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THE LEVEL OF IL-10, IL-17 AND S.IGA IN PATIENTS WITH *ENTAMOEBA HISTOLYTICA*

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ABSTRACT
The study was carried out during the period from the beginning of (November / 2013 - November / 2014) for detection of *Entamoeba histolytica* in patients with age range from (3-60) year who attended to AL-Yarmouk teaching hospital and AL-Tifil central hospital. The diagnosis done by microscopic examination. A total of 200 suspected patient there was120 infected with the parasite diagnosed by the direct examination method, a blood sample was taken from each one, as well as (60) healthy controls were involved. The study included the measurement of Interleukin -10, Interleukin-17 and Secretory- IgA (S.IgA) activities by Enzyme linked immuno sorbent assay (ELISA). The results indicated: The prevalence of *Entamoeba histolytica* by using microscopic examination was 145 (72.5%) in comparison to 120(82.75%) by using Triage (Micro parasite panel test). The level of Interleukin -10, Interleukin-17 and Secretory IgA increased significantly ($P<0.05$) in sera patients in comparison to healthy control, but there is no-significant ($P>0.05$) differences between the gender in both groups.

KEYWORDS: *Entamoeba histolytica* , IL-10 , IL-17 , S.IgA.

INTRODUCTION
Entamoeba histolytica is a protozoan parasite that causes amoebic dysentery and liver abscess. The disease is still one of the major health problems and predominantly affects individuals of lower socioeconomic status who live in developing countries.[1, 2, 3] Infections can be intestinal, extra intestinal, or both. Most cases are intestinal and asymptomatic. Symptoms, when occur, are multiple and varied, ranging from mild abdominal discomfort and diarrhea (often with blood and mucus) alternating with periods of remission or constipation, to severe illness with fever, chills, and significant bloody or mucoid diarrhea (”amoebic dysentery”). Amoebic colitis may be confused with inflammatory bowel disease such as ulcerative colitis.[4]

IL-10 is encoded by the IL-10 gene [5]. IL-10 signals through a receptor complex consisting of two IL-10 receptor-1 and two IL-10 receptor 2 proteins.[6] Cosequently, the functional receptor consists of four IL-10 receptor molecules. IL-10 binding induces STAT3 signalling via the phosphorylation of the cytoplasmic tails of IL-10 receptor 1 + IL-10 receptor 2 by JAK1 and Tyk2 respectively.[6] In humans, IL-10 is encoded by the IL10 gene, which is located on chromosome 1 and comprises 5 exons [5] and is primarily produced by monocytes and to a lesser extent lymphocytes, namely type 2 T helper cells (Th2), mastocytes, CD4+ CD25 + Foxp3+ regulatory T cells, and in a certain subset of activated T cells and B cells. IL-10 can be produced by monocytes upon PD-1 triggering in these cells[7]. IL-10 is released by cytotoxic T-cells to inhibit the action of NK cells during the immune response to viral infection.[5]

Interleukin 17 is a cytokine that acts as a potent mediator in delayed-type reactions by increasing chemokine production in various tissues to recruit monocytes and neutrophils to the site of inflammation, similar to Interferon gamma. IL-17 is produced by T-helper cells and is induced by IL–23 which results in destructive tissue damage in delayed-type reactions.[8] Interleukin -17 as a family functions as a pro inflammatory cytokine that responds to the invasion of the immune system by extracellular pathogens and induces destruction of the pathogen’s cellular matrix. Interleukin 17 acts synergistically with tumor necrosis factor and interleukin-1.[9] IgA is produced in mucosal linings than all other types of antibody combined.[10] Between three and five grams are secreted into the intestinal lumen each day.[11] This accumulates up to 15% of the total immunoglobulin produced in the entire body.[12]

IgA has two subclasses (IgA1 and IgA2) and can exist in a dimeric form called secretory IgA (sIgA). In its

secretory form, IgA is the mainimmunoglobulin found in mucous secretions, including tears, saliva, sweat, colostrums and secretions from the genitourinary tract, gastro intestinal tract, prostate and respiratory epithelium. It is also found in small amounts in blood. The secretory component of sIgA protects the immunoglobulin from being degraded by proteolytic enzymes, thus sIgA can survive in the harsh gastrointestinal tractenvironment and provide protection against microbes that multiply in body secretions.[13] S.IgA can also inhibit inflammatory effects of other immunoglobulins.[14]

MATERIALS AND METHODS

Studied groups

The study carried out during the period from (November 2013- November 2014), the age of patients extended from (3 – 60) years, two studied groups were involved:

- Suspected patients: Blood and stool samples were obtained from a total of 200 patients clinically suspected with amoebic dysentery that had been examined and defined as suspected cases by specialized physician; the samples were collected from (Al-Yarmouk teaching hospital & Al-Tifl central hospital) in Baghdad.

- Healthy Control: Blood and stool samples from a total 60 healthy control group were involved from Al-Yarmouk teaching hospital staff and from different places in Baghdad; they were examined and defined as healthy, with no history of amoebic dysentery.

Samples collection

Stool sample from each patient was collected in a clean, dry tight cover container and examined with a half an hour. The samples were examined for the presence of *E. histolytica.*

Stool sample examination

Macroscopic examination

It was performed by observing the consistency of stool, presence of blood, mucous and other substances.

