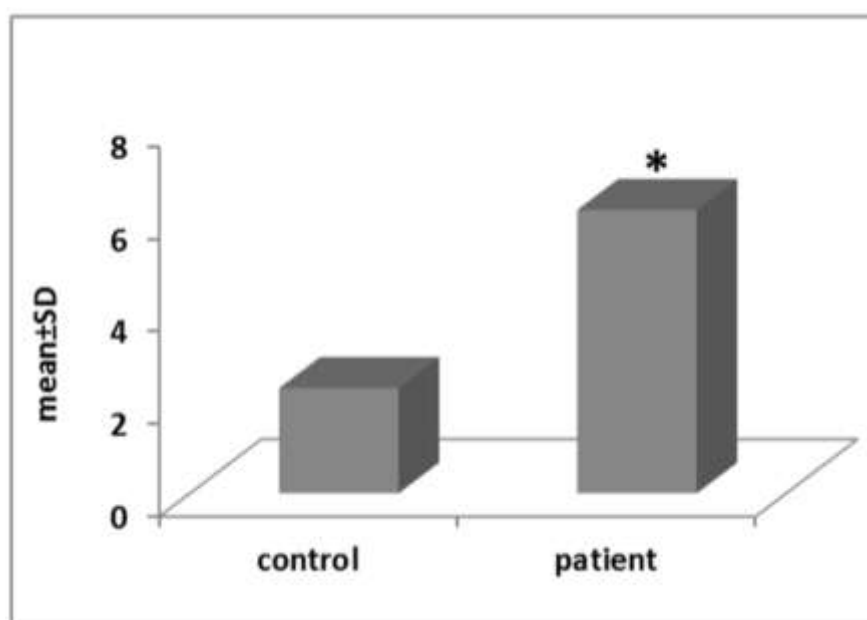


Figure 3:- The histogram represents IL-8 levels in patients newly infected with *Entamoeba histolytica* and control group. Data represented as Mean $\pm$ SD, t-test, $p < 0.005$.

Discussion:-

Previous studies showed an interesting heritage of information regarding the cellular immunology of *E. histolytica*, however the underlining mechanisms are still not fully explained. Interleukins have a key role in regulation of immune cells, our study focus on measuring the levels of three interleukins: IL-1, IL-2 and IL-8. Our results showed a significant increase in IL-1 α in serum of patients compared with the healthy control group as in table 1 and figure 1, ($p < 0.005$). These results were in agreement with Eckmann et al 1995, who showed an elevation in the IL1 alpha induced by *E. histolytica*. Also showed an extracellular pathogen, induce the production of cytokines and chemokines from intestinally derived cell lines (Eckmann et al 1995). Coincubation of *E. histolytica* with cultured epithelial cell lines caused epithelial cell secretion of IL-8, growth-regulated oncogene (GRO) the granulocyte, macrophage colony-stimulating factor (GM-CSF), IL-1 α , and IL-6 (Eckmann et al 1995). IL-8 production by HeLa cells was mediated primarily through the effect of IL-1 α , that was released from epithelial cells lysed by *E. histolytica* trophozoites, reviewed by Skappak et al, 2014.

IL-2 is a monomeric glycoprotein produced by activated CD4 $^{+}$ T-cells, CD8 $^{+}$ T-cells and dendritic cells (Gaffen and Liu 2004). IL-2 is a pro-inflammatory cytokine, that is secreted by Th-1 cells, and it effectively participates in the activation of T cells to produce the cytokines tumor necrosis factor alpha (TNF- α) and interferon gamma (IFN- γ); IL-2 can also enhance the cytolytic activity of natural killer cells (NK) (Gaffen and Liu 2004; Capobianco et al,

2016). Therefore, IL-2 is used therapeutically to stimulate the immune system (Al-Oumashi 2012). IL-2 also contributes to the development of regulatory T-cells, which controls the apoptosis of activated T cells, Furthermore, IL-2 influences cell survival, differentiation (Campbell et al 1999) and the formation of immune memory cells and acts as a negative regulator of immune activation (Gaffen and Liu 2004). Recent study results revealed a significantly high levels of IL-2 in patients, that newly diagnosed with *E histolytica* infection compared with the healthy control group, $P < 0.005$ as in table 2 and figure 2. These results were in agreement with Al-Oumashi *et al*, 2012 who, showed a significant increase in IL-2 in patient. Furthermore, our results is correspondent to Campbell et al, 1999 showed a significant elevation in level of IL-2 during *E histolytica* infection.

In addition, Sharma and Vohra, 2005 results concurred our results, since it revealed that, parasitic infection induced secretion of chemokines, cytokines and other inflammatory compounds are expressed in intestinal epithelium.

IL-8 also known as NAP-1 for Neutrophil-activating peptide is a chemoattractant protein for neutrophils. IL-8 is a pro-inflammatory mediator secreted by different cells such as monocytes, neutrophils, endothelial cells, fibroblast after activation via mitogen-stimulated T-lymphocytes (Bickel 1993). IL-8 is a key cytokine, which is expressed in scales of psoriasis patients, in synovial fluid of patients suffering from rheumatoid arthritis and gout (Diaz-Valencia et al 2015). The current study revealed a significant increase in IL-8 in patients diagnosed with *E histolytica* compared with the healthy control group, $P < 0.005$ as in Table 3 and figure 3.

Our results were coincident with YU et al, 1997 stated that tissue infiltration by neutrophils during the acute inflammatory response is dependent on the local release of soluble chemoattractants such as IL-8, we hypothesize that *E. histolytica* can cause the induction of IL-8 by colonic epithelial cells in the absence of cell-cell contact. The secretory response (IL-8 secretion) to *E. histolytica* may play a role in initiation of an acute inflammatory response before amebic invasion. (Dey and Chadee 2008). Based on the obtained results, it is possible to reach the following conclusions: IL-1 α , IL-2 and IL-8 levels in patient's revealed a significant increase in patient group in comparison to healthy control group.

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