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A study the role of Toxoplasmosis, Cytomegalovirus and anti-phospholipids antibodies in cases of abortion among women in Hilla city

A Thesis

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By

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A study the role of Toxoplasmosis, Cytomegalovirus and anti-phospholipids antibodies in cases of abortion among women in Hilla city

By
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Abstract

This study was conducted to detect the role of *T.gondii* and CMV in cases of abortion and detect the possible association between the two infections and detects 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.

Sera and biopsies were collected from aborted and controlled women. Latex agglutination test (LAT) and enzyme linked immunosorbent assay (ELISA) were used to assess the presence of specific antibodies against *T.gondii* and CMV. While biopsies were processed for histological examination with hematoxylin – eosin stain.

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

Among 28 sera from aborted women (Some negative sera and others positive sera for anti-*Toxoplasma* and/or anti-CMV antibodies) subtested for detection anti-phospholipids (aPL) antibody by using ELISA, it was observed that only 3(10.7%) sera were positive.

The histologic examination (hematoxylin – eosin stain) of 50 biopsies showed the presence of *T. gondii* cyst in 7(14%) biopsies and giant cell formation as well as owl's eye for CMV in 8(16%) biopsies.

Introduction

Abortion is the termination of pregnancy before the fetus is capable of survival. It has been established that *Toxoplasma gondii*(*T. 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 congenital, 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).

Aims of study

The aims of this study are to:

1-Investigate of *Toxoplasma* infection in pregnant women with abortion.
2-Investigate of CMV infection in pregnant women with abortion, who are *Toxoplasma* infection positive or negative.
3-To show the presence of anti-phospholipids antibody in aborted women, who are positive for anti-*Toxoplasma* and anti-CMV antibodies or negative.
4-Investigate of histopathological changes in the placenta due to *Toxoplasma* and CMV infection.

Materials & Methods

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

Serum sample was taken from each patient and 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 anti-phospholipids IgM antibody following Aeskulisa kit instructions in ELISA of IgM (Catalog Number: REF-7204).While placental biopsies were taken from 50 patients only using sterilized scissors and forceps. Then specimens were put in a sterile test tube and covered with 10% formalin as a preservative matter until the time for histopathology test (Drury and Wallington,1980). Each biopsy specimen was

used for histological examination with hematoxylin–eosin stain following the procedure that given by Qais *et al.*(1990).

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

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. These findings are shown in figure (1).

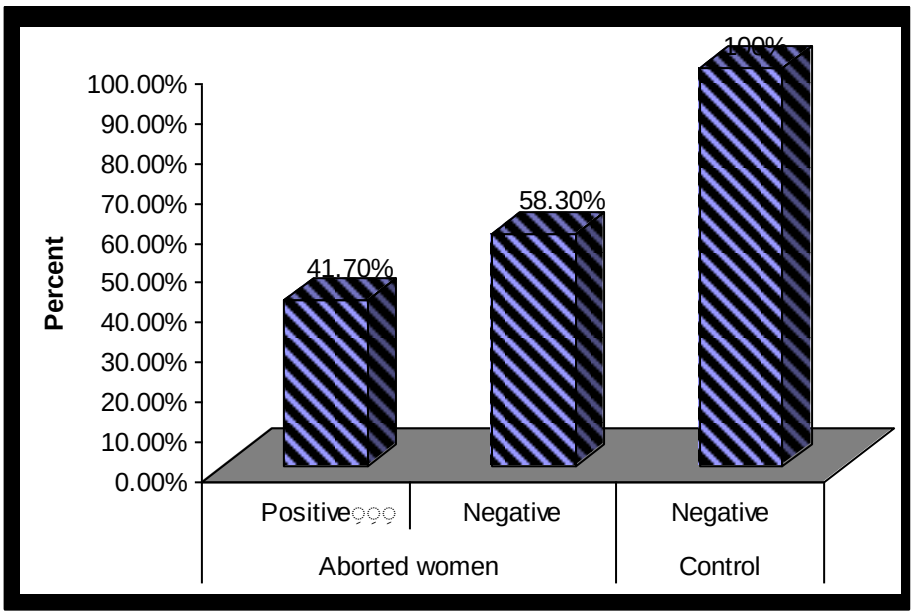

Figure (1): Distribution of the samples subjected to Toxoplasma Latex agglutination test.

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.

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).

The concentrations of anti-CMV (IgG and IgM) antibodies in 44 aborted women are listed in table (1).

Table (1): The concentration of anti-CMV (IgG& IgM) antibodies in aborted women detected by ELISA technique.

No.	IgG(IU\ml)	IgM(IU\ml)	No.	IgG(IU\ml)	IgM(IU\ml)
1	**2.8**	**1.6**	23	**2.7**	0.3
2	**2.9**	0.8	24	**2.3**	0.3
3	0.3	0.3	25	**2.3**	**1.3**
4	**2.6**	0.3	26	**2.9**	**3.0**
5	**2.2**	**1.4**	27	0.3	0.1
6	**2.5**	0.4	28	**1.9**	0.4
7	**3.0**	0.5	29	**2.0**	0.7
8	**3.0**	0.4	30	0.2	0.8
9	**2.6**	**1.3**	31	**3.5**	0.4
10	**2.2**	0.4	32	**1.8**	0.3
11	**2.5**	0.3	33	**2.5**	**1.5**
12	**3.0**	0.8	34	**2.0**	0.5
13	**3.0**	0.6	35	**1.9**	0.5
14	**2.6**	0.5	36	**2.1**	0.6
15	0.3	0.8	37	**2.1**	0.3
16	**3.0**	0.6	38	**2.2**	0.2
17	**3.0**	0.4	39	0.5	0.5
18	**2.7**	**1.4**	40	**2.2**	0.5
19	**2.5**	0.1	41	0.8	0.6
20	0.8	0.8	42	**2.3**	0.4
21	0.6	0.4	43	0.4	0.8
22	**3.0**	**3.0**	44	**3.5**	0.8

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 be refer to reactivation of a previous latent infection as a result of immune suppression that may be occurs during pregnancy or presence of other infection that lead to reactivate 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 be 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(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).

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 (2).

Table (2): 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 (3): 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 (3), 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 (Wohrle *et al*.,2000). 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).

From a total number of 50 placenta biopsies which were taken from aborted women, 15 (30%) have been found with histopathologic changes as a result to infection with *T.gondii* and CMV; out of these 15 biopsy, 7(47%) were from aborted women with positive *Toxoplasma* results and 8(53%) from aborted women with CMV.

Figure (2) shows cyst of *T.gondii* in placenta section stained by H&E stain which was taken from aborted women with Toxoplasmosis. Figure (3) shows the giant cell formation and Owl's eye in placenta section stained by H&E stain which was taken from aborted women infected with CMV; which are considered as a characteristic of CMV infection.

There were many morphological changes which have been detected in placenta section from women with *Toxoplasma* infection. One of the changes was the presence of highly significant lesion seen in the decidua and decidual cells by parasite *T. gondii*, probably because these cells are an important source of nutrient substances. Fibrosis of the villous stroma was frequently seen in placenta affected by toxoplasmosis. This lesion was mainly attributed to a reduced blood perfusion in the villi. The villous stroma serves to support the overlying trophoplasts, provides the environment for placental vascular development and contains lymphocyte cells that act as a secondary barrier against fetal infection (Barros *et al*.,2003).

Histopathological changes are considered as parameters indicative of the specific CMV proteins in the tissue sections. In addition to the pathological changes, giant cell formation which is a main indication due to its relation with the presence of CMV. Katlama (1993) revealed that the diagnosis of CMV disease should be assessed on the association of clinical symptoms with the presence of inclusions in biopsy specimens.

