Study the role of *Toxoplasma gondii*, Cytomegalovirus and anti-phospholipids antibodies in cases of abortion among women in Hilla city.

*-Mohammed S. Al-Saeed**
*-Mohammed A. Muhsin**
-Gayedaa J. AL-Juburi, Biology Department, Science College, Al-Qadisiya University, Iraq.
Babylon university\College of Medicine, Iraq.

الخلاصة

**أجريت هذه الدراسة لتحديد دور المقوسات الكوندية*(T.gondii)* وفيروس تضخم الخلايا(CMV) في حالات الإجهاض وتحديد العلاقة المحتملة بين الإصابتين وتحديد دورالأجسام المضادة الذاتية للدهون المفسفرة(APL) وعلاقتها مع الإصابة بالطفيلي والفيروس المذكورين لدى النساء المراجعات لمستشفى الولادة والأطفال والمستشفى التعليمي في مدينة الحلة للفترة من تشرين الثاني 2006 إلى نيسان 2007. من مجموع 120 نموذج مصلي ظهر50 (41.66 %) موجبا للأجسام المضادة للمقوسات الكوندية.بينما من 44 نموذج مصلي ،وجد 35 (79.5%) و8 (18.8 %) موجبة النتائج للأجسام المضادة لفيروس تضخم الخلايا(CMV) من نوع گاما و ميو على التوالي. أما نساء المجموعة الضابطة(السيطرة) فقد كانت النتائج سالبة لداء المقوسات وفيروس تضخم الخلايا. من أصل 28 نموذج مصلي من النساء المجهضات(بعضها سالب والبعض الآخر موجب للأجسام المضادة للطفيلي والفيروس) أعيد فحصها لتحديد وجود الأجسام المضادة للدهون المفسفرة بواسطة فحص الـ ELISA وجد 3 نماذج فقط (10.7 %)موجبة.

Abstract

This study was conducted to detect the role of *T.gondii* and CMV and the possible association between them in cases of abortion. Also the role of auto antibodies against phospholipids (APL) and it's association with the mentioned parasite and virus among women who have consulted the-Children and Maternity Hospital and General Teaching Hospital in AL-Hilla city, during the period from November 2006 to April 2007.

Out of 120 samples, 50(41.66%) were positive for anti-*Toxoplasma* antibodies. While out of 44 sera, 35(79.5%) and 8(18.8%) were positive for anti-CMV IgG and IgM antibodies respectively. All control samples have been found to be negative for both *Toxoplama* and CMV.

Twenty eight sera from aborted women (Some negative sera and others positive sera for anti-*Toxoplasma* and/or anti-CMV antibodies) were subtested for detection of anti-phospholipids (aPL) antibody by using ELISA, it was observed that only 3(10.7%) sera were positive.

Introduction

Abortion is the termination of pregnancy before the fetus is capable of survival. It has been established that *Toxoplasma gondii and* Cytomegalovirus (CMV) have direct effect on the fetus leading to spontaneous abortion, still birth or congenital anomalies (Jones *et al*., 2001 and Stagno, 2001). The risk and severity of the fetus infection depend partly on the timing of the mother's infection. *Toxoplasma* is transmitted either by consumption of raw or undercooked contaminated meat or exposure to *T. gondii* oocysts (a form of the organism passed in cat feces). On the other hand, CMV is transmitted by close contact between infected subjects, via blood or blood products, sexual intercourse, or congenitally, the prevalence rates of CMV and *Toxoplasma* in different countries vary between 40–100 % and 20–70 % respectively (Novotná *et al.*,2005).Congenital CMV infection is mostly noted as a cause of hearing loss and mental retardation, while congenital toxoplasmosis is known for its association with chorioretinitis, visual impairment, hydrocephalus, and mental retardation. Postnatal acquired infections in immunocompetent subjects are probably life long but usually harmless and asymptomatic. However, latent CMV or *Toxoplasma* infections can be activated in immunocompromised patients (Arribas *et al*.,1996). However, the effect of anti-phospholipid (aPL) antibodies on the pregnancy outcome of patients with recurrent spontaneous abortion is well known, 15% of women with a history of three or more consecutive miscarriages have been positive for aPL antibodies (Rai *et al.*,1995).

