Evaluation of Interferon –Gamma Concentration on Toxoplasmosis Women With a History of Abortion

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Abstract

Seventy-two women were included in this study with a history of single or repeated abortion,wich was referred to by the physician for the detection of anti Toxoplasma antibodies,and 26 healthy-looking controls .Venous blood was collected from these women,and serum was obtained for the performance of the ELISAtest for the detection of anti-Toxoplasma IgG and IgM.

Fifty –eight (80.6%) women displayed the presence of anti Toxoplasma antibodies. Thirteen (31.7%) had IgG antibodies ,28 (68.3%)had IgG and 17 (29.3%) had both IgM and IgM. This result indicates the high prevalence of this disase among women with abortion (80.6%) .this may be due to the wide sources infection .

The estimation of the levels of cytokines IFN- γ was also done by using the ELISA technique.there was a significant incrase in their levels in comparison with the control.this indicates that there is a high cellular immune reaction during the diseace.

Introduction

Toxoplasmosis is a classic Zoonosis. A wide range of vertebrate animals serve as hosts and while human infections are common, serious complication occur primarily in immunocompromised hosts. Human infections are caused by accidental ingestion of oocysts, shed in to environment by cast,or tissue cysts contained in undercooked meat. Infections in healthy adults are generally benign , although

toxollamic retinitis is frequently a cause of serious eye disease in otherwise healthy adults (1). More profound disease occurs in immunocompromised hosts (2) or as the result of congenital infections (3). Primery infection during pregnancy can result in abortion or fetal defects (4) ,toxoplasma infection stimulates both the humoral and cell-mediated immunity wich are essential for host control of intrea-cellular infection (5).the acute phase of infection is marked by elevated levels of IFN-γ and IL-12 as well as other pro inflammatory cytokines such as TNFα ,granulocyte macrophage colony stimulating factor,IL-6and IL-1. in addition, neutrophils,like macrophages, are capable of producing both pro-inflammatory and anti-infammatory cytokines during early infection (6).cell-mediated immune responses involving $CD4^+$ and $CD8^+$ T cells and NK cells play a protective volein in T. gondii primary infection (7). Interferon-γ (IFN-γ) and $cd8^+$ T-cell have been shown to play crucial roles in protective immunity against T. gondii in both acute and chronic infection. Studies have indicated that IFN-γ can exert protection against T. gondii by a non-independent mechanism .this alternative mechanism wold be mediated by the induction of antigen-specific $CD8^+$ cytotoxic T lymphocyte (CTL), because IFN-γ up regulates the major histocompatibility complex (MHC) class 1 expression on antigen-presenting cell (APC). Leading to stimulation of $CD8^+$ T cells (8,9). Both $CD4^+$ and $CD8^+$ cytotoxic T lymphocytes are part of the human response to T.gondii infection in murine models of T .gondii, $CD8^+$ cells play a pivotal role in defencs against acute infection and control of chronic infection (10).

Materials and Methods

Seventy-two women who had abortion were selected for this study. They were referred to different hospitals and private laboratories around Baghdad ,indicating the possibility of having toxoplasmosis by the physician. Venous blood was collected from those women for serum collection during the period between August 2004 and January 2005. The serum samples were divied into three groups according to the presence or absence of specific anti-Toxoplasma antibodies :-

The group included (28) serum samples containing IgG , another group included (13) serum samples containing IgM and Group

included (17) serum samples IgG and IgM giving atotal of 58. the other 14 patients were excluded from this study due to the absence of specific anti Toxoplasma antibodies in their sera .

Twenty-six healthy looking age-matched women were selected as controls .venous blood was collected for serum collection wich was tested for anti- toxoplasma antibodies. Six of these, revealed the presence of IgG and so were excluded from this study leaving a total of twenty controls. For all tests performed .the procedure was repeated twice for each sample and the mean of the results was noted.

This test was preformed by the use of two kits (Omega Diagnostics Company, Scotland), one for the detection of IgG antibodies against T. gondii antigens in the patients serum,and the other for the detection of IgM antibodies against T.gondii antigens in the patients serum.

