A study of some immunological features in aborted women infected with *Toxoplasma gondii* and Cytomegalovirus in Hilla city

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Abstract

This study was conducted to determine the role of *T.gondii* and CMV in cases of abortion in pregnant women and detect the possible association between the two infections. The occurrence of two pathogens in relation to the age has been studied. The infections reaching peak in the age group (25-29) years old .We found that the highest prevalence of Toxoplasmosis and CMV infection recorded for women in the first trimester of pregnancy. Sera and placental biopsies were collected from aborted and controlled women. Enzyme linked immunosorbent assay (ELISA) were used to assess the presence of specific antibodies against *T.gondii* and CMV in addition to the estimation of the concentration of INF-γ at systemic and mucosal levels to determine the humoral and cellular immunity. *T.gondii* and CMV infections and the synergism infections between two organisms induce both humoral and cellular immune responses both at mucosal and systemic levels.

Introduction:

Abortion is defined as termination of pregnancy resulting in expulsion of an immature fetus. A fetus of less than twenty weeks gestation or a fetus weighting less than 500 gm is considered an abortus (1). Recurrent abortion or recurrent pregnancy loss (RPL) (medically termed habitual abortion) is defines as three or more successive spontaneous abortions (2). Number of maternal infections can lead to a single or recurrent pregnancy loss, including : Toxoplasmosis which is a systemic disease caused by the protozoan *Toxoplasma gondii* (*T.gondii*) infection that acquired by pregnant women through ingestion of cysts, undercooked meat or oocysts that may contaminate soil, water, and food. Meat (primarily pork and lamb) is an important source of the infection in humans (3, 4). Human Cytomegalovirus (CMV) infection is the most common cause of congenital malformation in developed countries, its clinical manifestations range from asymptomatic infection to severe fetal damage(5). CMV will lead to complicated pregnancies. It has been reported that the risk of fetal damage is greater if the primary infection occurs during the first trimester of pregnancy (6). The placenta and the fetus can be infected either by transplacental transmission or by an ascending infection from the vagina (7,8).

Serologic confirmations of *T.gondii* and CMV antibodies are indicative of exposure to them and have widely become accepted as a mean to determine immune status and susceptibility to infection. It causes mucosal as well as systemic humoral and cellular immune responses (9).

The aim of this study was studying some of immunological changes that associated with recurrent abortion through detection of antibodies and INF-γ cytokine for acute and chronic infection for more causes of abortion spreading including *T. gondii* and Cytomegalovirus.

Materials & Methods:

1- Patients:

The study was conducted from May to August 2012 . One hundred and fifteen (115) aborted women were selected randomly to determine the role of *T. gondii* and CMV in their abortions and the possible association between these two infections . Patients were from visitors and hospitalized of Maternity and pediatrics hospital in Hilla city. The age of patients ranged between (20 - 40) years old. Blood samples and placental biopsies were taken from each patient.

A questionnaire form were provided to each patient to inquire about important points relevant to the study included age, number of previous abortions, type of trimester, social and economic status.

Ten women were selected randomly as a control group. They were seven pregnant women in different periods of pregnancy and the rest are not pregnant women. They were almost similar to patients in age ranges, occupation, social and economic status and their residence.

2- Collection of blood samples& biopsies:

blood samples were collected from all tested women, after the sera were obtained , they stored at –20°C until they were tested by ELISA for quantitative determination of INF-γ cytokine and anti *T.gondii* IgG and IgM with anti Cytomegalovirus IgG and IgM antibodies at systemic levels (10).

Biopsies were taken from the placenta of all tested women using sterilized scissors and forceps. Then the specimens were put in a sterile Petri dish . Under strict aseptic condition the placental tissues were cut and minced with cold normal saline and homogenized with proportion of 2 ml of cold normal saline for 1 gm of placental tissues. Immunoglobulin titer was determine by centrifugation of placental suspension for 30 min at 3500 r.p.m. then equal volumes of Poly ethylene glycol (PEG 6%) were add to supernatant and left over night at 4 C then centrifuged for 15 min. at 3500 r.p.m. the pellet was dissolved in formal saline then centrifuged at 3000 r.p.m for 10 min. for determination of INF-γ cytokine and anti *T.gondii* IgG and IgM with anti Cytomegalovirus IgG and IgM antibodies at mucosal levels (10).

3- Immumological Study:

Anti *T.gondii* IgG, IgM antibodies and anti Cytomegalovirus IgG, IgM were detected by using specialized kits which were provided from BioCheck instructions (Spain) and Biokit Company (Spain) respectively. INF-γ was assayed using ELISA kit (provided from Immuno. tech. A. Beckman, Coulter Company).

