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SERUM LEVELS OF IL-6 & IMMUNOGLOBULIN'S (IGA. IGM .IGE) EXPRESSION IN HUMAN INFECTED WITH AMOEBIASIS

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ABSTRACT

In this work (70) serum samples of patients suffered from diarrhea and infected with *Entamoeba histolytica* and (30) non infected considered group control were examined to evaluate the IL-6 concentration by ELISA method also measured the concentration of IGA,IGM,IGE by Enzyme linked immune sorbent assay between two groups first group patients composed (60) child infected and second group composed (24) child non infected. Recent this study revealed that there was an increasing in concentration of the studied cytokine (IL-6) in patient serum (407.78) pg./ml in a comparison with control group with ahighly significant differences ($p< 0.05$). Moreover, the present results showed that mean concentration of IGE,IGA in patients with intestinal amoebiasis was more than those of control group with significant differences ($p<0.05$), while there was a significant differences in concentration of IGM between two groups, (Although there is an increasing in it`s concentration in some patients more than the normal values).The data revealed significant differences ($p<0.05$) in concentration two f IL-6 between two studied group. The immune system response to amoebiasis infection was detected through expression of interleukins such as il-6.

KEYWORDS: Amoebiasis , Immunity, cytokines, IgA, IgM, IgE.

INTRODUCTION

Entamoeba histolytica is the etiological agent of invasion amoebiasis and its the third leading parasitic cause of mortality in the whorld (Gaucher&Cadee 2003).It is estimated annually that about 480 million people develop clinical amoebiasis and at least die. (Walsh, 1988). *E.histolytica* infection appears to involve the initial attachment of amoebic trophozoite to intestinal epithelial cell, followed by lysis of these cell and subsequent invasion into the submucosa (seydel et al.2000). It is known that mucosal cell act as the first line of defence against pathogens trying to invade the human body. local immune mechanisms particulary secretory antibodies are thought to have acentral role in this defence (Tomasi,1983).

Amoebic antibodies, indicated of past or present invasive infection, were found in 1% of general hospital patient, 2% of random serum specimens and 4% of healthy school child in the America (Walsh ,2000). over 90% of individual infected with E.histolytica are asymptomatic. Human with amoebic liver abscess exihibt robust parasite–specific T-cell proliferation and amoebicidal INF-GAMMA production (salata et al. 1986). On the other hand the cell mediated immunity of Th1 immune response is of paramount important in host defense against *E. histolytica*.

At the intestinal level (Houpt et al.,2002) revealed that amoeba- specific mesenteric lymph node CD4+T-cell C3H amoebic colitis produced high levels of Th1 cytokines.

Yet fundamental questions of whether humoral especially (systemic) or cellular (Th1) immunity or both induced during the course of intestinal amoebiasis, so in this work atrying to measure aserum IL-6(which represent aTh1-cytokine) in patients with intestinal amoebiasis were made and determining the systemic humoral immunity by measuring the concentration of serum IgA, IgM. IgE.

MATERIAL AND METHODS

A-samples collection

Serum sample were collected from(100) out patients suffering from diarrhea and visited the thi-qar hospitals and infected with *Entamoeba histolytica* (patients group) and (24) healthy person and not infected with the same parasite (control group). Age of studied groups ranged (2-10) years. Infection was assessed by the examination of the stool samples by using direct wet film preparation which included.

1.Normal saline preparation (paron et al.1994).

2.Lougols iodine preparation (John &Petri-jr.,2006).

B-Serum IL-6 measurement
IL-6 concentrations were asses by enzyme linked immune sorbent assay (ELISA) using akit from (Immunotech-elb science company, America)

C- Serum immunoglobulines determination
Serum IGA,IGE,IGM were determined byEnzyme linked immunosorbent assay (Elisa) which done by using kit produced (Alab science –company – America).

D-/ Statistical analysis
the data analyzed by using analysis of variance test(T).

Detection of Interleukin-6(IL-6) by Elisa method
Following the instructions of il-6 cytokine kit, catalog number E-EL-H0102, frozen samples serum were brought to room temperature and mixed thoroughly with out foaming. One hundred ml of assay diluent for il-6 was added to each well, 100 ml of control and samples were added to each per well.The covered plate with afresh sealer was incubated for 90 minutes at room temperature 37c and shaker at 5000 rpm. The plate was washed by wash buffer (350) into each well then the content of well was aspirated (washing was reapted three times). 100 ml of interleukin conjugate was added to each well. The covered plate was incubated for 30 minutes at room temperature 37c and shaker at 5000 rpm then washed as In up step five time, 90 ml of substrate solution was added, the covered plate was incubated for 15 minutes at 37c. Then stop solution was added (50ml) to each well ,the absorbence of each well was recorded by using elisa microplate reader at 490 nm.