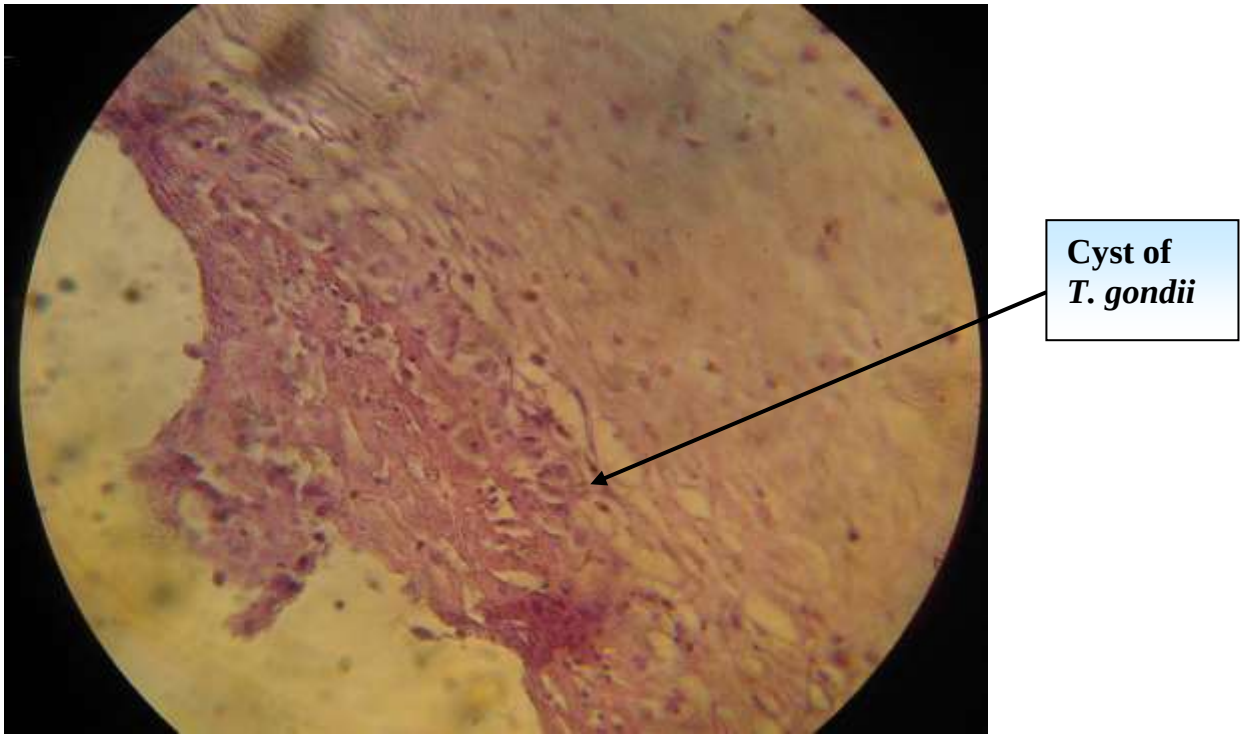

Figure (2): Light microscopic appearance of placenta from infected women with Toxoplasma showing cyst formation (H&E X 100).

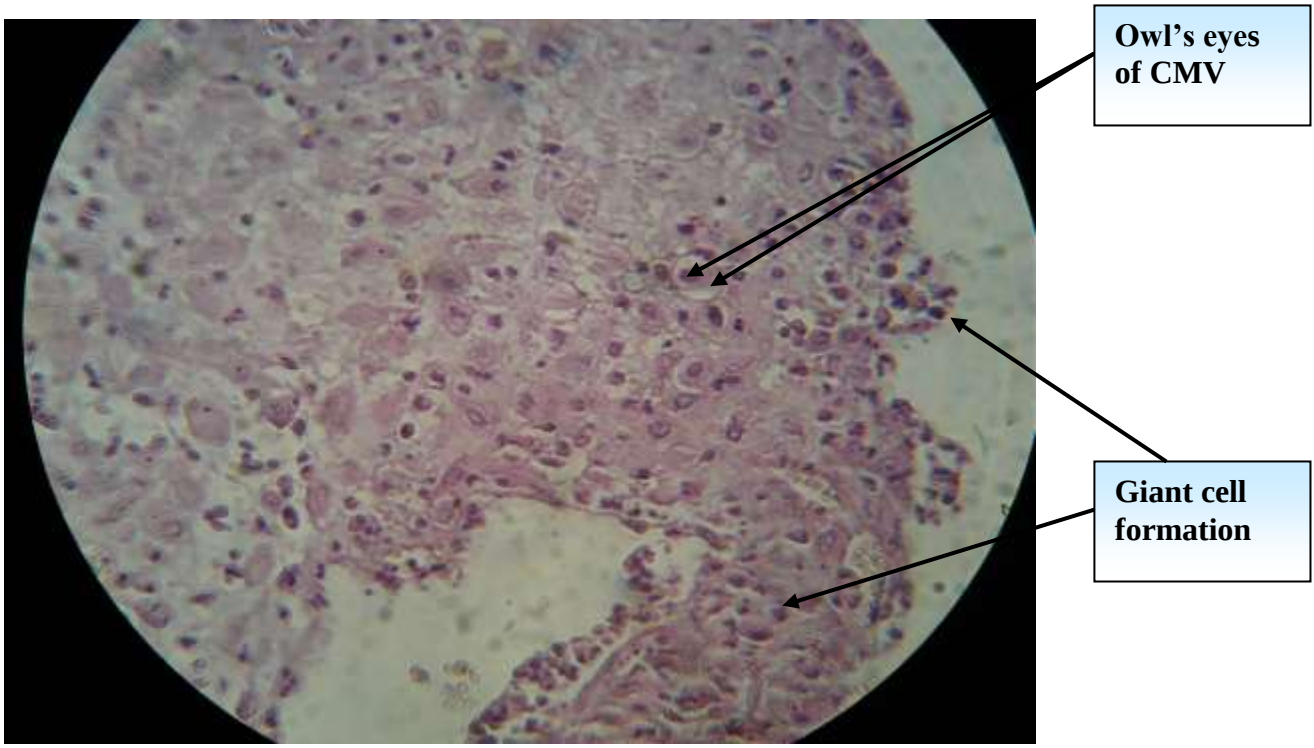

Figure (3): Light microscopic appearance of placenta from infected women with CMV showing Owl's eye and Giant cell formation (H&E X 100).

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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 ٢٠٠٦ إلى نيسانِ ٢٠٠٧.

جمعت المصول والخزع من النساء المجهضا ت والسيطرة وتم إستخدام فحص تلازن اللاتكس وفحص إرتباط الخميرة للإدمصاص المناعي(ELISA) لتخمين وجود الأجسام المضادة النوعية للمقوسات الكوندية وفيروس تضخم الخلايا .أما الخزع فقدعوملت للفحص النسيجي بصبغة الهيماتوكسلين-ايوسين.

من مجموع ١٢٠ نموذج مصلي ظهر ٥٠ (٤١,٦٦ %) موجبا للأجسام المضادة للمقوسات الكوندية.بينما من ٤٤ نموذج مصلي ،وجد ٣٥ (٧٩,٥%) و٨ (١٨.٨ %) 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 ٢٨ 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 ٣ نماذج فقط (١٠,٧ %)موجبة.

كما أظهرت نتائج الفحص النسيجي ل ٥٠ خزعة وجود الأكياس النسيجية للمقوسات الكوندية في ٧ (١٤%) وتكوين الخلايا العملاقة لفيروس تضخم الخلايا في ٨ (١٦%).

1.1. Introduction

Abortion is the termination of pregnancy before the fetus is capable of survival(Roizen *et al.*,1995).It has been established that *Toxoplasma gondii*(*T. 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. Studies suggest that when mothers are infected with CMV or

Toxoplasma, especially in the first trimester of pregnancy, frequently results in severe damage to the nervous system of the fetus or abortion (Britt,1996 and Jones *et al.*,2003).The prevalence rates of CMV and *Toxoplasma* in different countries vary between 40–100 % and 20–70 % respectively (Novotná *et al.*,2005).

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 congenital (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 (Ross *et al*.,2006). Postnatal acquired infections in immunocompetent subjects are probably life long but usually harmless and asymptomatic (Jones *et al*.,2001 and Stagno,2001). However, latent CMV or *Toxoplasma* infections can be activated in immunocompromised patients, e.g. those with AIDS, or immunosuppressed transplant recipients (Da-Cunha *et al*.,1994 and 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).

Aims of study

The aims of this study are to:

1-Investigate of *Toxoplasma* infection in pregnant women with abortion.

2-Investigate of CMV infection in pregnant women with abortion, who are *Toxoplasma* infection positive or negative.

3-To show the presence of anti-phospholipids antibody in aborted women, who are positive for anti-*Toxoplasma* and anti-CMV antibodies or negative.

4-Assess the association between *T. gondii* and CMV infections with some variables such as: a-age; b- residency; c-employment; d- trimester of pregnancy; and e- number of abortions.

5-Investigate of histopathological changes in the placenta due to *Toxoplasma* and CMV infection.

1.2. Literatures Review

1.2.1 Abortion

An abortion is the spontaneous or induced loss of early pregnancy. Early pregnancy is considered any pregnancy less than twenty weeks of gestation, defined by the inability of the fetus to survive outside of the uterus. The term miscarriage is often used to denote spontaneous abortion (Henshaw *et al*.,1999).

1.2.2. The role of maternal infections in spontaneous abortion:-

Infections acquired in uterus or during the birth process are a significant cause of fetal and neonatal mortality and an important contributor to early and later childhood morbidity

(Klein and Remington,1995). The original concept of the TORCH prenatal infections was to describe five infections with similar presentations, including rash and ocular findings; these five infections are: (Toxoplasmosis, Other (e.g. Syphilis), Rubella, Cytomegalovirus (CMV), Herpes simplex virus) (Epps *et al*.,1995).

Different theories have been postulated to explain exactly how an infectious agent leads to miscarriage (Brent and Beckman,1994). These include the following:-

- Toxic metabolic byproducts, endotoxin, exotoxin, or cytokines may have a direct effect on the uterus or the fetoplacental unit.
- Fetal infection may cause fetal death or severe malformation incompatible with fetal viability.
- Placental infection may result in placental insufficiency, with Subsequent fetal death.
- Induction of a genetically and anatomically altered embryo or fetus may occur because of viral infection (e.g. rubella, CMV, varicella-zoster, HSV, syphilis,) during early gestation.