4-Statistical analysis:

The results were analyzed statistically using statistical software (SPSS version 17) as test was used analysis of variance in one direction (ANOVA one way) also used less significant difference (LSD) to search the existence of differences moral between different transactions and the

level of probability ($P \leq 0.005$) and proven results arithmetic mean ± standard deviation (11).

Results:

The enzyme-linked immunosorbent assay (ELISA) is the most commonly available serologic test for measuring antibodies to *Toxoplasma* and CMV.

The most infectious agent that cause abortion was Cytomegalovirus (37.40%). There was relationship between *T. gondii* and CMV infection (Table 1) with positive correlation between the age group and the type of infection that had the age between (25-29) years was the most infected in aborted women at different cases (Table 2). The first trimester of pregnancy was more severe in abortion in women infected with *T.gondii* and CMV (Table 3). The development of immune responses at mucosal humoral and cellular levels were higher than those of systemic levels in infected women for different cases compared with control group at probability level ($p \geq 0.005$) which detected by measuring anti- *T.gondii* antibodies and anti- CMV antibodies with the correlation between them (Table 4). The cellular immunity represented by INF-γ also was higher at mucosal level than that of systemic level compared with control group at probability level ($p \geq 0.005$) (Table 5).

Table(1): Profile of organisms diagnosed in aborted women

Organisms	Number (No.)	Percentage (%)
Toxoplasma gondii	**40**	**34.78**
Cytomegalovirus	**43**	**37.40**
Mixed infection (*Toxoplasma gondii* and Cytomegalovirus)	**32**	**27.82**
Total	**115**	**100**

Table(2): Distribution of infection according to the age groups

Age group (years)	*Toxoplasma gondii*		Cytomegalovirus		Mixed infection	
	No.	%	No.	%	No.	%
20 ≥	5	4.35	4	3.48	0	0
20-24	9	7.83	8	6.96	8	6.96
25-29	11	9.56	14	12.17	12	10.42
30-34	6	5.21	7	6.09	7	6.09
35-39	5	4.35	8	6.96	5	4.35
≤ 40	4	3.48	2	1.74	0	0
Total	40	34.78	43	37.40	32	27.82

Table (3): Distribution of infection according to the trimester of pregnancy

Type of trimester	*Toxoplasma gondii*		Cytomegalovirus		Mixed infection	
	No.	%	No.	%	No.	%
First	18	15.65	22	19.13	9	7.83
Second	13	11.30	17	14.79	18	15.65
Third	9	7.83	4	3.48	5	4.35
Total	40	34.78	43	37.40	32	27.82

Table (4): Concentrations of systemic and mucosal anti *Toxoplasma* IgG and IgM and anti Cytomegalovirus IgG and IgM antibodies in tested women

Type of infection	Mean of IgG concentrations(IU/ml)				Mean of IgM concentrations(IU/ml)			
	Systemic M±S.D.	P value	Mucosal M±S.D.	P value	Systemic M±S.D.	P value	Mucosal M±S.D.	P value
Toxoplasma gondii	116±9.78	0.000[a]	124±10.89	0.100[a]	54±5.89	0.000[a]	67±5.67	0.020[a]
Cytomegalovirus	40±4.04	0.020[d]	85±9.67	0.040[d]	27±1.65	0.020[d]	78±6.98	0.040[d]
Mixed infection	36±3.67	0.010[b]	54±6.98	0.020[b]	38±3.98	0.020[b]	18±1.98	0.000[b]
Control	5±0.34	0.000[c]	7±.0.32	0.001[c]	0.5±0.02	0.000[c]	0.6±0.21	0.000[c]

Table (5): Concentrations of systemic and mucosal of INF-γ in tested women

Types of infection	Rang of systemic INF-γ concentrations(Pg/ml) M±S.D.	P value	Rang of mucosal INF-γ concentrations(Pg /ml) M±S.D.	P value
Toxoplasma gondii	25.00±3.80	0.010[a]	34.46±5.39	0.000[a]
Cytomegalovirus	24.25±2.36	0.030[b]	30.20±3.92	0.020[b]
Mixed infection	38.37±6.01	0.000[c]	42.56±8.51	0.000[c]
Control	19.04±1.09	0.000[d]	21.46±2.08	0.000[d]

Discussion:

Toxoplasma gondii and Cytomegalovirus are important microbiological agents causing abortion, still birth, premature delivery and congenital malformation (12,13). Many authors have observed an increasing risk of fetal death, in particular spontaneous abortion, with increasing maternal age. The association of age of the mother and the increased likelihood of chromosomal abnormalities is manifested by the age related increase of trisomy 21 and cytogenetic studies on

preimplantation embryosin in addition to exposure to the environmental pollution, if these pollutants accumulate in the body it may have teratogenic effect on the developing embryo or fetus, because that during pregnancy the immunity of the body reduce so the latent infection will reactivated (14). Other studies showed that elderly persons seem to be well protected against symptomatic disease due to accumulation of CD28 effector cytotoxic T lymphocytes. This is a characteristic feature of all age groups but is most pronounced in elderly persons(15).

