IL-5 As Indicator of Acute Toxoplasmosis

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ABSTRACT:

BACKGROUND:
Exposure to *Toxoplasma gondii* may result in either chronic infection or acute infection, the latter being asymptomatic or symptomatic. *Toxoplasma* infection stimulates humoral immune response ,in addition to cellular mediated immunity. Also cytokines have a role in resistance to *T.gondii* like IL-5 which has protective role during infection with the parasite.

OBJECTIVE:
This study aimed to assess the correlation between IgM and IgG *Toxoplasma* antibodies and IL-5 in the course of acute toxoplasmosis .

PATIENTS AND METHODS :
45women suspected of having toxoplasmosis were tested by ELISA IgM and IgG *toxoplasma* antibodies , and the level of serum IL-5 was tested using ELISA technique only on 32 women .

RESULTS:
The results showed that there are higher titer of IgM and IgG-*Toxoplasma* antibodies and higher levels of serum IL-5 in women with *Toxoplasma* infection .

CONCLUSION:
Serum IL-5 can be used as indicator to predict the presence of acute toxoplasmosis in pregnant women at high risk to toxoplasmosis.

KEY WORDS: toxoplasmosis, IgG, IgM, IL-5.

INTRODUCTION:

T. gondii is an important human pathogenic parasite, that posses an important public health problem and is widely distributed even in human population of developed countries[4]. It was found in high prevalence in Iraq , especially in the rural areas, due to the habits in favour of acquiring the disease [5].*Toxoplasma* infection during pregnancy may lead to severe if not fatal infection of the fetus [6,7,8] . Toxoplasmosis infection stimulates humoral immune response and cell mediated immunity , in addition to the role of cytokines [5].

T.gondii is strongly stimulates type1 cytokines during infection , that are induced early during *T.gondii* infection , that will induce abortion early in pregnancy [5,9,10]. Toxoplasmosis infection stimulates humoral immune response as antibody production , in addition to cell mediated immunity ,which is essential for the host and control of intracellular infection [1]. Also , IL-5 was investigated in mice infected with *T.gondii* [2]which was believed that IL-5 have a role during the early stage of oral infection with *T.gondii* [2] and it is suggested that IL-5 have a protective role against *T.gondii* infection and may play a role in the production of IL-12[3] .

MATERIALS AND METHODS:

Blood samples were collected from 45 women suspected of having toxoplasmosis during , the period from May 2008 to November 2009 , that attending Baghdad Teaching Hospital and AL-Zahraa Teaching Hospital, in Baghdad .All sera were investigated for the presence of IgM and IgG–*Toxoplasma* antibodies, because of limited resources serum IL-5 was measured only on 32 women by ELISA –kit. The kits that were used : ELISA –kit for detection of IgM and IgG –*Toxoplasma* antibodies provided by BioCheck, Inc. Foster city, USA. Serum IL-5 was detected by ELISA provided by Invitrogen / Belgium. Final diagnosis was based on a positive results of real-time PCR assays and /or positive of both *recom*Line assays and IgM –*Toxoplasma* antibodies detected by ELISA (unpublished data).

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Statistical analysis:
Statistical analysis were computer assisted using Statistical Package for Social Science (SPSS) version 13 .Frequency distribution for selected variables was done first . All outcome quantitative variables with exception of age were non normally distributed, and were thus conveniently described by median and inter-quartile range and non – parametric statistical tests of significance were used.

RESULTS :

To investigate the correlations between serum titer of IgM and IgG–*Toxoplasma* antibodies and serum levels of IL-5 , which can be used as an indicator of the stage of *Toxoplasma* infection Table(1) shows the median values of the three parameters (IgM, IgG, and IL-5) in women with final diagnosis.

Table 1: The median values of selected parameters in subjects with established diagnosis of toxoplasmosis and those negative for such a diagnosis.

	Final diagnosis of Toxoplasmosis		
Antibody titer	Negative	Positive	P
IgM *Toxoplasma* antibody titer			<0.001[S]
Range	(0.156 - 2.9)	(0.9 - 2)	
Median	0.381	1.084	
Interquartile range	(0.222 - 0.623)	(1 - 1.289)	
No.	33	12	
IgG *Toxoplasma* antibody titer			0.24[NS]
Range	(0.005 - 2.654)	(0.317 - 18.1)	
Median	0.8	1.075	
Interquartile range	(0.381 - 2.187)	(0.843 - 2.237)	
No.	33	12	
Serum IL5 pg/ml			0.001[S]
Range	(4 - 300)	(16 - 90)	
Median	7.5	62	
Interquartile range	(4.3 - 22.3)	(50 - 77.5)	
No.	20	12	

The median values of IgM –*Toxoplasma*-antibodies titer was statistically significant it is higher in the group of women with positive diagnosis (1.084), compared to those with negative diagnosis of toxoplasmosis which was 0.381, there were statistically significant differences between the two groups (P<0.001) . The median value of IgG was 1.075 among women with positive diagnosis of toxoplasmosis, while in women with negative diagnosis the median values was 0.8, there was no statistically significant differences between the two groups (P=0.24) . The median value of IL-5 levels was 62 in women that had toxoplasmosis , while in women that do not had toxoplasmosis was 7.5.

There was statistically significant differences in the median values of serum IL-5 between the two groups (P=0.001) .Table (2) shows the Receiver Operating Characteristic (ROC)analysis that was done to each parameter in the present study , which was useful in evaluating the ability of continuous marker in discriminating between two states of binary out-come such as diseased /not diseased. The resulting ROC curve and its functional such as the Area Under the Curve(AUC), have simple analytical forms, which provide the best working parameter for diagnosis the more valid the test.