1-2-2 -A. Toxoplasmosis

Toxoplasmosis is a disease caused by *Toxoplasma gondii*, a protozoan parasite mainly transmitted to humans via three routes: a) ingestion of raw or undercooked contaminated meat; b) exposure to *T. gondii* oocysts (a form of the organism passed in cat feces), through cat litter or soil (e.g., from gardening or unwashed fruits or vegetables), or contaminated water; and c) congenital in which maternal infection is passed transplacentally via blood to the fetus (Jones *et al.*,2001) .

A.1.Classification

The organism is classified according to Tenter *et al*(2002) as follows:

Kingdom: Protista

Phylum: Apicomplexa

Class: Sporozoa

Subclass: Coccidia

Order: Eucoccidida

Suborder: Eimeriorina

Family: Emierridae

Subfamily: Toxoplasmatinae (cyclosporinae)

Genus: *Toxoplasma*

Species*: gondii*

According to Levine's taxonomy (1977), there are three main important species belonging to genus *Toxoplasma*. These are:- *T. gondii, T. hammondi and T. bahaiensis*

A.2.Morphology of Toxoplasma : ***The organism exists in three life forms:-***

1-Tachyzoites

The term "tachyzoite" (tacho=speed in Greek), this term replaces the previously used term trophozoite. Aggregates of numerous tachyzoites are called groups (Dubey,1998). Tachyzoite are crescent or oval in shape and are approximately 2 to 4 μm wide and 4 to 8μm long (Katz et al.,1998). Tachyzoites can invade and multiply in all mammalian cells except nuclear erythrocytes. Tachyzoites cannot survive desiccation, freezing and thawing, or extended exposure to gastric digestive juices (Remington et al.,1995).

2-Oocyst

Oocysts (Sporozoites) are spherical or ovoid, about 10 to 12 μm in diameter and contain sporoblast, which divide into two sporocysts, then four sporozoites develop inside sporocyst, the mature oocyst containing eight sporozoites (Paniker,2002). They result from the parasite's sexual cycle, which takes place in the epithelial cells of the cat intestine and are exerted in the feces for periods varying from 7 to 20 days. Cats shed 1-100 million oocysts after the first infection; they must first

undergo sporulation to become infectious, a process that takes 2-3 days in temperate climates and longer in cold climates. Oocysts can remain infectious in warm, moist soil for 1 year or more (Leblebicioglu, 2004).

3. Bradyzoites and cysts

The term "bradyzoite" in Greek (Brady=slow), used to describe the organism multiplying slowly within a tissue cyst (Dubey,1998). Bradyzoites in tissue cysts are crescent –shaped organisms and they accumulate in large number in the host cell, they become surrounded by a tough wall and are called tissue cysts(Gerald* et al.*,1996). Cysts can exist in any organ, but are commonly found in myocardium, skeletal muscle, and the brain. The bradyzoites contained within tissue cysts are most resistant to pepsin than other forms. Cyst is round or oval, 10-200µm in size and contains numerous bradyzoites (Dubey* et al.*,1990).

A.3. Life Cycle

T. gondii has a complex life cycle consisting of three stages: a) tachyzoite during the acute stage of infection, this form of the parasite invades and replicates within cells; b) bradyzoite during latent infections, this form of the parasite is present in tissue cysts; and c) sporozoite this form of the parasite is found in oocysts, which are environmentally resistant (Wilson and McAuley,1999). Oocysts are only produced in the definitive

host, members of the family Felidae. When passed in feces and then ingested, the oocysts can infect humans and other intermediate hosts. They develop into tachyzoites, which are the rapidly multiplying trophozoite form of *T. gondii*. They divide rapidly in cells, causing tissue destruction and spreading the infection. Tachyzoites in pregnant women are capable of infecting the fetus. Eventually tachyzoites localize to muscle tissues and the CNS where they convert to tissue cysts, or bradyzoites. This is thought to be a response to the host immune reaction. Ingestion of cysts in contaminated meat is also a source of infection, as bradyzoites transform back into tachyzoites upon entering a new host (Cohen *et al*.,2004 and John *et al*.,2006).

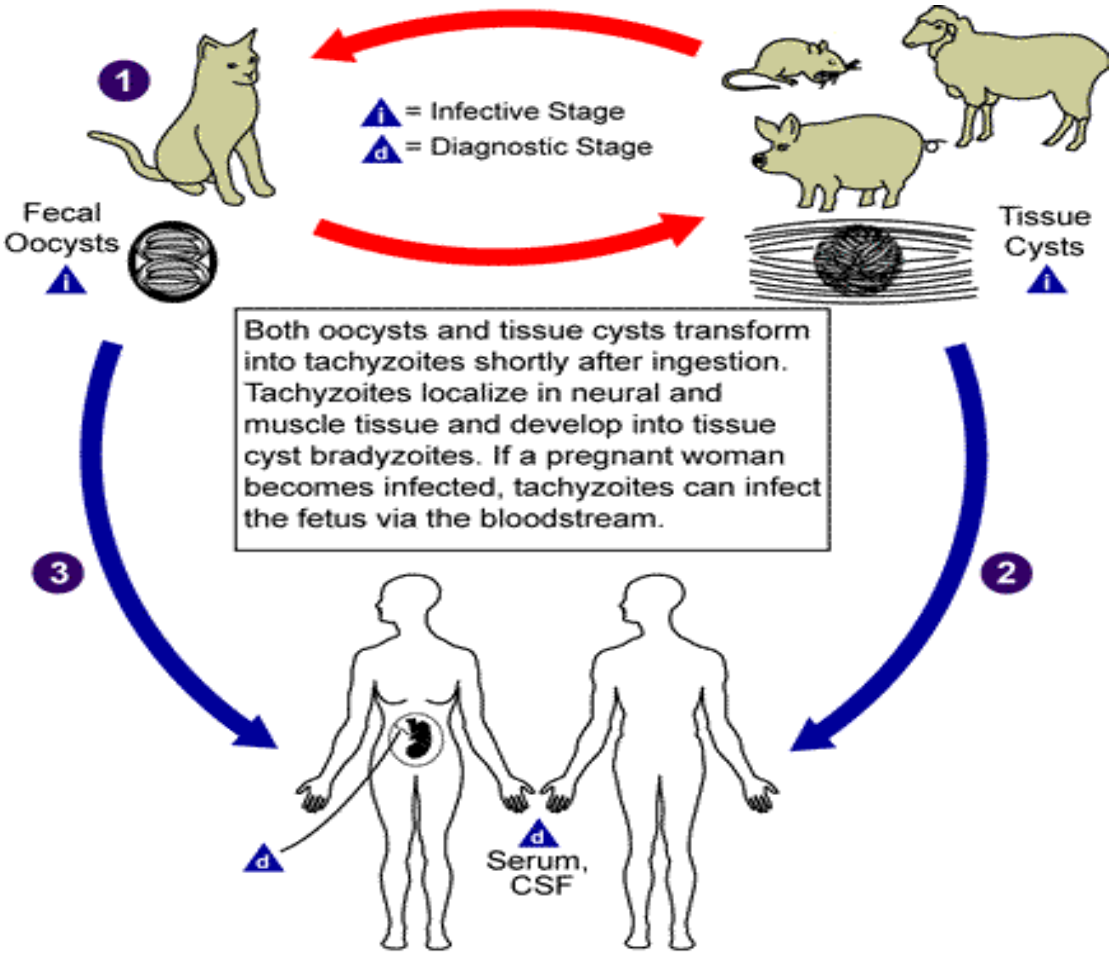

Figure (1-1): Life Cycle of *T.gondii* (Cohen *et al*.,2004;John and Petri,2006).

A.4. Distribution of Toxoplasmosis

It is estimated that between 30% and 65% of all people worldwide are infected with Toxoplasmosis. However, there is a large variation between countries: in France, for example, around 88% of the population is carriers, probably due to a high consumption of raw and lightly cooked meat (David,2003). Germany, the Netherlands and Brazil also have high prevalences of around 80%, over 80% and 67%

respectively (Montoya and Liesenfeld,2004). In Britain, about 22% are carriers, and South Korea's rate is only 4.3% (Carl,2006).In Arab countries, In Jordon, there was a study by Jumaian(2005), the prevalence rate was 47.1%. In Kuwait, the prevalence rate 58.2% was reported in a study by Al-Nakib *et al*.(1983) for women with reproductive age. In Saudi Arabia, a study by Al-Qurashi *et al*.(2001) in population of both sexes, the overall prevalence was 25.0%. In Iraq, in Baghdad, a study by Al-Shaway(1994) for women with history of single abortion and women with history of habitual abortion had found that the prevalence rate was 22.9% and 15.5% respectively. Also the prevalence reported by Abbas in 2002 for women with history of abortion, women with history of habitual abortion and women with history no abortion were 21.5%, 48.9% and 7.5% respectively. In Basra, the overall prevalence as reported by Al-Hamdani and Mahdi (1996) in women with habitual abortion was 18.5%. In Mosul, a study by Al- Khaffaf (2001), who found an overall prevalence rate of (69.2%).

A.5. Clinical manifestation:

Clinical toxoplasmosis can be categorized into four groups: 1) acquired infection in the immunocompetent patient; 2) acquired or reactivated in the immunodeficient patient; 3) ocular; 4) congenital (Garcia *et al*.,2004).

