Association between TORCH agents and recurrent spontaneous abortion

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Abstract

Background:Toxoplasmosis, rubella, cytomegalovirus (CMV), and herpes simplex virus (HSV) (TORCH), that can cause illness in pregnant women and may cause birth defects in their newborns. These entire infectious agents induce a shift of immune response during pregnancy from Th2 to Th1 and apoptosis which can be observed clinically as an abortion process.

Objective: To find out the significance of TORCH infection in patients with recurrent spontaneous pregnancy loss.

Materials and method: A total of one hundred and nineteen women, ranged from the mean age (23.9 − 28.5)years, were enrolled in the current study and were further classified into three categories: Group A- Recurrent spontaneous abortion (RSA): n= 62 women, with a mean age of (28.5 + 0.68);Group B- non- recurrent spontaneous abortion (non-RSA): n= 34 women, with a mean age of (26.4 ± 0.85)and group C- Control (successful pregnancy): n= 23 women, with a mean age of (23.9 ± 0.88). From each patient and control, blood sample was collected. Enzyme linked immune sorbent assay (ELISA),using anti CMV/IgG and IgM ,Rubrlla/IgM/IgG ,HSV/IgM and Toxoplasma/IgM/IgG was used.

Results: the current study revealed a significant difference in the levels of each of *Toxoplasma gondii* as well as Cytomegalovirus specific circulating IgM antibodies between group A and group C ($p< 0.05$) based on their respective enzyme linked immuno sorbent assay (ELISA)

Conclusion: In TORCH infections, there was a significant difference between RSA and control in acute infection of *T.gondii* and in the primary infection of CMV.

Key words: Torch,Recurrent Spontanious Abortion,Elisa

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Introduction

Recurrent spontaneous abortion (RSA) is one of the important complications in pregnancy, its incidence is 0.5–1%, and the etiology of RSA is varied, and includes maternal or paternal chromosomal aberrations, uterine anatomic abnormalities, endocrine disorders, infections, and reproductive autoimmune defects. However, the etiology is undetermined in 40–60% of women with recurrent abortion [1-3]. About half of the concepts of RSA have an abnormal karyotype [4], even though the risk for a spontaneous abortion in a subsequent pregnancy is increased when a normal embryonic karyotype is found in abortus material [5]. Infection of the uterine lining or endometrium with slow growing bacteria has also been associated with pregnancy loss in 5–10% of women with RSA. Certain infectious agents have been identified more frequently in cultures from women who have had a spontaneous pregnancy loss; these include *Ureaplasma urealyticum*, *Mycoplasma hominis*, and *Chlamydia*. Other less frequent pathogens include *Toxoplasma gondii*, Rubella, HSV, Measles, CMV, Coxsackie virus and *Listeria monocytogenes*, though none have convincingly. At all any severe maternal infection which leads to bacteraemia or viraemia can cause miscarriage. TORCH {toxoplasmosis, rubella, cytomegalovirus (CMV), and herpes simplex virus (HSV)}, that can cause illness in pregnant women and

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may cause birth defects in their newborns. These entire infectious agents induce a shift of immune response during pregnancy from Th2 to Th1 and apoptosis which can be observed clinically as an abortion process [6]. Thus, the aim of our study is to find out the significance of TORCH infection in patients with pregnancy loss.

Materials and methods

A total of One hundred and nineteen women attending the Obstetrics and Gynecology department of Al-Kadhimyia Teaching Hospital in Baghdad between December 2004 and August 2005 were the subject of this study.women, ranged from the mean age (23.9 − 28.5)years, were enrolled in the current study and were further classified into three categories: Group A- Recurrent spontaneous abortion (RSA): n= 62women, with a mean age of (28.5 + 0.68);Group B- non-recurrent spontaneous abortion (non-RSA): n= 34 women, with a mean age of (26.4 ± 0.85)and group C- Control (successful pregnancy): n= 23 women, with a mean age of (23.9 ± 0.88).