Aim of study

The aim of this study was to investigate *Toxoplasma* infestation in pregnant women with abortion, investigate CMV infection in pregnant women with abortion, who are *Toxoplasma* infestation positive or negative and to show the presence of anti-phospholipids antibody in aborted women, who are positive for anti-*Toxoplasma* and anti-CMV antibodies or negative.

Materials & Methods

In this study, 120 aborted women and 20 healthy pregnant women as a control were selected randomly from visitors and in hospital patients of Maternity and Children Hospital and Hilla Teaching Hospital.

Blood sample was taken from each patient and centrifuged for about 5min at 3000 r.p.m. to obtain serum sample. After the serum samples were collected, they were stored at –20°C in the laboratory of Public Health until they were tested by latex agglutination for anti *T. gondii* antibody following manufacturer's instructions in Toxocell latex from Biokit co. (Spain) and by ELISA technique for quantitative determination of anti Cytomegalovirus IgG and IgM antibodies following BioCheck instructions in ELISA of IgG (Catalog Number: BC-1089), and IgM (Catalog Number: BC-1091), also the same technique was used for detection of anti-phospholipids IgM antibody following Aeskulisa kit instructions in ELISA of IgM (Catalog Number: REF-7204).

The chi-square (X^2) test was used as a test of significance and regression was also used. Differences were recorded as significant whenever the probability (P) was less than 0.01 under confidence level of 0.99 and P value > 0.01 was considered a non-significant difference (Hopkns, 2002).

Results&Discussion

1- Distribution of the tested sera by *Toxoplasma* Latex agglutination test (LAT):-

From aborted women, 50(41.7%) sera out of 120 were positive for anti- *Toxoplasma* antibody with titer ≥20 IU/ml that considered as a positive titer according to (Dunford and Johnson, 1991). While 70(58.3%) sera from 120 aborted women as well as all control sera were negative.

This result may be due to the availability of optimum environmental conditions for survive and spread the parasite in addition to the presence of more than one risk factor influencing the occurrence of toxoplasmosis as the habits of people and the sanitary conditions and many sources of infection, including the ingestion of sporulated oocyst in soil (e.g. during gardening), eating under cooked meat contaminated with cysts, eating unwashed raw vegetables or unpadded fruits.

2- Distribution of the examined sera for anti-CMV antibody by ELISA technique:

Forty four aborted women were found carrying specific anti CMV IgG and IgM antibodies. However, according to BioCheck kit, Inc., the concentration of anti CMV (IgG and IgM) antibodies which is ≥1.2 IU/ml was considered as a positive concentration. This has been reported by (Voler and Bidwell, 1985).

Anti CMV IgG antibody was positive in 35(79.5%) aborted women which indicates previous exposure and only 8(18.18%) aborted women were considered positive for anti-CMV IgM antibody who were positive for anti-CMV IgG antibody at the same time, these findings might point to acute infection or reactivation of latent infection, if the patient is simultaneously positive to anti-CMV IgG antibody. Whereas, all healthy women appeared negative results for both anti-CMV (IgG &IgM) antibodies.

The presence of both anti-CMV (IgG and IgM) antibodies during pregnancy may be used as a presumptive evidence of primary infection as mentioned by Gaytant *et al*.(2002) or may refer to reactivation of a previous latent infection as a result of immune suppression that may occur during pregnancy or presence of other infection that reactivates latent infection and as mentioned by Brooks *et al*.(2004), where recurrent infection may follow reactivation of latent (endogenous virus), or re-infection with another (exogenous strain).