1. Acquired in the immunocompotent patients

Adults with normal immune function who are infected with *T. gondii* are usually asymptomatic or have self-limited symptoms (e.g. fever, malaise, and lymphadenopathy) (Jones *et al*.,2001).Once infected, these individuals usually develop an immune response against toxoplasmosis (Remington *et al*.,2001; Kravetz and Federman, 2005).

2. Acquired or reactivated toxoplasmosis in immunocompromised patients

Immunocompromised patients such as those with AIDS, malignancy, autoimmune disease and its therapy, and solid organ or bone marrow transplants are at risk for severe toxoplasmosis. Common presenting symptoms of toxoplasmosis include fever, constant headache and subtle changes in mental status including confusion, memory loss, and ill-defined personality or behavior changes may predate presenting symptoms by weeks (Ziefert *et al.*, 2002).

3. Ocular toxoplasmosis

T.gondii can cause severe chororetinitis in susceptible individuals. The disease was believed to be caused by congenital

infection with the parasite but is now known to be also a consequence

of infection acquired after birth (Vallochi *et al.*,2002).

4. Congenital toxoplasmosis

Congenital transmission of *T. gondii* occurs when immunologically susceptible mother acquires the infection during gestation (Greco *et al.*,2003). The classic triad of signs suggestive of congenital toxoplasmosis includes chorioretinitis, hydrocephalus, and intracranial calcifications (Greco *et al.*,2003).

Women infected with *T. gondii* before conception rarely transmit the parasite to their fetus, but those who become acutely infected or have reactivation of *T. gondii* during pregnancy (i.e., because of immunosuppression) can transmit the organism transplacentally. The risk of congenital disease is lowest (10 to 25 %) when maternal infection occurs during the first trimester and highest (60 to 90 %) when maternal infection occurs during the third trimester (Dunn *et al.*,1999; Remington *et al.*,2001). However, congenital disease is more severe when infection is acquired in the first trimester (Remington *et al.*,2001). The overall risk of congenital infection from acute *T. gondii* infection during pregnancy ranges

approximately from 20 to 50 %(Remington *et al.*,2001). Extrapolation from regional studies suggests that ~400–4,000 cases of congenital toxoplasmosis occur each year in the United States(Lopez *et al.*,2000).

Premature infants with toxoplasmosis may develop CNS and ocular disease in the first three months of life. In contrast, *T. gondii*infected full-term infants more often have milder disease, with hepatosplenomegaly and lymphadenopathy in the first two months of life (Montoya and Remington,2000). Although most infants infected in utero are born with no obvious signs of toxoplasmosis on routine newborn examination, up to 80% develop learning or visual disabilities later in life (Wilson *et al*.,1980; Carter and frank,1986).

The measurement of *T. gondii* IgG avidity in the postnatal of congenital toxoplasmosis is usefulness; this fact verified by Buffolano *et al*.(2004) who found IgG avidity values in serum samples from infants with congenital infection and uninfected infants and it has been found that IgG avidity values soon after birth in all born to mothers with toxoplasmosis acquired during gestation reflected maternal values in the large majority of the samples. Low or borderline IgG avidity values were systematically found in the cohort of congenitally infected subjects. After birth, IgG avidity values slowly increased over

time for up to 2 years in congenitally infected subjects. On the contrary, IgG avidity values in the uninfected infants remained stable over time. The presence of low IgG avidity in a newborn can be considered a marker of maternal seroconversion in the second or third trimester of gestation and, as a consequence, an indicator of risk for congenital toxoplasmosis (Buffolano *et al*.,2004).

A.6.Immunity

The vast majority of infected humans develop a good immune response following initial *T. gondii* infection, where both humoral and cellular immune components have a role, but the cellular response appears to be critical (Frenkel,1989). The presence of antibodies is not sufficient for protection, as shown by the ability of the parasite to persist in the presence of high antibody titers, this is because *Toxoplasma* antibodies do not enter cells in which tachyzoites are proliferating(Hynemann,1982).

Cell mediated immunity is believed to be the primary mechanism of defense against Toxoplasmosis (Fatoohi *et al*.,2002). *Toxoplasma* antigens have been isolated (p30 and p22 surface protein antigens) and lymphocyte proliferation has been demonstrated with the p22 membrane antigen, these antigens may be responsible for inducing the inflammatory

response (Nussenblatt *et al*.,1998). Interferon gamma (INF-gamma) is important in macrophage activation and parasite killing. INF-gamma is essential for protection against *Toxoplasma* (Olle *et al*.,1996). Cytokines such as IL-4, IL-12, macrophage chemotactic and activating factors (MCAF) have protective effects against Toxoplasmosis (Suzuki *et al*.,1996; Scharton-Kersten *et al*.,1996 and Araujo *et al*.,1997).

A.7.Diagnostic tests:-

I. Non serologic tests:-

1-Isolation of the parasite

Parasite can be isolated with limited success by inoculating patient tissue or body fluids into either mice or tissue culture cells (Murray *et al*.,2003).

2-Skin test (Toxoplasmin)

A skin test showing delayed hypersensitivity reaction to *Toxoplasma* antigens may be useful as a screening test, but it is not useful in congenital and acute toxoplasmosis (Feldman,1996).

3-Polymerase chain reaction (PCR)

PCR amplification for the detection of *T.gondii* DNA in body fluids and tissues as successfully diagnosed congenital, ocular, cerebral and disseminated toxoplasmosis (Brezin *et al*.,1994 and Cinque *et al*.,1997), via enzymatic replication, without using a living organism. As PCR is an in vitro technique, it can be performed without restrictions on the form of DNA, and it can be extensively modified to perform a wide array of genetic manipulations (Sambrook and Russel,2001). A single organism can be detected directly from a

crude cell lysate or as few as to parasites in the presence of 100.000 leucocytes (Savva *et al*.,1990).

II. Serological test:-

1-Dye test (DT)

Sabin and Feldman have developed the methylene blue dye test (DT) for detection IgG antibodies to *Toxoplasma* (Remington *et al*., 2001).

2-Latex agglutination test (LAT)

This test is used normally for the diagnosis of immunoglobulin IgM and IgG, titers rise rapidly during the acute disease (Barbara *et al*.,2000).

3-Indirect fluorescent antibody technique (IFAT)

The IFA measures the same IgG antibodies as the Sabin-Feldman

dye test, neutralizing antibodies usually appear one to two weeks after infection and reach highest titer in 6 to 8 weeks and gradually decline over months to years (Fadul *et al*.,1995).

4-Indirect hemagglutination test (IHAT)

In this test, soluble antigens absorbed to tanned RBC, patient serum is incubated with sensitized cells. If the patient has antibodies to *T. gondii*, RBC will agglutinate (Wilson *et al*.,1990).

5-Enzyme linked immuno sorbent assay (ELISA)

ELISA test is adapted for the detection of *Toxoplasma* specific IgG and IgM. This test was initiated for the first time by Van-Waeman and Schurs(1971) in Holland. Cunningham *et al*.(2001) described ELISA as most diagnostic in case of congenital toxoplasmosis to detect IgM in the acute infection and IgG in the previous infection. Specific IgM and low avidity IgG is associated with more recent infection of the immunocompetent individual (Johnson and Sayles,2002). For most, the prevalence of specific IgG and IgM antibodies was

sufficient to define a serum sample as being from acutely infected individuals, while the absence of specific IgM antibodies was sufficient to define a serum sample as being from chronically infected individuals (Wilson *et al.*,1997).

6-Immunosorbent agglutination assay (ISAGA)

This test uses formalin -fixed organism or antigen-coated latex particles to detect IgG or IgM antibodies (Jorge and Quiñonez, 2004).

7-Complement fixation test (CFT)

In this test the cell wall antigen is used but it is not widely used because of its technical difficulties (Fatoohi,1985).

1-2-2-B. Cytomegalovirus (CMV):-

Cytomegalovirus (CMV) is a viral genus of the Herpesviruses group: In human it is commonly known as human herpesvirus 5 (HHV-5), CMV belongs to the Betaherpesvirinae subfamily of Herpesviridae which includes herpes simplex virus types 1 and 2, varicella-zoster virus (which causes chickenpox and shingles), and Epstein-Barr virus(Rayan and Ray,2004).These viruses share the properties of latency and reactivation (Demmler,1996;Nelson and Demmler,1997).

Although distinct serotypes of CMV have not been defined, the virus can be subdivided into 4 subtypes based on variation in glycoproteinB. The subdivisions appear to correlate with tropism in vivo, and some proof exists that variation in this protein may influence the virulence of CMV (Gaytant *et al*.,2002).

B.1. Structure

Complete particles have a diameter of 120-200 nm and consist of a core containing double stranded DNA, an icosahedral capsid with 162capsomere, an amorphous tegument or matrix, and surrounding phospholipids-rich envelope. An electron microscope feature of CMV includes virions morphologically indistinguishable from those of other herpesviruses, a high ratio of defective viral particles, and the

presence of spherical particles called dense bodies (Murray *et al.,* 2003) . The viral envelope is formed as assembled nucleocapsids bud from the inner surface of the nuclear membrane (Murray *et al.*,2003).