Serum levels of IFN-γ were measured by means of enzyme immmunossay using ELISA kits (Mabtech AB, Sweden).

Statistical analyses included calculation of the mean ± standard error (SE) the confidence interval that puts a lower and upper limits to the mean and considered upper 99% confidence limit.The t-test was adopted to check for any significance of difference between infected and controls. The correlation coefficient (r)was calculated to reflect an association between parameters as a quantitative description to the relation. All statistical analyses were carried according to (11)

Results

The result showed that 58 of 72 women (80.6%) have antibodes against T. gondii (Table-1).Although the other 14 of 22 women (19.4%) had abortion (single or repeated), they were negative for toxoplasmosis using ELISA (Table-1). Samples from 26 healthy-looking women were collected as controls and tested for IgG and IgM specific antibodies for T. gondii by using the ELISA kit .The results indicated that 20 women (76.9%) were negative to the presence of IgG and IgM of T. gondii in their serum, while the remaining six women had T. gondii antibodies . The mean level of IFN -γ in those same patients was also found to be significantly ($p \leq 0.0001$)higher when compared with the control group, the difference being more than tow folds (Table-2). The mean level of IFN-γ in that same group was also found to be significantly ($p \leq 0.0001$) higher when compared to the

control group,the size of the difference being more than two folds (Table-3) the mean level of IFN-γ in patients who had both IgG and Igm was also found to be significantly ($p \leq 0.0001$) higher when compared to the control group (table-4) .(Table-5) shows the levels of IFN-γ in women with IgM, IgG and both IgG and IgM respectively ,compared to the control group.All three groups shwoed a significantly higer ($p \leq 0.0001$) level when compared with the control group.In addition, those with IgM had a significantly ($p \leq 0.05$) lower level of IFN-γ when compared to those with IgG and both IgG and IgM The F-test reflected a highly significant difference between the group ($p \leq 0.001$, F=6.3). The correlation coefficient between IgM and IFN-γ was found to be positive (r= 0.55) and significant ($p \leq 0.05$) and between IgG and IFN-γ was also negative (r=0.03) but both were not significant ($p > 0.05$) as shown in table (6)

Discussion

Toxoplasmosis a worldwide prevalent disease (12). In Iraq, studies also indicated this fact (13). In the present study,the incidence of this disease was also found to be relatively high (80.6%) in women with sigle or repeated abortion .The percentage of women with past or chronic toxoplasmosis (with IgM class)was also found to be relatively high (68.3%) and those with acute toxoplasmosis (with IgG class)was high (31.7%) while those with both IgG and IgM was 29.3% in Iraq ,similar results were obtained (14.15). these results are also similar to those in other countries like Egypt 81.4% (16),France 17% Ibadan and Nigeria 87% (17). Other studies had variable results as 25% in Saudai Arabia (12) .Specific IgM antibodies were reported in 60.4% of women in USA (18) and 60.2% of women in iraq (14). The results differed in Saudi pregnant women being 35% (19). The high prevalence of this disease in iraq could be due to the high number of risk factors and many sources of infection. These include the ingestion of sporulated oocysts in soil (e.g during gardening). eating under cooked meat contaminated with cyst,eating un washed raw vegetables or unpadded fruits (20,21) one of the other sources of infection ,the animals that are consumed by human,were also found to be infected in addition to the trensplacental trensmission (22). Serological surveys found the highest prevalence of Toxoplasma specific antibodies in rabbits 22.2% (23), in pigs 3.3%, 17.3% in adult swine (24), 33% in