From each patient and control blood sample was collected and serum was seperated for the estimation of antibodies against TORCH infection.

Enzyme Linked Immuno Sorbent Assay (ELISA) Was used according to the instruction **for the detection of:**

CMV/IgG/IgM(BioCheck, Inc. Foster City, CA).

Rubella/IgM/IgG (BioCheck, Inc. Foster City, CA).

HSV/IgM(BioCheck, Inc. Foster City, CA).

Toxoplasma/IgM/IgG (BioCheck, Inc. Foster City, CA).and results were regesrited as mean optical density (OD) readings. The mean gestational age (GA) at the time of abortion for group A and B was (13.94± 0.550) and (15.18±0.937) weeks; respectively, but the mean gestational age at the time of delivery in group C was (38.65±0.135) weeks.

Statistical Analysis

The ANOVA analysis program, chi-square and the relationship between the indicators was measured qualitatively by using the correlation coefficient.

Results

There was a significant difference (p<0.05) in the serum level of *Toxoplasma gondii* specific IgM among the three investigated patients groups (Table **1)**. The number of positive acute infection of *T.gondii* was 15(24.2%) in group A (RSA) and 5(14.7%) in group B (non-RSA).

Table 1: Prevalence of TORCH infection in the three studied groups.

Variable	Results	Groups			Total (n=119)	Chi-Square *P* value
		A (n=62) No (%)	B (n=34) No (%)	C (n=23) No (%)		
***T.gondii* /IgG**	**Negative**	52 (83.9)	29 (85.3)	21 (91.3)	102	**0.693**
	Equivocal	5 (8.1)	3 (8.8)	0	8	
	Positive	5 (8.1)	2 (5.9)	2(8.7)	9	
***T.gondii* /IgM**	**Negative**	44 (71)	25 (73.5)	23 (100)	92	**0.023***
	Equivocal	3 (3.3)	4 (11.8)	0	7	
	Positive	15 (24.2)	5 (14.7)	0	20	

Rubella /IgG	**Negative**	52 (83.9)	24 (70.6)	19 (82.6)	95	**0.153**
	Equivocal	6 (9.7)	3 (8.8)	0	9	
	Positive	4 (6.5)	7 (20.6)	4 (17.4)	15	
Rubella /IgM	**Negative**	59 (95.2)	31 (91.2)	23 (100)	113	**0.177**
	Equivocal	0	2 (5.9)	0	2	
	Positive	3 (4.8)	1 (2.9)	0	4	
CMV /IgG	**Negative**	56 (90.3)	33 (97.1)	20 (87)	109	**0.565**
	Equivocal	3 (4.8)	1 (2.9)	1 (4.3)	5	
	Positive	3 (4.8)	0	2 (8.7)	5	
CMV /IgM	**Negative**	51 (82.3)	28 (82.4)	23 (100)	102	**0.128**
	Equivocal	0	1 (2.9)	0	1	
	Positive	11 (17.7)	5 (14.7)	0	16	
HSV /IgM	**Negative**	56 (90.3)	29 (85.3)	23 (100)	108	**0.464**
	Equivocal	1 (1.6)	1 (2.9)	0	2	
	Positive	5 (8.1)	4 (17.4)	0	9	

*=significant (p<0.05)

A significant difference was noticed between group A and group C (p<0.001) concerning acute infection with *T.gondii* and in acute infection with CMV (p<0.05); but no significant difference (p>0.05) in the mean values of ODs was noticed with the other infections and groups of patients (Table2).

Table 2 : Comparison between positive TORCH infections in the studied groups.