B .2.Replication

Cytomegalovirus (CMV) characterized by highly cell associated and replicates slowly (Collier *et al*.,1998). It attaches to specific receptors on cell and enters by fusion of envelope with plasma membrane. Nucleocapsid moves to nucleus, viral DNA is uncoated at nuclear pores and then transcribed by cellular RNA polymerase so that the five sets of viral genes are sequentially activated: Immediate-early gene products stimulate synthesis of second wave of early gene products; those are involved in genome replication and include the DNA polymerases. After DNA replication the remaining late gene products are expressed and are involved in assembly. In the nucleus viral DNA is inserted in capsids` and resulting nucleocapsids attach to sites on inner nuclear membrane where envelope proteins are present and budding takes place between inner and outer nuclear membranes. Enveloped virus particles are transported through the cytoplasm and released by reverse phagocytosis (Green-Wood *et al*.,2002).

B.3.Transmission and epidemiology

Transmission of CMV occurs from person to person. Seroprevalence is age-dependant: 58.9% of individuals aged 6 years and over are infected with CMV while 90.8% of

individuals aged 80 years and over are positive for CMV (Staras *et al*.,2006). Despite being thought to lie completely latent, virus can be isolated from 61%

of saliva samples, 10% of uterine secretions, and 37% of urine samples, and is also found in blood, semen, vaginal secretions, milk, and stool (Gautheret *et al*.,1997; Kashden *et al*.,1998 and Tierney *et al*.,1999). Transmission can occur between persons by intimate contact including kissing and through transfusion of infected blood or blood products or transplantation of infected organ. CMV is most prevalent between those who have multiple sexual partners (Zhang *et al*.,1995 and Crumpacker,2000).

Vertical transmission to the infant can occur in utero by transplacental passage of maternal blood borne virus (2%), or at birth by passage through an infected maternal genital tract (10 to 20%) or postnatal by ingestion of CMV positive human breast milk (50%) (Stagno,1990).

B.4. Pathology and pathogenesis

Primary infection with CMV results in the establishment of persistent or latent infection (Murray *et al*.,2003). Recurrent infections

may follow reactivation of latent (endogenous) virus, or re-infection with another (exogenous) strain (Brooks *et al*.,2004).The pathogenesis of CMV viewed as a lytic viral

infection resulting from a failure of host immunity to control effectively viral replication and spread. However, it is almost certainty that CMV exerts its pathogenic potential through non lytic mechanism as well; these may include modulation of normal host cell functions by limited expression of its genome, and perhaps by affecting neighboring cellular functions through the induction of cytokine production (Collier *et al*.,1998). Other pathways of host cell damage may include the generation of host derived immunopatholological responses that results in significant organ damage, such as a mechanism has been afforded as an explanation of the severe pneumonia associated with CMV infection in bone allograft recipients (Grundy and Shanley,1987). Histological findings include large refractile cells (Cytomegalic) with so-called Owl's eye intranuclear inclusions (Collier *et al*.,1998).

B.5. Clinical manifestations:-

1-Congenital CMV infection

Primary CMV infection of women during pregnancy results in transmission to the fetus transplacentally in ~30–40% of maternal infections (Fowler *et al*.,2003;Cannon and Pellet,2005) which can

cause damage to the central nervous system, kidneys, endocrine glands, gastrointestinal tract, lungs, and liver. Long-term sequelae include cerebral palsy, mental retardation, and hearing loss (Demmler, 1991 and Stagno,2001). Severe disease with manifestation at birth can appear in 5% of in utero infected children and these children can excrete CMV in a saliva and urine for 12 to 40 months (Stagno, 2001). IgG antibody appears 2 to 3 weeks following a primary infection and persists for life in both children and adults (Crumpacker, 2000). The birth prevalence rate of congenital CMV infection varies between ~0.6–1.5% in the United States (Stagno *et al*.,1982 and Stagno *et al*.,1986).

Cytomegalovirus can cross the placenta and cause both fetal and placental infections. Studies have shown that infants congenitally infected with CMV as a result of primary infection of their mothers are more likely to have overt sequelae than those infected from reactivated infections of the mothers (Schopfer *et al.*,1978 and Stagno *et al*.,1982).Transmission of CMV infection to the fetus has been identified in all trimesters of pregnancy. Abortion can result from ascending CMV endometritis and the virus has been isolated from post-abortion uterine discharge (Dehner and Askin,1975).

2-Infection in the immunocompotent patients

Adults with normal immune function infected with CMV are usually asymptomatic or might experience mild flu-like symptoms, or even mononucleosis (Britt and Alford,1996 and Stagno,2001).Once a human is infected, the virus passes into a latent state (Britt and Alford, 1996).

3- Infection in the compromised patients

When an individual is immunosuppressed by HIV or immunosuppressive drugs, unnoticed smoldering CMV infections or virus previously lying latent may cause severe illness or death(Crumpacker,2000).Common manifestations include retinitis (which may cause blindness), hepatitis, cholangitis, encephalitis, pneumonitis, gastrointestinal ulcerations, colitis, abdominal pain, fever, and weight loss (Hirsch,1994).

B.6. Immunity:-

I-Humoral response

IgM antibodies are produced following initial infection and generally persist for 3-4 months. IgG antibodies appear at the same time, peak about 2 or 3 months after infection, and persist for many years and often for life. Although, the antibody

response is directed against many virion proteins, neutralizing antibodies are primarily

directed against the envelope glycoproteins, especially gB and gH. Although, neutralizing antibodies play no role in control of established CMV infection, there is good evidence that they can prevent infection (Parslow *et al*.,2001).

II-Cell mediated immunity

Cell-mediated immunity is considered the most important factor in controlling CMV infection. Patients deficient in cell-mediated immunity are at greatest risk for CMV disease (Griffiths and Emerym, 1997). CMV-specific $CD4^+$ and $CD8^+$ lymphocytes play an important role in immune protection after primary infection or reactivation of latent disease. Studies of bone marrow transplant patients have revealed that patients who do not develop CMV-specific $CD4^+$ or $CD8^+$ cells are at higher risk for CMV pneumonitis. Additionally, no cases of CMV pneumonia have been reported in allogeneic marrow transplant patients receiving infusions of CMV-specific $CD8^+$ cells (Walter *et al*.,1995).

B.7.Laboratory diagnosis:-

I. Virus isolation

CMV can be isolated from a variety of body fluids and tissues; however, urine, respiratory secretions (e.g., saliva, throat washings, and bronchoalveolar lavage fluid) and anticoagulated whole blood are most common for diagnostic purpose (Murray *et al.*,2003). The specimens have been treated with one or more of the following methods:-

(a)Histopathology

Histological examination of Wright-Giemsa or hematoxylin-eosin stained biopsy specimens can be useful in the diagnosis of localized CMV organ disease (Deibel *et al*.,1974).

(b) Tissue immunofluorescence

Some biopsy samples (e.g. liver, lung) may contain cells infected with CMV, which can be visualized by staining frozen sections with antisera to CMV (Zuckerman *et al*.,2000).

(c)Cell culture

Human fibroblast best support the growth of CMV and therefore are used for diagnostic purposes (Murray *et al.*,2003).

(d) Electron microscopy (EM)

Samples of urine from infants infected congenitally or perinatally contain high titers of CMV, by using EM technique; it has been possible to demonstrate this viruria (Ho,1991).

(e) Polymerase chain reaction (PCR)

PCR is currently the most widely used molecular method for the detection of CMV DNA and mRNAs (Solano *et al*.,2001) in all the different cell lineages and organ systems in the body (Gershon *et al*.,1997).

II-Serologic diagnosis:-

(a) Enzyme immuno assay (EIA)

This technique could detect soluble virus, Specific proteins by producing a color change, visible to naked eye, whenever CMV is present (Zuckerman *et al.*,2000).

(b)Immunoflouresence assay (IFA)

IFAs are very useful and inexpensive methods that offer the advantages of speed, sensitivity, and simplicity for the qualitative and quantitative detection of CMV antibodies (Murray *et al*.,2003).

(c)Passive latex agglutination test

This method is highly sensitive and specific, and it may serve to determine the immune status of patients or blood donors (Beckwith *et al*.,1985 and Hursh *et al*.,1989).

(d)Other serologic tests:-

Complement fixation(CF), immuno adherence hemagglutination, indirect hemagglutination and the neutralization test. Some of these procedures have the disadvantage of being insensitive, poorly standardized, and relatively labor. Intensive and time (Murray *et al*., 2003).