Table 2: The ROC (Receiver Operating Characteristic) analysis area for selected parameters when used to diagnose toxoplasmosis

	Area	P
IgM- *Toxoplasma* antibodiy titer by ELISA	0.889	<0.001[S]
Serum IL5	0.86	0.001[S]
IgG- *Toxoplasma* antibodiy titer by ELISA	0.616	0.24[NS

The values ROC areas of IgM-*Toxoplasma* antibodies and serum IL-5 were statistically significant differ from the value of ROC area of IgG- *Toxoplasma* antibodies. Since, the value of ROC-area of IgM –*Toxoplasma* antibodies was 0.889 , which was statistically significant (P<0.001). Also, the value of ROC of IL-5 was 0.86, which was statistically significant (P=0.001). Serum IgG *Toxoplasma* antibodies had ROC value 0.616 that was not statistically significant(P=0.24) . On the other hand , Table (3) shows the median value of the parameters (IL-5 and IgM) in chronic toxoplasmosis.

Table 3: The median values of IL-5 and IgM in women with and without chronic toxoplasmosis

	IgG *Toxoplasma* antibody test (Chronic reaction) by ELISA		
	Negative	Positive	P
Serum IL5			0.46[NS]
Range	(4 - 1200)	(3 - 300)	
Median	16	16	
Interquartile range	(5 - 56.5)	(4 - 46)	
No.	37	40	
r=-0.085 P=0.46[NS]			
IgM *Toxoplasma* antibodies titer			0.02
Range	(0.096 - 2.9)	(0.153 - 2)	
Median	0.298	0.394	
Interquartile range	(0.211 - 0.48)	(0.254 - 0.645)	
No.	79	51	

r : linear correlation factor

Table 4: Linear correlation between IL-5 and IgM- *Toxoplasma* antibodies

	IL-5	
IgM-*Toxoplasma* Abs.(acute reaction)	r=0.44	P<0.001

From the data presented in Table (3), the median values of IL-5 were the same value, that was 16 in the two groups of women that had/ and those did not had toxoplasmosis .There was no statistically significant linear correlation between serum IL-5 and IgG –*Toxoplasma* antibodies (r=0.085, P=0.46).The median values of IgM –*Toxoplasma* antibodies was 0.394 in women that had chronic toxoplasmosis ,while in that did not had chronic toxoplasmosis the median value was 0.298, there was statistically significant differences between the median values of the two parameters (P=0.02). There was no statistically significant correlation between IgM –*Toxoplasma* antibodies and IgG –*Toxoplasma* antibodies (r =0.156 , P=0.08) .While Table(4) shows that there was statistically significant linear correlation between IgM *Toxoplasma* –antibodies and serum IL-5(r=0.441 , P<0.001).

DISCUSSION :

The results of the present study shows that the median value of IgM-*Toxoplasma* antibodies in women with toxoplasmosis that was 1.084 , while in women that did not had toxoplasmosis the value was 0.381, there was statistically significant differences between the two groups (P=0.001) . The results reflect that the first group of women had acute toxoplasmosis . ELISA technique for detection of IgM of *T. gondii* must be used within *Toxoplasma* serologic profile that include : Sabine-Feldman dye test , IgM-ELISA, IgA-ELISA and differential agglutination test , IgE-ELISA, IgE immunosorbent agglutination assay[11], which are routinely used to assess whether infection was

acquired during pregnancy, and the patients' physician should be contacted to provide interpretation of the results and to discuss management of both the patient and her fetus. When serologic results suggest recent infection treatment with spiramycin is recommended [12,13,14]. Also , the median value of IgG –*Toxoplasma* antibodies was 1.075 in women with toxoplasmosis while the value was 0.8 in women that did not had toxoplasmosis , since the diagnosis of *T.gondii* infection is most commonly made by detecting IgG and IgM antibodies in the blood ,however these tests can estimate the time of infection precisely enough to properly manage the risk to the fetus of maternal infection[18,13,1].The serologic tests are of limited effect in detecting acute infection , because peak concentrations of specific immunoglobulins are found week after parasitemia has subsided . Furthermore reactivation of the disorder in infected individuals and immunocompromised patients is rarely accompanied by significant –titer rise[18] . The results presented in Table (1) shows that the median value of IL-5 was higher (62) in women infected with *T.gondii* than in women that did not had toxoplasmosis that was only 7.5 .These results were compatible with that recorded by Zhang and Denkers [3] who found that mice deficient for IL-5 production displayed increased susceptibility to the parasite by elevated parasite numbers within the brain and increased weight loss during infection. On the other hand, the results of the present study ,were not in accordance with that reported by Nickdel *et al.* [2] who found that IL-5 gene deficient mice infected with *T.gondii* were less susceptible to infection than wild type. This leading to believe to the detrimental role of IL-5 during early phase of oral infection with *T.gondii* which is associated with increased small intestine pathology ,eosinophilia and reduced IL-12 and INF-γ [2].The result shows that there was statistically positive correlation between IgM *Toxoplasma* antibodies and serum IL-5 (r=0.441 , P< 0.001) this explain the protective role of IL-5 during the early stage of oral infection with *T.gondii* ,and may play a role in the production of IL-12 production[2] . The results shows also that there was negative correlation between IgG-*Toxoplasma* antibodies in women with /without toxoplasmosis and serum IL-5(r=0.0085 , p=0.46) ,which was not statistically significant. The median values of IL-5 was 16 in the two groups of women with and without chronic toxoplasmosis , the difference between the two groups was 0.46 which was not statistically significant , suggests the role of IL-5 only during acute reaction of *T. gondii* [2] .

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