Detection of Immunoglobulin's (IgA,IgM,IgE) by Elisa.
The kit on(IgA. IgM. IgE) depended linked immune sorbent assay (Elisa), which is quantitative sandwich immune assay allows good determination for concentration of (IgA,IgM,IgE) in the sample, following the instruction of biological (IgA.IgM.IgE) kit Elab science-America. catalog NO(96T-1814). Recommended that all samples and slandard be assay in duplicate .Added 100 ml of standard and sample per well and cover with plate seasle incubate for 90 minutes at 37c.Remove the liquid of each well but don't wash it.add 100ml of biotin anti body to each well cover with plate sealer provided incubate for 2 h at 37c. Aspirate each well and wash it repeating the process four time for a total four washes. wash by filling each well with buffer 350ml using as asqurit bottle multi channel pipette ,mani fold dispenser or outo washer and let is stand for 3 minutes ,complete removal of liquid. Add 50 ml of HRB to each well cover with the plate sealer incubate for 45 m at 37c. Reapet the aspiration was process for five times. Add 90 ml of TMB substrate solution to each well incubate for 30 m at 37c. Add 50 ml of stop solution to each well, gently tap the plate to ensure through mixing. This allows the operator to produce a standard curve of optical density versus (IGA,IGM,IGE) concentration (mg/ml) The concentration of these immunoglobulines in the samples was then determined by standered curve and straight line equation.

RESULTS
Concentration of il-6 in serum
Elisa results documented that there was ahighly significant differences ($p<0.05$) in concentration of il -6 between patient s(407.78)pg/ml and control group (286.57) pg/ml (table 1).

Table (1): concentration of il-6 pg/ml in sera of patients infected with Entamoeba histolytica and control group.

Groups	No of samples	Mea Pg/ml	S.D	Df	T-value	p-value
Patients	70	407.78	138.42	98	1.654	0.01
Control	30	286.57	57.855			

SD=standarddeviation
df=degreefredom

Serum concentration of immunoglobulin's
Serum concentration of immunoglobulin's by enzyme linked immune sorbent assay work of sera from infected and control groups significant differences ($p< 0.05$) in concentration of IGE between two groups (290.33) mg/100 ml, respectively (Table 2).

Table (2): concentration of IGE (mg/100ml) in sera of patients with Entamoeba histolytica and control group.

Groups	No of samples	Mean	S.D+_	D.F	T-value	p-value
Patients	60	290.33	134.5	82	8.954	90.87
Control	24	1.568	2.994			

The present data represented in table (3) showed the singnificant differences ($p<0.05$) in mean concentration of IGA between patient (290.33) mg/ml and control group (1.568) mg/ml.

Table (3): concentration of IGA (mg/ml) in sera of patients infected with entamoeba histolytica and control group.

Groups	No of samples	Mean	S.D+_	Df	T-value	p-value
Patients	60	185.33	49.22	82	-927-	0.01
Control	24	11.88	1.014			

In the cases of IGM results demonsrated high siginifcant in concentration of immunoglobulin of (60) infected person (613.44) mg/ ml and (24) non infected (3.9213) mg/ml (Table 4).

Table (4): concentration of IGM (mg/ml) in sera of patients infected with entamoeba histolytica and control group .

Groups	No of samples	Mean	S.D+_	D.F	T-value	p-value
Patients	60	613.44	107.250	82	9.631	34.143
Control	24	3.9213				

DISCUSSION

The immune and inflammatory response have acentral role against *Entamoeba histolytica* there by i the cytotoxic activity of the immune cells and their ability to produce awide range of cytokine which play acrucial role in the defence like interferon gamma, tumor necrosis factor al-pha and interleukins(ILS) which recruit the inflammatory process against parasite (Yamamoto et al., 1993 and paul, 1999).

The serum concentration of one of TH2 cytokine or IL-6 in patients with intestinal amoebiasis was evaluated, the results indicated that most of patients have ahigh concentration in their sera in ac comparsion with the control group and these results were aslso indicated by (Campbell & chadee., 1997; Sanchez–Guillen et al., 2002).

IL-6 represented as one of the cytokines which produced by (TH2) and act as a cofactor in activation of humoral immunity by activation of B-cells and T-cells proliferation and differentiation (Talamas-Rohan Mosmann & sad ,2000).

In addition to the role of cellular immune respons which document by increasing of serum IL-6 which recorded in the present study, the humoral immune response was also studied, the recent work demonstrated that IgE in sera of infected person was more than of control group ,and these results were also astablished by (Arinapour & Mohapatra 2003) whom indicated the increasing of IgE concentration of this immunoglobulin in patients with amoebic liver abscess and intestinal amoebiasis. The most accept able explanation for these increasing in IgE may be due to increasing of IL-6 concentration which help in secreting of this immunuoglobulin (Hoffman et al .,2000 ; Ximene et al., 1990) also recorded that in patient s with intestinal amobiasis the anti amoebial IgE increasing four times in comparsion with the control group .(Kaur et al., 2004) noticed that the increasing of the IgE isotype associated with stage of amoebiasis infection whereas(Haque et al. 2006) indicated that anti -amoebal IgA confer protection against repeated infection although its concentration elevated in serum.

(SRID) results also showed that the patients had a high concentration of IGA when compared with control group and these results identical with the results of (AL-Kubassi, 2002) and this may be attributed to the fact that the IGA had an important activity in Humoral immunity against parasite which part icipating in reducing the infection and preventing the repeated infection (Haque et al.,2003).

Generally the elevated level of IGE and IGA which document in patient of the recent study may be due to the systemic sensitization of B-Cells (Valenzuela et al.,2001).

IgM is recorded asignificant differences between two studied groups and this was also reported by (AL-kubassi., 2002; Arinapour & Muhapatra., 2003), the latest worker showed that IgM increases in patient with hepatic liver abscess and patients with intestinal amoebiasis, thepositive results of IGM may be due to the fact that the patients of present study is in different stage of infestation (acute or chronic) (Valenzuela et al .,2001). Finally both humoral and cell mediated immune response have been observed in patients with intestinal amoebiasis. and other infection.

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