1.2.2. C. Antiphospholipid (APL) antibodies

Antiphospholipid antibodies are autoantibodies which are directed against negatively charged phospholipids, essential components of cell membrane which have an important role in cell-cell membrane fusion (James *et al*.,1999). Anti-phospholipid antibodies include the lupus anticoagulant (even in the absence of systemic lupus erythematosus) and antibodies against cardiolipin and phosphatidylserine. Clinically, aPLs are associated with thrombocytopenia, thrombosis and recurrent miscarriage, they are also implicated in the etiology of other pregnancy complications (e.g fetal growth deficiency and pre-eclampsia) if the pregnancy progresses into the third trimester (James *et al*.,1999). Uteroplacental thrombosis considered to be the major cause of fetal loss (Out *et al*.,1991). However, there is also evidence that aPLs impair trophoplast function directly by

other mechanisms (Rote *et al*.,1992; Rai and Regan,1996). Another hypothesis is that complement activation is a central mechanism of pregnancy loss in anti-phospholipid antibody syndrome(Girardi *et al*.,2003). Anti-cardiolipin antibodies and lupus anticoagulant; one or the other is present in 5% to 15% of women with recurrent pregnancy loss (Reindollar,2000). Patients with high levels of anti-cardiolipin antibodies who have had a fetal death seem to be at high risk of future loss (Kiwi,2006).The

successful pregnancy rate is only 10-15% in untreated women carrying aPL and recurrent miscarriage is often the initial manifestation (Rai *et al*.,1995).The first pregnancy loss, which may follow an initial successful pregnancy, characteristically occurs in the second trimester (James *et al*.,1999). Subsequent losses are more often in the first trimester (Rai and Regan,1996). The association with systemic lupus erythematosus (SLE) is highly variable and often relatively weak at the time of initial presentation. However, women with established SLE are often positive for aPL and are at risk of recurrent miscarriage (James *et al*.,1999). The biologic effects mediated by the human aPL antibodies include (1) reactivity with endothelial structures, which disturbs the balance of prostaglandin E2/thromboxane production; (2) interaction with platelet PLs, with consequent

up-regulation of platelet aggregation; (3) dysregulation of complement activation; and (4) interaction of aPL with phosphatidylserine exposed during trophoblast syncytium formation, which raises the possibility of a more direct effect of these auto-antibodies on placental structures (Piona *et al*.,1995; Vogt *et al.*, 1996 ; Machin ,1996 and Pierro *et al*.,1999).

1.2.2 .D. The rest three agents of TORCH infections are:-

D.1. Other (e.g. Syphilis)

Congenital syphilis occurs by spirochete *Treponema pallidum*, infection can result in stillbirth, hydrops fetalis, or prematurity and associated long-term morbidity (Gutman,1998).

D.2. Rubella

Rubella (German measles) is caused by a togavirus (Banatvala *et al*.,2004). An infant acquiring the infection in utero may be normal at birth but many infants with this congenital infection are growth retarded and have radiolucent

bone disease, hepatosplenomegaly, thrombocytopenia, and purpuric skin lesions (Sheridan,2002).

D.3. Herpes simplex virus (HSV)

HSV is transmitted to an infant during birth primarily through an infected maternal genital tract or by an ascending infection, when primary genital herpes occurs in pregnancy; the risk of fetal and neonatal involvement is high, especially with infection in the third trimester (Brown *et al.*,1997).

1.2.3. C-reactive protein (CRP)

C-reactive protein (CRP) is one of the major acute phase proteins (APP) and is widely distributed in nearly all vertebrates (Sarikaputi *et al*.,1991).During infections and under stressful conditions, human and animal mononuclear series cells, including monocytes and macrophages, secrete cytokines; such as: IL-1, IL-6, tumor necrosis factor-a, and interferon, which stimulate the liver to rapidly synthesize large amounts of CRP (Sarikaputi *et al*.,1991; Baumann and Gauldie,1994 and Godson *et al*.,1996). C-reactive protein is the serum APP that responds most quickly to infections. It recognizes phosphocholine on the

cytoplasmic membrane of a cell, activates the complement cascade, and stimulates macrophages to engulf infectious agents (Baumann and Gauldie,1994). C-reactive protein can regulate the immune system during the early stage of an infection. It plays a role in destroying infectious agents, minimizing tissue damage, and facilitating tissue repair and regeneration (Sarikaputi *et al*.,1991 and Horadagoda *et al*.,1999).Women who have very high levels of the inflammatory marker C-reactive protein early in pregnancy run the risk of delivering before term (AJCN.,2005).In addition to that CRP has also been associated with adverse pregnancy outcomes, including pre-eclampsia (Pitiphat *et al*.,2005). Abortion is not associated with increased systemic inflammation as measured by maternal serum C-reactive protein (Boggess *et al*.,2005).

Healthy persons generally have C-reactive protein levels no higher than 10 mg/L (Jaye and Waites,1997). CRP is released by the body in response to acute injury, infection, or other inflammatory stimuli thus its levels are elevated with *T. gondii* infection(Udvarnoki *et al*.,2007). Zhu *et al*.(1999) hypothesized in the Journal of the American College of Cardiology that CMV may stimulate an inflammatory response, reflected by elevated CRP levels. In patients with viral infections, the C-reactive protein value is usually less than 20 to 40 mg/L. However, this distinction is not absolute. C-reactive protein values greater than 100 mg/L can occur in

uncomplicated infections caused by adenovirus, cytomegalovirus, and the viruses that cause influenza, measles and mumps. C-reactive protein determination cannot be used to classify the precise microbial etiology of an infection (Jaye and Waites,1997). Also C-reactive protein level elevated in patients with autoantibodies against phospholipids (Miesbach *et al*.,2005).

3.1. Distribution of the tested sera by Toxoplasma Latex agglutination test (LAT):-

A total of 120 sera obtained from aborted pregnant women admitted to a gynaecological emergency in Maternity and Children Hospital, Hilla Teaching Hospital and 20 sera from healthy women (control) were examined for anti-*Toxoplasma *(IgG) antibodies by using latex agglutination test.

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 (Gray* et al.*,1990; Dunford and Johnson, 1991). While 70(58.3%) sera from 120 aborted women as well as all control sera were negative. These findings are shown in figure (3-1).

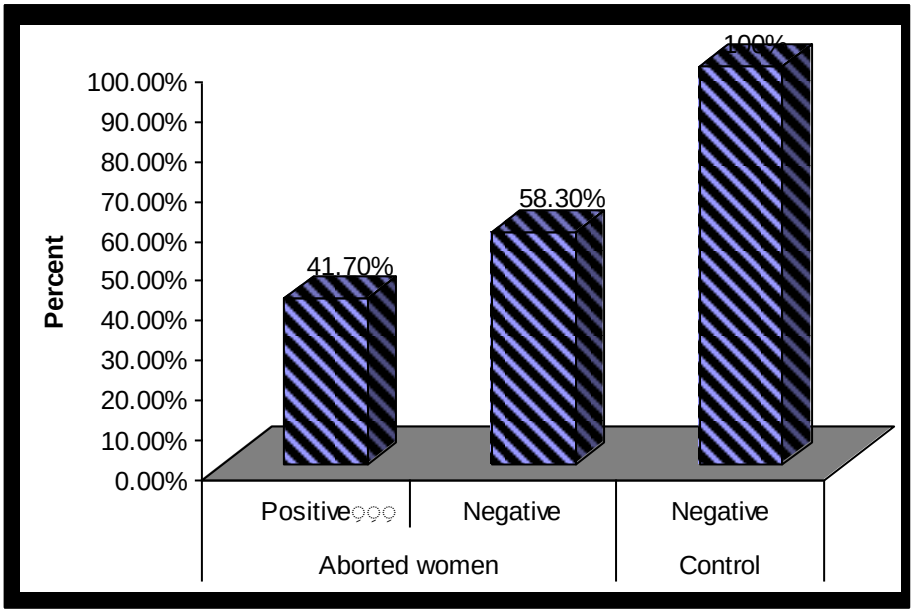

Figure (3-1): Distribution of the samples subjected to Toxoplasma Latex agglutination test.

This result in comparison to other Iraqi studies carried out in other cities is nearly similar to that reported in Basra,(35%) by Hassony* et al.*(1987),in Baghdad,(38.8%) by Niazi* et al.*(1988),in Kirkuk,(36.6%) by Othman(2004). But it is lower than that reported in Mosul,(69.2%) by Al-Khaffaf(2001) and in Baghdad,(60.21%) by Abbas(2002) and (80.6%) by Al-Sorchee(2005). This may be due to the difference in 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. Comparing the present study with those of other Arab countries; it is higher than that reported in Egypt,(14.57%) by Hamadto* et al.*(1997) and in Saudi Arabia,(25%) by Al-Quraishi(2001).This may attributed to the low level of health education about the ways of prevention of the disease in concomitant with a general decline on the sanitary in our community in comparison to that in Egypt and Saudi Arabia.

In other areas of the world, the yielded result is nearly similar to that reported in Spain,(38.9%) by Ashrafunnessa* et al.*(1998). But it is higher than that in Britain, (22%) and in

South Korea, (4.3%) as reported by Carl(2006).The high prevalence of this disease in Iraq may be due to the high number of risk factors and many sources of infection, these include 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 (Population and public health branch,2001).

3-2.Distribution of the examined sera for anti-CMV antibody by ELISA technique:

All 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 (Cremer, 1985; Voler and Bidwell, 1985).

The concentrations of anti-CMV (IgG and IgM) antibodies in 44 aborted women and 6 healthy women (control) are listed in table (3-4) and table (3-5) respectively. 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 were considered negative for both anti-CMV (IgG &IgM) antibodies, although they were carried low level of specific antibodies as shown in table (3-5).