Variable	**Groups**	**n=119**	**MeanOD readingngs ± SE**	**F test** ***P* value**	**Sig. between groups**	
					groups	***P* value**
***T.gondii* IgG**	**A**	**62**	0.55 ± 0.04	>0.05	**A –B**	0.914
	B	**34**	0.05 ±0.54		**A –C**	0.814
	C	**23**	0.06 ±0.53		**B –C**	0.899
***T.gondii* IgM**	**A**	**62**	0.05 ± 0.74	>0.05	**A –B**	0.608
	B	**34**	0.06±0.65		**A –C**	**0.000****
	C	**23**	0.03 ±0.53		**B –C**	0.317
Rubella IgG	**A**	**62**	0.04 ±0.59	>0.05	**A –B**	0.151
	B	**34**	0.06 ±0.69		**A –C**	0.493
	C	**23**	0.08 ± 0.53		**B –C**	0.080
Rubella IgM	**A**	**62**	0.04 ±0.45	>0.05	**A –B**	0.837
	B	**34**	0.04 ±0.43		**A –C**	0.315
	C	**23**	0.03 ± 0.51		**B –C**	0.284
CMV IgG	**A**	**62**	0.03 ±0.57	>0.05	**A –B**	0.275
	B	**34**	0.04 ±0.51		**A –C**	0.619
	C	**23**	0.06±0.53		**B –C**	0.678

CMV IgM	**A**	**62**	0.05 ± 0.64	>0.05	**A –B**	0.808
	B	**34**	0.06 ±0.58		**A –C**	**0.029***
	C	**23**	0.04 ± 0.47		**B –C**	0.388
HSV IgM	**A**	**62**	0.04 ± 0.51	>0.05	**A –B**	0.925
	B	**34**	0.06 ±0.48		**A –C**	0.999
	C	**23**	0.03 ± 0.51		**B –C**	0.954

*= significant different (p<0.05); **= highly significant different (p<0.01);
SE= standard error.

The incidence of TORCH infection in the first; second trimester abortion and control was compared. There was no significant difference (p>0.05) in the mean values of infection by*T.gondii* (IgG); CMV (IgG); HSV (IgM), between 1st or 2nd trimester abortion and control and between 1st and 2nd trimester abortion, there was no significant difference (p>0.05) between infection and trimester of abortion, except in acute infection with CMV and *T.gondii* when we compared between first trimester abortion and control, as shown in table 3.

In addition, it was found marginally significant difference (0.05<p<0.1) in the mean of acute and chronic infection of Rubella between first and second trimester abortion and when compared between first trimester abortion and controls. Furthermore, this study showed a significant difference (p<0.05) in the mean of acute infection of CMV between first trimester abortion (0.7±0.06) and control (0.5±0.04).

Table 3: Comparison between TORCH infection in first and second trimester abortion and control.

Variable	Group	n=119	Mean OD ± SE	F test *p* value	Sig. between groups	
					groups	*P* value
***T. gondii* (IgM)**	**1st**	**53**	0.7±0.06	>0.05	**1st –2nd**	0.786
	2nd	**43**	0.7±0.05		**1st –C**	0.017*
	C	**23**	0.5±0.03		**2nd – C**	0.079[a]
CMV (IgM)	**1st**	**53**	0.7±0.06	>0.05	**1st –2nd**	0.138
	2nd	**43**	0.6±0.06		**1st –C**	0.028*
	C	**23**	0.5±0.04		**2nd – C**	0.334
Rubella (IgG)	**1st**	**53**	0.7±0.04	>0.05	**1st –2nd**	0.086[a]
	2nd	**43**	0.6±0.05		**1st –C**	0.082[a]
	C	**23**	0.5±0.08		**2nd – C**	0.750
Rubella (IgM)	**1st**	**53**	0.6±0.05	>0.05	**1st –2nd**	0.062[a]
	2nd	**43**	0.5±0.03		**1st –C**	0.073[a]
	C	**23**	0.4±0.03		**2nd – C**	0.078[a]

1st= first trimester abortion; 2nd=second trimester abortion;
*=a significant difference; [a]= marginally significant difference.For HSV no notable results was found

