

Serum levels of IL – 4 , Immunoglobulines and complements during human amoebiasis

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IL -4

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				60	
		IL -4			<i>Entamoeba histolytica</i>
	C4	C3	IgM	IgA	IgG
IL -4)				/	(29 -12) (
	IgA	IgG			(P < 0.01)
		IgM			(P< 0.05)
		C3		(P< 0.05)	
		C4			

Abstract

In this work (60) serum samples of patients suffered from diarrhea and infected with *Entamoeba histolytica* were examined to evaluate the IL – 4 concentration by ELISA method and the concentration of IgG , IgA , IgM , C3 and C4 by single radial immunodiffusion . Recent study revealed that there was an increasing in concentration of the studied cytokine (IL – 4) in patients serum (29.11) pg/ml in comparasion with control group with a highly significant differences (p < 0.01) . Moreover , the present results showed that the mean concentration of IgG and IgA in patients with

intestinal amoebiasis was more than those of control group with significant differences ($p < 0.05$), while there was no significant differences in concentration of IgM between two studied 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 of C3 between two analyzed group . whereas a highly significant differences ($p < 0.01$) in concentration of C4 was established between patients and control groups .

Introduction

Entamoeba histolytica is the iteological agent of invasion amoebiasis and it is the third leading parasitic cause of mortality in the world (Gaucher & Chadee , 2003) . It is estimated annually that about 480 million people develop clinical amoebiasis and at least 40.000 die . (Walsh , 1988) .

E. histolytica infection appears to involve the initial attachment of amoebic trophozoites to intestinal epithelial cells , followed by lysis of these cells and subsequent invasion into the submucosa (Seydel *et al.* 1997) . It is known that mucosal cells act as the first line of defence against pathogens trying to invade the human body . Local immune mechanisms , particularly secretory antibodies are thought to have a central role in this defence (Tomasi , 1983) .

Amoebic antibodies , indicated of past or present invasive infection , were found in 1 % of general hospital patients , 2 % of random serum specimens and 4 % of healthy military recruits in the United States (Walsh , 1986) . Over 90 % of individual infected with *E. histolytica* are asymptomatic .

Human with amoebic liver abscess exhibit robust parasite – specific T – cell proliferation and amoebicidal IFN – γ production (Salata *et al.* 1986) . On the other hand the cell mediated immunity of Th1 immune response is of paramount importance in host defence and resistance to invasive *E. histolytica* infection . Campbell and Chadee (1997) established that Th1 cytokines are important in host defence against *E. histolytica* in the gerbil model of amoebic liver abscess .

At the intestinal level , Houpt *et al.* (2002) revealed that amoeba – specific mesenteric lymph node CD4⁺ T – cell from C3H / HeJ mice with amoebic colitis produced high levels of Th2 cytokines .

Yet fundamental questions of whether humoral especially (systemic) or cellular (Th2) immunity or both induced during the course of intestinal amoebiasis , so in this work a trying to measure a serum IL – 4 (which represent a Th2 – cytokine) in patients with intestinal amoebiasis

were made and determining the systemic humoral immunity by measuring the concentration of serum IgG , IgA , IgM , C3 and C4 .

Materials and Methods

a - / Samples collection :

Serum samples were collected from (60) out patients suffering from diarrhea and visited the Basrah hospitals and infected with *E. histolytica* (patients group) and (24) healthy person and not infected with the same parasite (control group) . Age of studied groups ranged from 10 - 20 years . Infection was assessed by the examination of the stool samples by using direct wet film preparation which included :-

- 1- Normal saline preparation (Baron *et al.* 1994) .
- 2- Lugols iodine preparation (Jhon & Petri – Jr. , 2006) .

b - / Serum IL- 4 measurement :

IL-4 concentrations were assayed by enzyme linked immunosorbent assay (ELISA) using a kit from (Immunotech – ABekman Coulter Company , France) .

C-/ Serum immunoglobulines and complements determination :

Serum IgG , IgA , IgM , C3 and C4 were determined by single radial immunodiffusion (SRID) which done by using Biomaghreb kit produced by Tunis .

d - / Statistical analysis :

The data analyzed by using analysis of variance test (ANOVA) .

Results

1 – Concentration of IL – 4 in serum .

ELISA results documented that there was a highly significant difference ($p < 0.01$) in concentration of IL – 4 between patients (29.11) pg/ml and control group (25.86) pg/ml (Table 1) .

Table (1) : Concentration of IL – 4 pg/ml in sera of patients infected with *E. histolytica* and control group

Group	Number of samples	Range	Mean Pg/ml	SD. ±	S.E.
Patients	60	22.98-59.99	29.11	5.22	0.67
Control	24	19.34-30.28	25.86	3.32	0.74

SD. = Standard deviation S.E. = Standard error

2 – Serum concentration of immunoglobulines and complements .

Single radial immunodiffusion work of sera from infected and control groups revealed significant differences ($p < 0.05$) in concentration

of IgG between two groups (1212.41) mg/100ml , (744.49) mg/100ml respectively (Table 2) .

