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Role of p-Selectin and Some Immunological Marker in Patient Infected with Amoebiasis.

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ABSTRACT

This study amid to evaluated of some biomarker such as p-selectin, IL-8andIgE in male infected with *Entamoeba histolytica*in compared with control group. From 30healthy male and 60 out patients stool samples were collected and diagnosis this parasite by using the wet amount microscope who have visited Al-Sadder teaching and Al-Hakeem Hospital in Al- Najaf Province during the period from August till march 2015.also serum sample collected from the same patients and control to estimated the IL-8, IgE and p-selectin. The current study detected significant decrease (P<0.05) in IL-8, IgE and p-selectinin *E. histolytica* infection patients in compared to control group**.**

Keywords: p-selectin, immunology, amoebiasis

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INTRODUCTION

Amebic diarrhea, colitis and amebic liver abscess that causes by a protozoan called *Entamoeba histolytica* parasite mostly in developing countries [1-3]. IL-8 has been important role in tissue injury and inflammatory bowel diseases example Crohn's disease and ulcerative colitis, its production from mucosal epithelial cells [4].

E. histolytica is a protozoan pathogen for human and may be can develop by using several of iron sources such as free iron, Lactferrin, transferrin, ferritin and hemoglobin, the infective stage of amoebiasis is mature cyst and transmitted by intake of contaminated water and foods through fecal-oral route, in the ileucecal the invasive stage or trophozoite is producing through excystation after the cysts are ingested and pass throughout the acidic pH of stomach[5].

Several host proteins example ferritin, Lactferrin, hemoglobin and transfer in may be used by *E. histolytica* trophozoites as source for iron because it is requires a high concentration of iron to survive[6].Interleukin-8is a potent chemokine or monocyte-derived neutrophil chemotactic factor causing neutrophils infiltration recruitment of T cells into local inflammatory sites in different localized diseases [7. In humans most iron is found within intracellular proteins and not soluble within the cell due to its toxicity[8].

Leukocytes are recruitment from the circulating to sites of localized inflammation via P-select in which up-regulated rapidly on the surface of inflamed vascular endothelial cells [9].

This study conducted to estimate the serum levels of P-select in, IL-8 and IgE in serum of patients infected with *E. histolytica* parasite.

MATERIAL AND METHODS

Specimens

From August till march, 2015, The collection of samples was approved by the institutional ethics committee of the faculty of science at the university of Kufa and all participants signed informed consent forms, stool samples collected in clean wide-mouth specimen bottles from 30 control male and 60 male patients who attended the clinics in'' AL-Sadder teaching and AL-Hakeem Hospital in AL-Najaf province'' and samples of blood from patients has been drawn into serum tubes and stayed for 30 min. at 25Cº. and centrifugation at 3000 rpm for 5 min.(Backman/counter, Germany**).** The serum preserved at -20Cºtill used for the determination of IL-8, IgE and p-selectin.

Specimen processing

According to [10] stool samples examined and processed by using X40 objective lens of light microscopy

Detection of human P-Selectin

Procedure

1- According to quantity to be tested sample and the standard, the quantity of the strips determined.
2- Set standard wells, test sample wells and blank wells
a-The Sample and Horseradish peroxide (HRP) do not add to blank wells.
B-for each standard wells 50 µl of standard solution was added.
C-40µl of sample diluent added to test sample wells and then 10µl of sample also added (The final sample dilution is five times and the final result calculation should be multiplied by five times).
d-Except blank well. Add to each well 50µ of horseradish peroxide (HRP). Then the plate is sealed and gently shake, then incubate for 60 minute at 37°C.
3- drying after eliminate the excess fluid from each well ,then filled with diluted washing liquid and mix up by shaking for thirty second , remove the washing liquid and then tap the plate into absorptive papers to dry .Repeat five time and then pat dry.

4- To each well added 50µl of chromogen solution A and then 50µl of chromogen solution B is also added, softly shake and incubated away from light for ten minutes at 37°C.
5-To stop the reaction 50µl of stop solution is added to each well (the color changes from blue to yellow).
6- At 450 nm wavelength, the optical density is measure after 15 minute from adding the stop solution.
7- By standard curve linear regression equation calculated the results, according to the corresponding optical density values and standards concentration.