dogs (25) and 70.6% of cats were –sero positive (26). Thid study showed significantly increased levels of IFN-γ in all three groups of women infected with T.gondii when compared with the control group,the difference being more than two folds. This indicates that T.gondii is an opportunistic intracellular parasite which induces a highly strong type-1 cytokine response (27) such as IFN-γ and IL-2 (28) during initial infection as a result of early T-cell as well as NK cell activation (5). Induction of IL-12 by macrophage is a major mechanism driving early IFN-γ synthesis (29). The latter cytokine, in addition to promoting the differentiation of Th1 effectros, is important in macrophage activation and acquisition of microbicidal function ,such as nitric oxide release (30).Levels of IFN-γ among groups(IgM,IgG and both) were found to be significantly different.this may indicate the effect of sex-and pregnancy associated hormones on the function of virtually all immune cells types For example, hormones can profoundly influence cell of the innate immune system, such as mast cells, eosinophils, macrophages, dendritic cells and NK cells. These cells do not only from the first line of defense against many organisms, but also play an important role in directing the developing adaptive immune response. the adaptive immune response involving T cells and B cells is also directly affected by these hormones (31). Levels of IFN-γ were decreased in women who had IgM. This may be due to the effects of female sex hormones such as estrongen and progesterone on NK cell activity which induced suppression of NK cell activity (32). In addition, the androgen effect downregulates IL-4, IL-5 and IFN-γ (33) but progesterone inhibits the development of type-1 response while promoting a Th2 response (34,31).IL-10 is derived from T-cells and prevalently from $CD4^+$ T cells the majority of the $CD4^+$ T cell producing IL-10 (7% of total $CD4^+$ T cells) also produce IFN-γ .these double IL-10 and IFN-γ producing cells and represent approximately one quarter of all the IFN-γ producing cells (35).the correlation coefficient between IgM and IFN-γ was found to be positive (r=0.55) and highly significant. This may indicate that CD_4^+ cells contribute significantly to protection .in fact,it also contributes to the protection against infection with highly virulent as helper cells for production of isotype- switched antibodies (36).the correlation between IL- 10 and IFN-γ in women

with IgM was negative (r=0.13) but not significant (unpublished data). This suggests that IL- 10 inhibition of IFN-γ production may be primarily due to its blocking production from accessory cells of the IFN-γ inducer NK SF/ IL- 12 as well as the costimulating molecule IL- Iβ (3). The correlation coefficient between IgG and IL-10 was negative (r=0.16) and between IgG and IFN-γ was also negative (r=0.03)but both were not significant .the correlation coefficient between IL-10 and IgM was negative (-0.70) but highly significant (unpublished data). This may be due to the androgens effect (31). The correlation coefficient between IL-10 and IFN-γ in women who had IgM and IgG was positive (r=0.43) and marginally significant (unpublished data) .these data suggest that IFN-γ is essential for resistance to acute and chronic T.gondii infection IFN-γ can activate macrophages to inhibit or kill T.gondii without collaboration of any other lymphokines (37.38) .in addition, IL-10 down regulation of Th1 cytokines during infection plays a role against immuno pathogenicity,and normally performs an important role in modulating the degree of the immune response. The over production of Th1 cytokines may be due to an absence in production of IL-10. However, levels of this cytokine were also extremely elevated during lethal infections (39.40)

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Table (1) Frequency distribution of T. gondii antibodies using ELISA test in women with a history of abortion compared with healthy- looking controls.

Type of case	ELISA positive	ELISA negative	Total
Singleor repeated abortion	58(80.6%)	14(19.4%)	72(100%)
Healthy-looking controls	6(23.1%)	20(76.9%)	26(100%)
Total	64(65.3%)	34(34.7%)	98(100%)

Table (2) the level of and IFN-γ (pg/ml) in women with a history of abortion with specific IgM antibodies detected by the ELISA

No.	IgM	IgG	IFN-γ	Control IFN-γ
1	160	-	2.0	1.2
2	150	-	1.6	1.4
3	161	-	2.4	1.6
4	185	-	2.6	1.6
5	286	-	2.8	1.2
6	264	-	2.0	2.0
7	226	-	1.6	1.4
8	149	-	2.0	1.6
9	212	-	2.1	1.6
10	222	-	2.3	1.6
11	274	-	2.5	1.4
12	130	-	2.0	2.0
13	188	-	2.1	1.4
14				2.0
15				2.0
16				1.6
17				1.4
18				1.6
19				1.4
20				1.2
M	200.5	-	2.18	1.56
± SE	14.3	-	0.09	0.05

M=Mean
SE=Standard Error .

Table (3)the level of IFN-γ (pg/ml) in women with a history of abortion with specific IgG antibodies, detected by the ELISA technique.