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 be refer to reactivation of a previous latent infection as a result of immune suppression that occurs

during pregnancy or presence of other infection may also lead to reactivate latent infection. Presence of anti-CMV (IgG and IgM) antibodies may also point to re-infection in addition to presence of previous infection as mentioned by Brooks *et al*.(2004), where recurrent infection may follow reactivation of latent (endogenous virus), or re-infection with another (exogenous strain). Because of latent nature of disease, previous infection does not confer immunity against infection in the infant as clarified by Nigro *et al*. (1999).

Table(3-1):The concentration of anti-CMV (IgG& IgM) antibodies in aborted women detected by ELISA technique.

No.	IgG(IU\ml)	IgM(IU\ml)	No.	IgG(IU\ml)	IgM(IU\ml)
1	**2.8**	**1.6**	23	**2.7**	0.3
2	**2.9**	0.8	24	**2.3**	0.3
3	0.3	0.3	25	**2.3**	**1.3**
4	**2.6**	0.3	26	**2.9**	**3.0**
5	**2.2**	**1.4**	27	0.3	0.1
6	**2.5**	0.4	28	**1.9**	0.4
7	**3.0**	0.5	29	**2.0**	0.7
8	**3.0**	0.4	30	0.2	0.8
9	**2.6**	**1.3**	31	**3.5**	0.4
10	**2.2**	0.4	32	**1.8**	0.3
11	**2.5**	0.3	33	**2.5**	**1.5**
12	**3.0**	0.8	34	**2.0**	0.5
13	**3.0**	0.6	35	**1.9**	0.5
14	**2.6**	0.5	36	**2.1**	0.6
15	0.3	0.8	37	**2.1**	0.3

16	**3.0**	0.6	38	**2.2**	0.2
17	**3.0**	0.4	39	0.5	0.5
18	**2.7**	**1.4**	40	**2.2**	0.5
19	**2.5**	0.1	41	0.8	0.6
20	0.8	0.8	42	**2.3**	0.4
21	0.6	0.4	43	0.4	0.8
22	**3.0**	**3.0**	44	**3.5**	0.8

Table (3-٢): The concentration of anti-CMV (IgG & IgM) antibodies in healthy women (control) detected by ELISA technique.

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 be refer to

No.	IgG(IU\ml)	IgM(IU\ml)
1	0.5	0.7
2	0.6	0.3
3	0.4	0.2
4	0.7	0.7
5	0.5	0.1
6	0.3	0.2

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 (Jones *et al*.,2001 and Stagno, 2001). However, latent CMV or *Toxoplasma* infections can be activated whenever immunesuppression occurs such as that occurs during pregnancy or as a result of infection with one of them or other infections. Thus the presence of anti-CMV IgM antibody in aborted women with anti-*Toxoplasma* antibody, who are at the same time positive for anti-CMV IgG antibody may explain CMV reactivation rather than recent infection, where CMV remains latent within the host, reactivating and shedding when the host's immune system is compromised as mentioned by Gregory and Taylor(2003) and this may refer to the role of *Toxoplasma* infection that can be played in suppressed immune system and reactivate other latent infection as CMV.Also it may be the same reason for the presence high concentration of anti -*Toxoplasma* antibody in sera which were positive for anti-CMV antibody, which can explain reactivation of latent *Toxoplasma* in the presence of the other infections suppressed immune system such as CMV.

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

T.gondii ***and CMV can negatively affect pregnancy outcomes. Preconception counseling about such effects can reduce the risks posed by these pathogens (Ross* et al*.,2006).***

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

This study also included the role of anti-phospholipid (IgM) antibody in abortion and if it is concomitant with anti-*Toxoplasma* and anti- CMV antibodies.

Twenty eight samples have been selected from aborted women, which include (19 samples from aborted women with *Toxoplasma* or CMV or with both infections and 9 from aborted women with no *Toxoplasma* or CMV infection). These selected cases which were tested for anti-phospholipids antibody by ELISA technique are more clarified in table (3-3).

Table (3-3): 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* & anti-CMV antibodies	5	-
Women patients with no anti-*Toxoplasma* &no anti-CMV antibodies	9	3
Total	**28**	**3**

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**

Table (3-4): The concentration of anti-phospholipid (IgM) antibody in aborted women detected by ELISA technique.

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	

Patient: Mean =5.089, Std. Error=0.699, Std. deviation=3.050
Control: Mean =10.867, Std. Error=3.129, Std. deviation=9.387

As shown in table (3-4), 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 (Wohrle *et al*.,2000). The concentrations were registered as 18.8, 21,28 MPL/ml. These cases were from control women in whom abortion has occurred with no history of Toxoplasmosis and CMV infection and also both are not detected in the sera of these patients. 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).

It is important to say that so far no similar work has been done in Iraq concerning toxoplasmosis in relation to CMV and anti-phospholipid antibodies in cases of abortion.

3-4 Histopathological changes:

From a total number of 50 placenta biopsies which were taken from aborted women and preserved until used for histopathology test, 15 (30%) have been found with histopathologic changes as a result to infection with *T.gondii* and CMV; out of these 15 biopsy, 7(47%) were from aborted women with positive *Toxoplasma* results and 8(53%) from aborted women with CMV.

Figure (3-5) shows cyst of *T.gondii* in placenta section stained by H&E stain which was taken from aborted women with Toxoplasmosis. Figure (3-6) shows Owl's eye formation; which are considered as a characteristic of CMV infection in section of placenta biopsy stained by H&E stain, inflammatory cells spread very little through the section when examined by light microscope. Figure (3-7) shows the giant cell formation and Owl's eye in placenta section taken from aborted women infected with CMV.

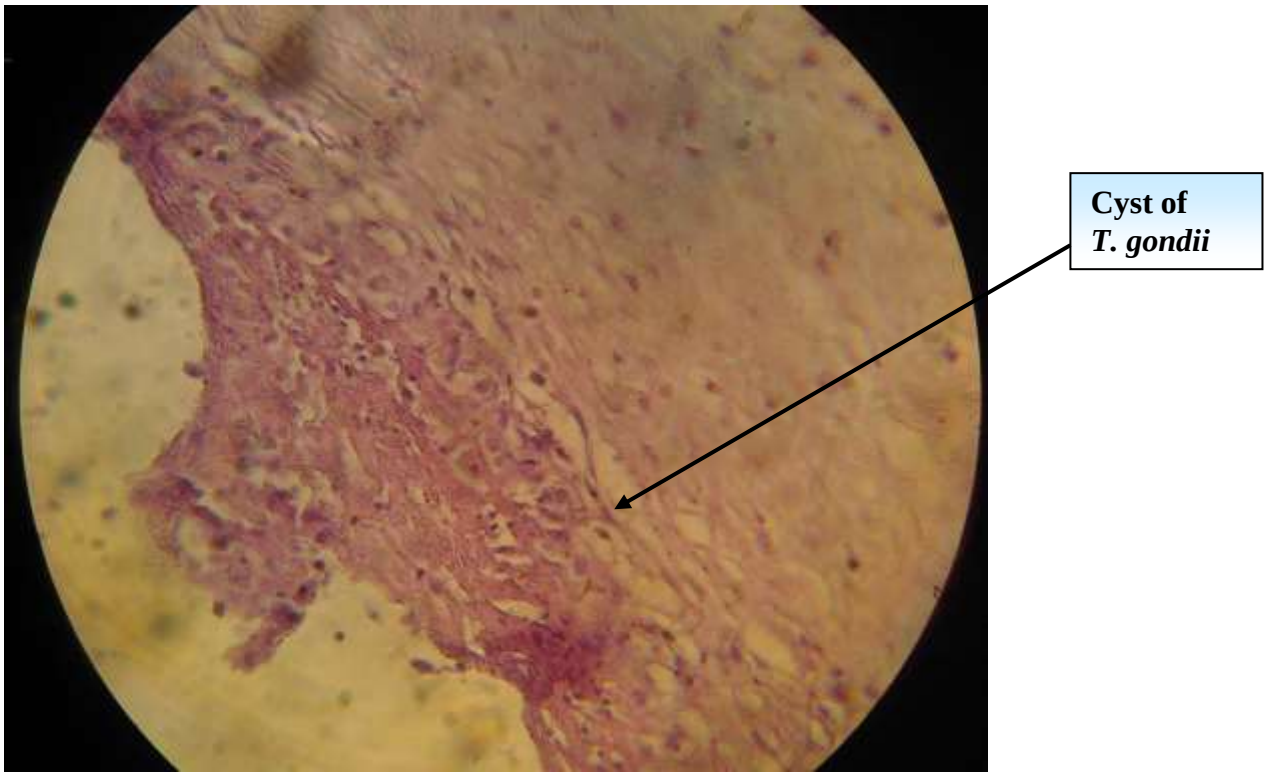

Figure (3-5): Light microscopic appearance of placenta from infected women with Toxoplasma showing cyst formation (H&E X 100).