Detection of IL-8

Procedure

1- According to quantity to be tested sample and the standard, the quantity of the strips determined.2- Set standard wells, test sample wells and blank wells
a-The Sample and Horseradish peroxide (HRP) do not add to blank wells.
B-for each standard wells 50 µl of standard solution was added.
C-40µl of sample diluent added to test sample wells and then 10µl of sample also added (The final sample dilution is five times and the final result calculation should be multiplied by five times).
d-Except blank well. Add to each well 50µ of horseradish peroxide (HRP). Then the plate is sealed and gently shake, then incubate for 60 minute at 37°C.
3-drying after eliminate the excess fluid from each well ,then filled with diluted washing liquid and mix up by shaking for thirty second , remove the washing liquid and then tap the plate into absorptive papers to dry .Repeat five time and then pat dry.
4- To each well added 50µl of chromogen solution A and then 50µl of chromogen solution B is also added, softly shake and incubated away from light for ten minutes at 37°C.
5-To stop the reaction 50µl of stop solution is added to each well (the color changes from blue to yellow).
6-Final measurement: set blank well zero. At 450 nm wavelength, the optical density is measure after 15 minute from adding the stop solution.
7- By standard curve linear regression equation calculated the results, according to the corresponding optical density values and standards concentration.

Detection of IgE

Procedure

1.Place into holder, the desired number of coated strips.
2.Into appropriate wells, pipette 25 µl of IgE standards, controls and samples.
3.To each well adding 100 µl of Biotin Reagent and shake the plate for 10-30 sec.
4.Incubate the plate after covering, for 30 minutes at room temperature (18-26°C).
5.Remove liquid from all wells. And using 300 µl of 1X wash buffer, wash wells. Blot on absorbance or towel paper.
6.100 µl of enzyme reagent add into each wells
7.Incubate the plate after covering, for 30 minutes at room temperature (18-26°C).
8.Remove liquid from all wells. And using 300 µl of 1X wash buffer, wash wells. Blot on absorbance or towel paper.
9.Add to each wells, 100 µl of TMB.
10. Incubate at room temperature for 15 minutes.
11. 50 µl of top solution are added to all wells. Shake the plate gently to mix the solution.
12. -ELISA Reader at 450 nm used to read absorbance within 15 minutes after adding stopping solution.

Statistical analysis

The software (Graph pad prism 5.04, USA) used to analyzed data of research, whereas (SE) as the mean ± standard error and one-way ANOVA used to appear the different between the patients and healthy groups. So significant difference as p-value < 0.05.

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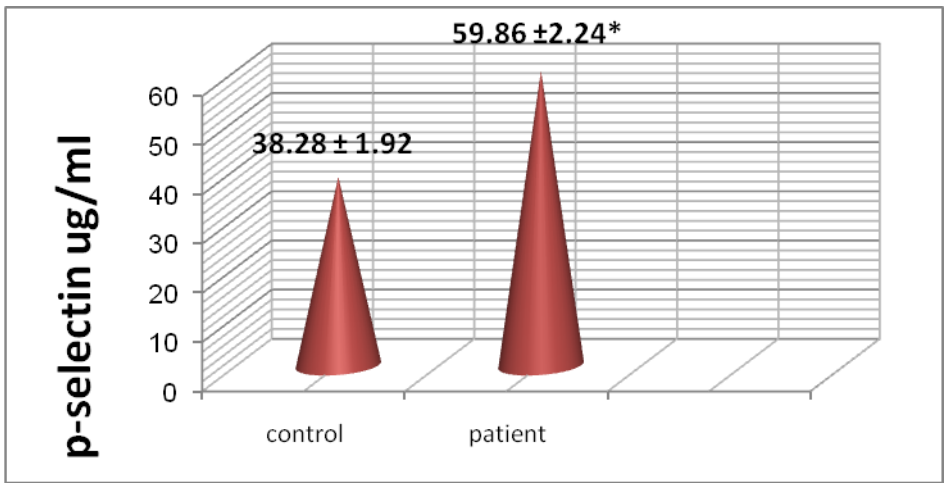
RESULTS

The current study revealed that concentration of p-selectin inpatients infection with *E. histolytica* was significant increase ($P< 0.05$) in male patients (59.86± 2.24) in compared with control group (38.28 ± 1.92), as seen in figure (1).