From a total number of 44 sera obtained from aborted women which were tested for anti- CMV(IgG and IgM) antibodies by using ELISA technique, there were 21(47.7%) positive cases for anti-*Toxoplasma* antibody and 23(52.2%) were negative by using latex test. Out of 21 positive cases for anti-*Toxoplasma* antibody, only 19(90.4%) cases were positive for anti-CMV IgG antibody .However, from these 19 cases, 5 cases carry anti-CMV IgM antibody also. This result might refer to *Toxoplasma* and CMV; both are opportunistic infection and possibly reactivated by the same factors, where acquired infections in immunocompetent subjects are probably life long but usually harmless and asymptomatic However, latent CMV or *Toxoplasma* infections can be activated whenever immunesuppression occurs such as that occurs during pregnancy where there are certain physiological changes in the body and weakness which lead to activation of latent infection which has a role in abortion(Jones *et al*.,2001 and Stagno, 2001).

According to these findings which are verified statistically, there was an association between *Toxoplasma* and CMV infections; the correlation coefficients was (r=0.10).

3- Distribution of the examined sera for anti-Phospholipids antibodies by ELISA technique:

Twenty eight samples have been selected from aborted women for detecting anti-phospholipids antibody using ELISA technique and these cases are more clarified in table (1)

Table 1: Shows the number of abortion cases tested for anti-phospholipid (IgM) antibody.

Case	No.	Positive results for anti-phospholipid antibody
Women patients with anti-*Toxoplasma* antibody	11	-
Women patients with anti-CMV antibody	3	-
Women patients with anti-*Toxoplasma* & anti-CMV antibodies	5	-
Women patients with no anti-*Toxoplasma* &no anti-CMV antibodies	9	3
Total	28	3

Table (2): The concentration of anti-phospholipid (IgM) antibody in aborted women detected by ELISA technique.

Concentration of aPL IgM antibody(MpL\ml)		
No.	Patients with Toxoplasmosis or CMV	Control(Abortion with no *Toxoplasma* or CMV)
1	10.5	7.7
2	4.0	9.2
3	5.8	2.1
4	3.3	18.8
5	4.4	3.0
6	11	4.0
7	4	21
8	7.2	28
9	2.3	4.0
10	1.5	-
11	1.1	-
12	3.6	-
13	3.7	-
14	3.0	-
15	2.5	-
16	3.8	-
17	6.5	-
18	8.2	-
19	10.3	-

As shown in table (2), it is noticed that three cases were positive for aPL antibody in which their concentrations are more than 15 MPL/ml, where the concentration of antibody that is (>15 MPL/ml) considered positive concentration according to Aeskulisa kit. The concentrations were registered as 18.8, 21, 28 MPL/ml. These cases were from control women, the first case with concentration (18.8 MPL/ml) was from woman with single abortion in the second trimester. Whereas the other two cases with concentrations (21 and 28 MPL/ml) were from women with repeated abortions in the first trimester. These findings indicate that auto antibodies for phospholipids are considered

as another cause of abortion. The first case is in accordance with the finding mentioned by James *et al.*(1999), where the first pregnancy loss in women with anti-phospholipids antibodies, which may follow an initial successful pregnancy, characteristically occurs in the second trimester. While subsequent losses occur more often in the first trimester in accordance with Rai and Regan (1996). First trimester loss of pregnancies is the most common type of miscarriage in women with anti-phospholipid antibodies. This may be due to defective implantation and subsequently causing defective placentation (Rai *et al.*,1995). On the other hand, negative cases indicate the presence of other causes of abortion rather than *Toxoplasma*, CMV and aPL antibodies.

According to the obtained results, there is no relationship between the presence of anti-phospholipids antibodies and anti-*Toxoplasma* or anti-CMV antibodies, which is verified statistically, the correlation coefficient(r) between anti-phospholipids and anti-*Toxoplasma* antibodies was (r=-0.139), also between anti-CMV and anti-phospholipids antibodies was (-0.074).

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