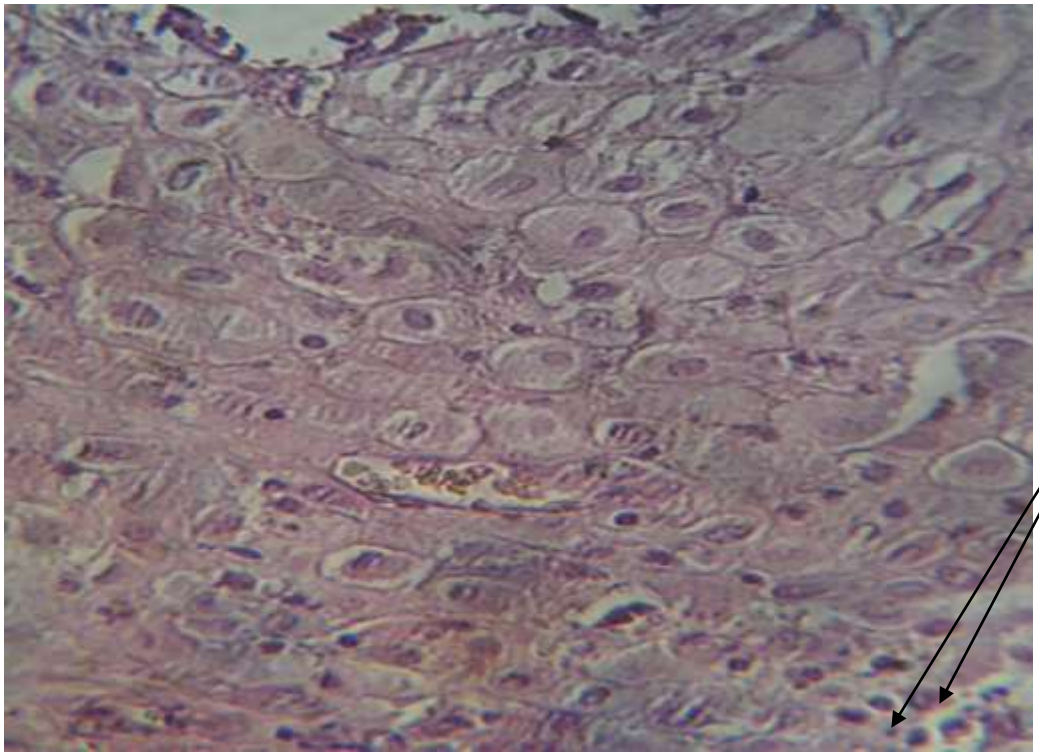

Figure (3-6): Light microscopic appearance of placenta from infected women with CMV showing Owl's eye formation (H&E X 100).

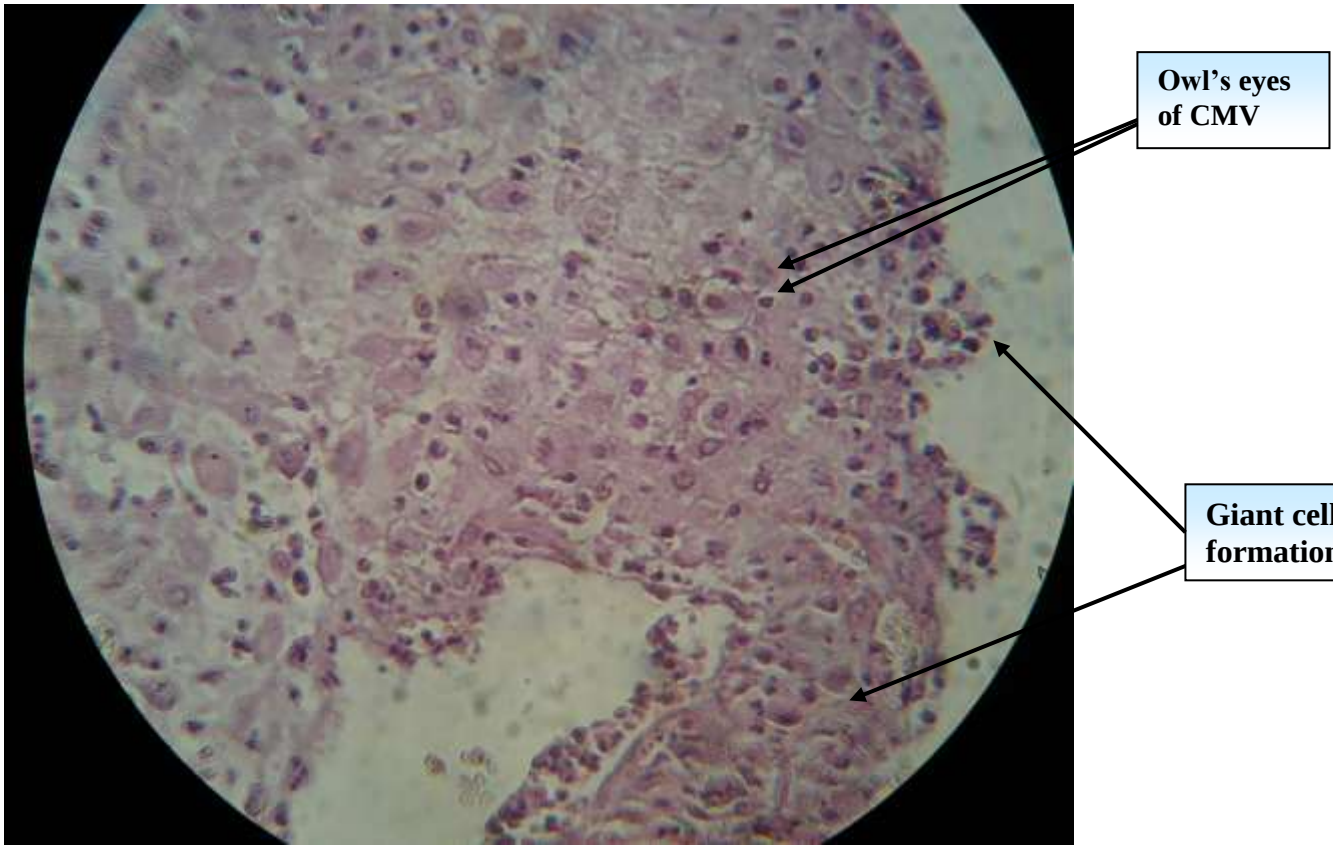

Figure (3-7): Light microscopic appearance of placenta from infected women with CMV showing Owl's eye and Giant cell formation (H&E X 100).

There were many morphological changes which have been detected in placenta section from women with *Toxoplasma* infection. One of the changes was the presence of highly significant lesion seen in the decidua and decidual cells by parasite *T. gondii*, probably because these cells are an important source of nutrient substances.

Fibrosis of the villous stroma was frequently seen in placenta affected by toxoplasmosis. This lesion was mainly attributed to a reduced blood perfusion in the villi. The villous stroma serves to support the overlying trophoplasts, provides the environment for placental vascular development and contains lymphocyte cells that act as a secondary barrier against fetal infection (Barros *et al*.,2003 and Chen and Aplin,2003).

Histopathological changes are considered as parameters indicative of the specific CMV proteins in the tissue sections. In addition to the pathological changes, giant cell formation which is a main indication due to its relation with the presence of CMV. Katlama (1993) revealed that the diagnosis of CMV disease should be assessed on the association of clinical symptoms with the presence of inclusions in biopsy specimens.

Conclusions

1. Anti- *T. gondii* (IgG) antibodies were found in 50(41.66%) cases from aborted women which are not found in healthy women (control) and this may be explain the role of *T.gondii* infection in most abortion cases.

2- Anti-CMV IgG and IgM antibodies were positive in, 35 and 8 sera respectively from 44 aborted women, whereas all control were negative and this refer to the role of CMV infection in some abortion cases.

3. The highest prevalence of *T.gondii* and CMV infections has been found in the age groups (20-29) years old.

4. The prevalence rate of *T.gondii* and CMV infections were higher in aborted women from rural areas than from urban areas.

5. There were significant differences between housewives and employed women, where the positive cases from housewives were more than those of employed women for both pathogens.

6. The highest prevalence of Toxoplasmosis has been found in the second trimester of pregnancy, whereas in the first trimester for CMV infection and in women with single abortion more than women with multiple abortions for both pathogens.

7. The specific anti-phospholipids antibody has been found in 3(10.7%) sera from aborted women and this may be refer to the role of aPL antibodies in some cases of abortion.

8. The presence of C-reactive protein has been detected in 34(34%) sera from aborted women.

9. The histological examination (hematoxylin-eosin stain) of biopsies taken from aborted women showed cyst of *T.gondii* and giant cell formation as well as owl's eye for CMV.

Recommendations

This study recommends:

1. As much as possible screening of married women before and during pregnancy for detection of anti-*Toxoplasma*(IgG & IgM) and anti-CMV (IgG & IgM) antibodies.

2. If available, using more developed and specific serological tests, as PCR, (IgG &IgM) ELISA for *Toxoplasma* and IFAT-IgM.
3. Health educating of pregnant women about the mode of *T.gondii* and CMV transmission and prevention of infection, like avoiding contact with cat feces, wearing disposable gloves during cleaning the garden, proper cooking of meat, washing of fruits and vegetables for prevention *Toxoplasma* infection and practicing good personal hygiene, specially hand washing with soap and water, after contact with diapers or oral secretions for prevention of CMV.

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