The severity of fetal infection is greatest with first and second-trimester infection, and congenital defects are rarely seen if the infection occurs after 20 weeks of gestation . This may be due to the fact that first trimester of the pregnancy is considered as a critical period in which the fetus is not well established in the uterus and it is threatened for abortion whenever the mother is exposed to any risk factor such as reactivation of latent infection as CMV that result from immunosuppressant concomitant with pregnancy which can lead to placental infection and next placental insufficiency, with subsequent embryonic death (9, 16). The damage seems to be more severe in infections occurring during the first half of the pregnancy, while infections in the second half would result in reduced morbidity (17). Persistence of encysted forms of Toxoplasma in chronically infected uteri and their subsequent rupture during placenta leads to infection of the baby in the first trimester and often results in recurrent abortion (4).

The highest number of positive cases for *T.gondii* antibody and CMV antibody was related to recurrent abortions. This may be due to the fact that primary infection with *Toxoplasma gondii* and Cytomegalovirus in pregnant women can lead to adverse outcome, which is initially unapparent or asymptomatic and thus difficult to diagnose on clinical grounds (18), So the parasite remain in latent state and again when the mother become pregnant where her immunity suppressed due to certain physiological changes in the body that occur during pregnancy, the parasite will reactivated and become the cause of her next abortion unless the treatment is received (4, 9).

The presence of specific Immunoglobulin M (IgM) may not be indicative of primary infection, since it is also produced during reactivation and reinfection (6,19). Prevalence of IgG anti CMV antibodies in women following spontaneous abortions suggest that abortion might result from fetal infection due to reactivation of chronic CMV infection during the course of pregnancy. Significant differences were found between the types of antibodies. This may be an indicative of chronic infection in women. The titer of IgG may be detectable till the end of life and may indicate the chronic stage (20).

The cellular systemic and mucosal immune responses were represented by significant increase in the concentrations of cytokines especially INF-γ. Hence, the epitopes of two organisms can be of T dependent type through the activation of Th1 & Th2 (9). This result agrees with other studies which showed significant increase in the level of IFN-γ in aborted women infected with *T.gondii* compared with healthy control (21, 22).

However , compartment of the common mucosal immune system is linked together, lymphocytes homing do occur between these compartments. Functionally, four views are known to explain the relationship between mucosal and systemic immune responses they are : Mucosal is distinct from systemic, Mucosal immunity induce both mucosal and systemic immune responses, Systemic immunity possibly induce mucosal immune responses, and systemic serum antibodies transduced to mucosal compartments , and this relationship was affected by several factors like host susceptibility to replicating or non replicating immunogens, and the nature of the host immune system (23).

From all of the above it can be concluded that *Toxoplasma gondii* and Cytomegalovirus infections and the synergism infections between two organisms induce both humoral and cellular immune responses both at mucosal and systemic levels.

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دراسة بعض المعايير المناعية عند النساء المجهضات المصابات بطفيلي داء المقوسات الكوندية وفايروس تضخم الخلايا في مدينة الحلة

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الخلاصة

أجريت هذه الدراسة لتحديد دور المقوسات الكوندية و فايروس تضخم الخلايا في حالات الإجهاض لدى النساء الحوامل وتحديد العلاقة المحتملة بين الإصابتين. تم دراسة تأثير العمر على نسبة حدوث الإصابتين واشارت النتائج إلى وجود نسبة إصابة بينهما ووصلت ذروتها عند الفئة العمرية (25–29) سنة. بينت الدراسة إن نسبة انتشار الإصابة بالمقوسات الكوندية وفايروس تضخم الخلايا كانت عالية في الثلث الأول من الحمل بينما كانت مرتفعة في الثلث الثاني من الحمل عند الإصابة المشتركة بينهما. جمعت المصول والخزع من مجموعة النساء المجهضات ومجموعة السيطرة وتم استخدام فحص ارتباط الخميرة للإدمصاص المناعي لتحديد وجود الأجسام المضادة النوعية للمقوسات الكوندية وفيروس تضخم الخلايا بالإضافة إلى قياس تركيز الحركي الخلوي الانترفيرون – كاما على المستويين ألجهازي والموضعي لتحديد الاستجابة المناعية الخلطية والخلوية. وجدت الدراسة الحالية ان الاصابة بالمقوسات الكوندية و فايروس تضخم الخلايا يحث استجابة مناعية خلطية وخلوية على المستويين ألجهازي والموضعي.