Microscopic examination

For each stool sample, wet mount preparation slide was examined by clean, dry slides by obtaining one drop of normal saline and small amount of stool from different places of stool by using clean wooden stick, especially when blood or mucous were noticed, then mixed gently with normal saline and covered with cover slip, the slide was examined under the low (10x) and high power (40x) of microscope.[15]

Blood samples

Five mL of Venus blood was obtained from each patient and collected in sterilized screw cap plastic tube, blood samples were left for 30 min. at room temperature, then centrifuge at 3000 rpm for five minute, then the serum for each sample was collected in Eppendorf tubes and stored in deep freeze at -20c° until the time for using. The current study included some Immunological aspects: One hundred twenty clinical patients of *E.histolytica* and (60) healthy control involved in the study. The level of IL-10, IL-17 and S.IgA examined by Enzyme Linked Immunosorbant Assay (ELISA) according to.[16]

Statistical analysis

The statistical Analysis (T - test) was used to compare between means in studied groups according to.[17]

RESULTS AND DISCUSSION

Interleukin -10 (IL-10)

The level of IL-10 show significant difference ($P<0.05$) in patients and control, while the results showed non-significant difference ($P>0.05$) between the gender in both groups. The level was (491.93 ± 12.45) mg/mL, (460.20 ± 14.47) mg/mL in males and females of patients group respectively and (110.05 ± 13.33) mg/mL, (187.33 ±18.01) in males and females of control group respectively Table (1).

The result in line with [18] who found that the malnutrition decreases T-cell function and cytokines production, also the ability of lymphocytes, to respond appropriately to cytokines. Another study done by [19], which detected that IL-10 serum levels were significantly increased only in amoebic liver abscess patients, but not in other groups, the invasion of the liver tissue by *E.histolytica*elicits the anti-inflammatory immune response. IL-10 is capable of inhibiting synthesis of pro-inflammatory cytokines such as IFN-γ, IL-2, IL-3, TNFα and GM-CSF made by cells such as macrophages and regulatory T-cells. It also displays a potent ability to suppress the antigen-presentation capacity of antigen presenting cells; however, it is also stimulatory towards certain T cells (Th2) and mast cells and stimulates B cell maturation and antibody production.[20]

Table 1: The level of IL-10 (pg /mL) in serum of *E.histolytica* patients and healthy control

Group	Mean ± S.D Gender	
	Male	Female
Patients	491.93 ± 12.45	460.20 ± 14.47
Control	110.05 ± 13.33	187.33 ±18.01
T-test value	38.51 *	50.06 *

* ($P<0.05$)

Interleukin -17(IL-17)

The level of IL-17 in patients serum infected with *E.histolytica* showed significant increasing (P<0.05) in comparison with healthy control, while the results between the gender showed no significant difference (P>0.05) in both groups. The level was (326.66 ± 5.58) mg/mL, (266.93 ± 9.47) mg/mL in males and females of patients group respectively and (225.04 ± 10.88) mg/mL, (192.93 ±12.29) mg/mL in males and females of healthy control group respectively Table (2).

These results were agreed with another study [21] who found that persistent *E.histolytica* infection is associated with elevated levels of Th-17 cytokines. The present study is disagreeing with other study shows that IL-17 exhibited little effect during the infection with *E.histolytica*.[22] The mechanism of IL-17 mediated protection involves neutrophil recruiting to sites, regulation of dendritic cells function and Th1 responses through cytokines and chemokines induced by IL-17.[23,24]

Table 2: The level of IL-17 (pg /mL) in sera of *E.histolytica* patients and healthy control group

Group	Mean ± S.D Gender	
	Male	Female
Patients	326.66 ± 5.58	266.93 ± 9.47
Control	225.04 ± 10.88	192.93 ±12.29
T-test value	21.88 *	33.08 *

*(P<0.05)

Secretory- IgA

The level of secretory IgA showed significant increasing (P<0.05) in patients with *E.histolytica* and healthy control, while the results indicated no significant difference (P>0.05) in the gender of both groups. The level of secretory- IgA was (5.46 ± 0.03) mg/mL, (5.42 ± 0.03) mg/mL in males and females respectively of patients group and (3.34±0.04) mg/mL, (3.23±0.03) mg/mL in males and females respectively of healthy control group Table (3) & Figure (3).

These results were in agree with previous study denoted that the highest level of specific secretory anti-amoebic IgA were observed in the patients infected with intestinal amoebiasis and liver amoebic abscess [25], another study was done by [26, 27] which found that in the infection with *E.histolytica* antigen specific secretory immunoglobulin A (IgA) antibodies have been found to mediate protection against intestinal infection by *E.histolytica*. Secretory -IgA serves as lines of defense against microorganisms through a mechanism called immune exclusion; S.IgA adherence selectively to Microfold cell (M. cells) in intestinal Peyer's patches, thus mediating the trans epithelial transport of the Ab molecule from the intestinal lumen to underlying gut-associated organized lymphoid tissue.

In Peyer's patches, S.IgA bind and is internalized by dendritic cells in the sub- epithelial dome region. When used as carrier for antigens in oral immunization. S.IgA induces mucosal and systemic responses associated with production of anti-inflammatory cytokines and limits activation of dendritic cells.[28]

Table 3: The level of S.IgA (µg /mL) in sera of *E.histolytica* patients and healthy control group

Group	Mean ± S.D Gender	
	Male	Female
Patients	5.46 ± 0.03	5.42 ± 0.03
Control	3.34 ± 0.04	3.23 ±0.03
T-test value	0.109 *	0.115 *

*(P<0.05)

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