Table (2) : Concentration of IgG (mg/100ml) in sera of patients infected with *E. histolytica* and control group

Group	Number of samples	Range	Mean mg/100ml	SD. ±	S.E.
Patients	60	2270.6-403.1	1212.41	456.61	58.95
Control	24	1342.2-291.7	744.49	315.74	64.45

N.V. = Normal value = (600 – 1650) mg / 100ml

The present data represented in table (3) showed the same significant differences ($p < 0.05$) in mean concentration of IgA between patients (205.31) mg/100ml and control group (97.92) mg/100ml (Table 3) .

Table (3) : Concentration of IgA (mg/100ml) in sera of patients infected with *E. histolytica* and control group

Group	Number of samples	Range	Mean mg/100ml	SD. ±	S.E.
Patients	60	66-817	205.31	107.51	13.88
Control	24	16.6-222.4	97.92	60.32	12.31

N.V. = (90 – 400) mg / 100ml

In the case of IgM results demonstrated no significant differences in concentration of this immunoglobuline of (60) infected person (144.46) mg/100ml and (12) non infected one (115.55) mg/100ml (Table 4) .

Table (4) : Concentration of IgM (mg/100ml) in sera of patients infected with *E. histolytica* and control group

Group	Number of samples	Range	Mean mg/100ml	SD. ±	S.E.
Patients	60	51.3-245.9	144.46	48.03	6.20
Control	12	55.6-1779	115.55	36.77	10.61

N.V. = (40 – 300) mg / 100ml

Statistical differences was recorded in mean concentration in sera of C3 in patients (136.83) mg/100ml when compared with it's mean concentration in sera of control group (84.73) mg/100ml with significant differences ($p < 0.05$) (Table 5) .

Table (5) : Concentration of C3 (mg/100ml) in sera of patients infected with *E. histolytica* and control group

Group	Number of samples	Range	Mean mg/100ml	SD. ±	S.E.
Patients	60	45.9-247.7	136.83	38.98	5.03
Control	24	29.5-151	84.73	35.00	7.14

N.V. = (80 – 160) mg / 100ml

In the case of C4 a highly significant differences ($p < 0.01$) were recorded between patients (32.65) mg/100ml and control group (21.17) mg/100ml (Table 6) .

Table (6) : Concentration of C4 (mg/100ml) in sera of patients infected with *E. histolytica* and control group

Group	Number of samples	Range	Mean mg/100ml	SD. ±	S.E.
Patients	60	13-57.7	32.65	10.22	1.32
Control	24	6.9-38.3	21.17	9.27	1.89

N.V. = (20 – 40) mg / 100ml

Discussion

The immune and inflammatory responses have a central role against *E. histolytica* infection thereby the cytotoxic activity of the immune cells and their ability to produce a wide rang of cytokines which play a crucial role in the defence like interferon gamma $IFN-\gamma$, tumor necrosis factor alpha $TNF-\alpha$ 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-4 in patients with intestinal amoebiasis was evaluated , The results indicated that most of patients have a high concentration of IL-4 in their sera in comparison with the control group and these results were also indicated by Campbell & Chadee (1997) and Sanchez – Guillen *et al.*(2002) .

IL-4 represented as one of the cytokines which produced by Th2 cells and act as a cofactor in activation of humoral immunity by activation of B – cells and T – cells proliferation and differentiation (Talamas – Rohana *et al.* , 1995 and Mosmann & Sad , 1998) .

In addition to the role of cellular immune response which documented by increasing of serum IL-4 concentration which recorded in the present study , the humoral immune response was also studied , the recent work demonstrated that IgG level in sera of infected person was more than of control group , and these results were also established by Arinapour & Mohapatra (2003) whom indicated the increasing in IgG concentration

of this immunoglobulin in patients with amoebic liver abscess and intestinal amoebiasis . The most acceptable explanation for these increasing in IgG may be due to the increasing of IL-4 concentration which help in secreting of this immunoglobulin (Hoffman *et al.* , 2000) . Ximenez *et al.* (1990) also recorded that in patients with intestinal amoebiasis the anti – amoebal IgG increasing four times in comparison with the control group . Kaur *et al.* (2004) noticed that the increasing of the IgG isotype associated with stage of amoebiasis infection whereas Haque *et al.* (2006) indicated that anti – amoebal IgG not 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 participating in reducing the infection and preventing the repeated infection (Haque *et al.* , 2003) .

Generally the elevated levels of IgG and IgA which documented in patients of the recent study may be due to the systemic sensitization of B – cells (Valenzuela *et al.* , 2001) .

IgM not recorded any significant differences between two studied groups and this was also reported by Al – Kubassi (2002) and Arinapour & Muhapatra (2003) , the latest worker showed that the serum IgM increases in patients with hepatic liver abscess but not in patients with intestinal amoebiasis , the negative results of IgM may be due to the fact that the patients of present study is in a different stage of infection (acute or chronic) (Valenzuela *et al.* , 2001) .

The increase in the concentration of C3 and C4 may be because the *E. histolytica* activate the complement system (Reed *et al.* , 1986 and Reed *et al.* , 1995) .

Gaucher & Chadee (2003) indicated that galactose / galactose N – acetyl cholin Gal / Gal NAC lectin of amoeba can activate the membrane attack complex of complement (C5b – C9)

Finally , both humoral and cell mediated immune responses have been observed in patients with intestinal amoebiasis .

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