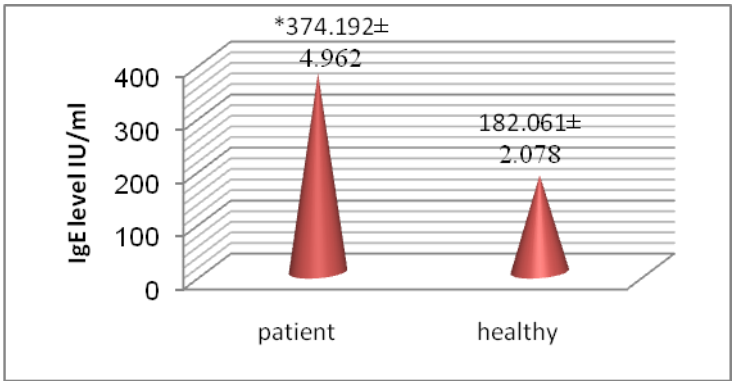


* Significant difference P< 0.05 between control group and patients

Figure 1: Concentration of p-select in (Ug/ml) Comparison between Patients Suffering from *Entamoeba histolytica* Infection and Control Group

The Immunoglobulin IgE in Patient and Control Group:

The results of present study as shown in figure (2) revealed that the concentration of immunoglobulin E was significant increase ($P< 0.05$) in male patients (374.192± 4.962IU/ml) compared with control group (182.061 ± 2.078IU/ml.



* Significant difference P< 0.05 between control group and patients

Figure 2: Concentration of IgE (IU/ml) Comparison between Patients Suffering from *Entamoeba histolytica* Infection and Control Group.

Interleukin – 8 (IL – 8):

Result of study revealed that concentration of (IL-8) in patients infected with *E histolytica* were significant increase ($P< 0.05$)(363.42 ± 9.09 pg /ml) in compared to the control group (245. 732 ±7.08 pg /ml) as seen in figure (3).

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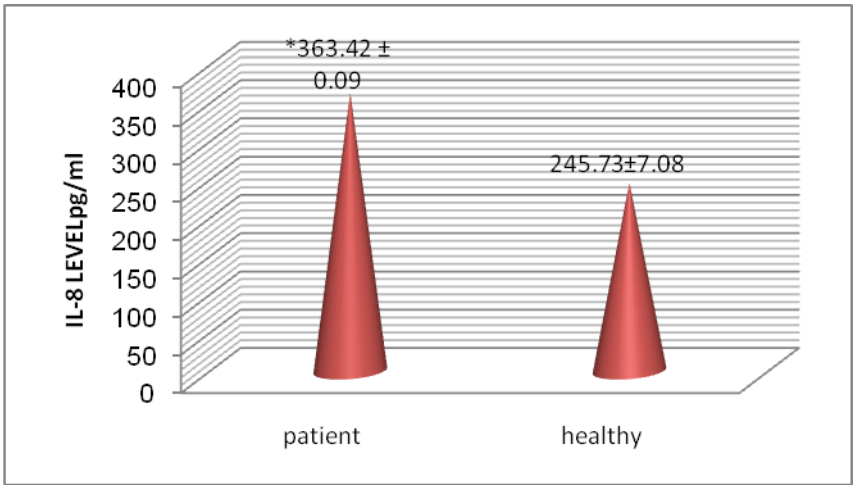

* Significant difference P< 0.05 between control group and patients

Figure 3: Concentration of IL-8 (pg/ml) Comparison between Patients Suffering from *Entamoeba histolytica* Infection and Control Group.

DISCUSSION

Study of results showed significant P< 0.05 increased of serum IL-8 and IgE in *Entamoeba histolytica* infection patients compared to healthy group. The increase in level of IL-8 may be due to cytotxity of parasite which caused stimulate produce the pro inflammatory cytokines (TNF, IL-1, IL-6 and IL-8)[11].In some inflammation cases such as inflammation caused by protozoan pathogen *E.histolytica* which occur by contact between trophozoite of this parasite and galactose-inhibitable amebic adherence protein and induced production of interleukin-8[12].Also may be due to induce the inflammation by invasive stage of the parasite to host and this case lead to Produce cellular cytokines such as IL- 8 , IL-1, TNF and IFN-gamma[13,14].

The increase in serum level of IgE may be due to increase the activation of eosinophils by increased the production of IL-5 and IL-4 which lead to increase the induction of IgE synthesis in patient infected with *E.histolytica* in compared with control group. which the eosinophilia is commonly visible in parasite infection , originally related to B cell, under the command of (IL-4 and IL-5), creating IgE in response to early revelation to an antigen or allergen, or due to increase in memory CD4 T cell by intestinal parasitic infections[15].In helminth and protozoan infection the height in serum levels of total IgE was reported in several studies, The increase total IgE level also caused by increased in secreted IL-13 which induce direct damage of the parasite through activation of the complement system, or increase IgE production[16].This results agrees with results of[17,18]. Which recorded that serum IgE high levels occur in tissue migration of larvae or harboring of parasites in tissues, IgE activates platelets and induces cytotoxic functions against parasites.

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