Discussion

In the current study there was a significant difference (p<0.05), in the serum level of *Toxoplasma gondii* specific IgM among the three investigated patients groups. In Iraq, a similar result was obtained by Abbas (2002) [7], showed that 21.5% of women with first abortion have positive only IgM by ELISA test. Al-Fertosi (2006) [8] and Salman (2006) [9] showed that 19.17% of women with single or repeated abortion by using ELISA test. In addition, there is more than one *T. gondii* strain with difference in virulence among isolates in the nature [10].This strains difference could be a potential explanation regarding to the high prevalence of toxoplasmosis.

In the present study, the relatively high frequency of toxoplasmosis in women with abortion could be due to the sample selection. The samples were collected from Al-Kadhimyia Teaching Hospital which is a reference hospital for the surrounding rural areas where they have habits in favor of acquiring toxoplasmosis by eating unwashed raw vegetables or unpadded fruits. In addition, in the rural cities there is close contact with cats and consequent exposure to sporulated oocysts by ingestion of these oocysts that contaminate soil during gardening, or eating undercooked meat contaminated with cysts. Moreover, the low level of education in the women about the risk factors for toxoplasmosis may play an important role in the high rate of infection [11].

Furthermore, in the current study showed a highly significant difference between group A and group C (p<0.001) in acute infection of *T.gondii*, but no significant different in the mean value between group A and B and between group B and C. It has been proposed that during pregnancy ,systemic maternial immune response is biased in favor of Th2 cytokine [12,13].Moreover,Th2 cytokines pattern of pregnancy induces the susceptibility to toxoplasmosis infection ,together with risk of placental infection and congenital transmission [14].Evidence from murine and human pregnancy showed that since Th1 type cytokine mediated pregnancy loss, a shift towards Th1-type immunity during *T.gondii* infection may help to explain pregnancy failure [15,16].Thus, a considerable amount of evidence suggests that Th1 cytokine might well be implicated in adversely affecting pregnancy, directly by interfering with trophoblast survival and function, and indirectly by activating cell-mediated immune effecters [17].

This study, showed a significant difference (p<0.05) in the mean value of acute infection of *T.gondii* between first trimester abortion and control ,and found marginally significant difference (0.05<p<0.1) in the mean of acute infection of *T.gondii* between second trimester abortion and control ;because when infection occurs in the first trimester, hormone levels are low and there is little Th2 bias, the chance of transmission to the fetus is low, although the chance of abortion is high [15] .

Conversely, infection during the third trimester, when there is a strong Th2 bias, is unlikely to induce abortion but more frequently results in congenital transmission. There is very likelihood that the Th1 response induced early during *T. gondii* infection will induce abortion early in pregnancy. In contrast, during the late stages of pregnancy, the strong Th2 bias and the diminished NK cell, macrophage, and CD8+ T-cell function may facilitate parasite survival and increase the likelihood of congenital transmission [15].The significant difference between groups might be associated with placental blood flow, the virulence and

amount of T. *gondii* acquired and the immunological ability of the mother to restrict parasitemia.

A significant difference between RSA (group A) and group C ($p<0.05$) in acute infection of CMV was seen but no significant different in the mean OD values between group A and B or between group B and C was noticed. There are many confounding studies about the association between CMV infection and pregnancy loss; the studies showed that HCMV can result in abortion or stillbirth [18, 19].

HCMV act as an immune modulator through elaborating an array of immune evasion strategies to avoid elimination from the host, and its viral proteins an involved in the regulation of cellular gene expression and induction of pro-inflammatory cytokine [20].

In the current study, there was no significant difference ($p>0.05$), in the serum level of HSV specific IgM among the three investigated groups.Lutwick *et al*., (2006) [21] reported, that in the world about one million pregnancies occur each year in women who have been infected with HSV-2, but complications occur in only .01% to .04% of all infected pregnant women [22].

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