No.	IgM	IgG	IFN-γ	Control IFN-γ
1	-	158	4.0	1.2
2	-	358	1.8	1.4
3	-	232	2.6	1.6
4	-	190	1.6	1.6
5	-	121	3.2	1.2
6	-	184	2.0	2.0
7	-	158	3.6	1.4
8	-	196	4.0	1.6
9	-	32	2.0	1.6
10	-	160	2.0	1.6
11	-	180	3.2	1.4
12	-	132	4.0	2.0
13	-	620	4.0	1.4
14	-	170	5.0	2.0
15	-	364	2.0	2.0
16	-	352	1.8	1.6
17	-	300	4.0	1.4
18	-	220	2.0	1.6
19	-	270	2.0	1.4
20	-	320	5.0	1.2
21	-	132	3.0	
22	-	160	1.8	
23	-	164	1.8	
24	-	132	2.0	
25	-	122	4.0	
26	-	125	1.3	
27		124	1.4	
28	-	142	2.0	
M	-	205.3	2.75	1.56
± SE	-	15.2	0.21	0.05

M=Mean.
SE=Standard Error.

Table (4) the level of IFN-γ (pg/ml) in women with a history of abortion with specific IgG and IgM antibodies, detected by the ELISA technique.

No.	IgM	IgG	IFN-γ	Control IFN-γ
1	130	257	1.6	1.2
2	135	189	3.2	1.4
3	250	175	2.4	1.6
4	200	189	2.4	1.6
5	145	190	1.6	1.2
6	130	209	8.0	2.0
7	145	103	3.6	1.4
8	200	130	3.2	1.6
9	140	210	2.0	1.6
10	175	194	4.0	1.6
11	153	250	1.8	1.4
12	170	250	1.8	2.0
13	220	245	4.0	1.4
14	153	180	8.0	2.0
15	130	174	1.6	2.0
16	150	187	1.6	1.6
17	180	139	1.8	1.4
18				1.6
19				1.4
20				1.2
M	164	192.4	3.09	1.56
± SE	8.64	10.5	0.49	0.05

M=Mean.

SE=Standard Error.

Table (5) Mean level of IFN-γ (pg/ml) in women with a history of abortion with anti T. gondii specific IgG ,IgM and both.

Interferom- γ				
	IgM	IgG	IgG &IgM	control
Mean	2.18	2.75	3.09	1.56
±SE	0.1 A	0.21 C	0.49 d	0.06 b

- the means that carry different letters are significantly different at 5 % level ($p \leq 0.05$) .

Table (6) The correlation coefficient (r-value) of IFN-γ with antibody titer in women infected with T.gondii with a history of abortion .

	p.IgM	p.IgG	p.IgM& IgG	
	IgM	IgG	IgM	IgG
IFN-γ	*0.55	0.03	-0.15	-0.08
IgG			-0.09	

**=Significat at the 1% level ($p \leq 0.01$).

- = Significat at the 5% level ($p \leq 0.05$).

P = patient

مجلة ابن الهيثم للعلوم الصرفة والتطبيقية المجلد20 (2) 2007

تقييم الانترفيرون كاما لنساء مصابات بداء المقوسات الكوندية تعرض للاجهاد

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الخلاصة

تضمنت الدراسة 72 امراة تشكي من الاجهاض المفرد والمتكرر مع 26 امراة سليمة كسيطرة.وكانت نتيجة لفحص ELISA ان 58 (80,6%) يحملن الضد IgG و 17 (3,29%) يحملن الضدين IgM,IgG .وهذه النتيجة دلت على النسبة العالية لوقوع المرض بين النساء الواتي يشكين من الاجهاض اذ كانت النسبة حوالي 80,6 % وتعود هذه النسبة العالية الى الطرائق المتعددة للاصابة بالطفيلي. تم في هذه الدراسة تشخيص تركيز الحركي الخلوي IFN-γ في مصول دم المريضات.وتبين من خلال الفحص بتقنية ELISA ان هناك نسبة ارتفاع معنوي عند المقارنة مع السيطرة في المصابات سواء الحاملات لاضداد IgM,IgG او كلاهما.يدل هذا على وجود استجابة مناعية خلوية عالية خلال الاصابة.