



جامعة بابل

كلية الطب

**دراسة دور داء المقوسات, فيروس تضخم
الخلايا والأجسام المضادة للدهون المفسفرة
في حالات الإجهاد في مدينة الحلة
رسالة**

**مقدمة إلى مجلس كلية الطب/جامعة بابل
كجزء من متطلبات نيل درجة الماجستير
في علم الأحياء المجهرية الطبية**

من قبل

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**University of Babylon
College of Medicine**

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

A Thesis

**Submitted to the Council of the College of Medicine - University
of Babylon In Partial Fulfillment of the Requirements
For the Degree of Master of Science
In Medical Microbiology**

By

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B.sc. Microbiology

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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 *Toxoplasma* and CMV.

The occurrence of two pathogens in relation to the residence, age and occupation has been studied. There was a higher infection among women from rural areas than from urban areas and reaching peak in the age group (20-29) years old and higher in housewives in comparison to employees(P value <0.01).

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

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.

Out of 100 samples from aborted women, 34(34%) of the samples were positive for C - reactive protein by using latex test.

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.

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Ghaida'a

DEDICATION

*To the memory of my
father*

To my mother

To my brothers

Appendix

Questionnaire form

Case No:

Date:

Address:

Name :

Age:

Occupation:

Animal contact: Cat

other diseases:

Gravity:

Abortion: number

Duration of pregnancy:

Obstetric history:

Abortion: months

No.	Age	Residence	Occupation	Trimester of pregnancy	No. of abortion
1	20yrs	Urban	Housewives	1 st trimester	A1
2	24 yrs	Urban	Housewives	1 st trimester	A1
3	23 yrs	Rural	Housewives	1 st trimester	A3
4	20 yrs	Rural	Housewives	1 st trimester	A1
5	22 yrs	Rural	Housewives	2 nd trimester	A1
6	26 yrs	Rural	Employed	2 nd trimester	A1
7	22 yrs	Rural	Housewives	1 st trimester	A2
8	27 yrs	Urban	Employed	2 nd trimester	A2
9	25 yrs	Urban	Housewives	1 st trimester	A1
10	20 yrs	Urban	Employed	2 nd trimester	A1
11	30 yrs	Rural	Housewives	2 nd trimester	A1
12	27 yrs	Urban	Employed	2 nd trimester	A1
13	22 yrs	Rural	Housewives	1 st trimester	A2
14	25 yrs	Urban	Housewives	1 st trimester	A1
15	35 yrs	Urban	Housewives	1 st trimester	A1
16	30 yrs	Urban	Housewives	1 st trimester	A4
17	27 yrs	Urban	Employed	1 st trimester	A4
18	24 yrs	Urban	Housewives	1 st trimester	A3
19	25 yrs	Rural	Employed	2 nd trimester	A2
20	21 yrs	Rural	Housewives	1 st trimester	A1
21	25yrs	Rural	Employed	3 rd trimester	A1
22	18 yrs	Rural	Housewives	3 rd trimester	A2
23	37 yrs	Urban	Housewives	2 nd trimester	A1
24	22 yrs	Urban	Housewives	1 st trimester	A1
25	32 yrs	Rural	Employed	1 st trimester	A1
26	37 yrs	Rural	Housewives	2 nd trimester	A1
27	23 yrs	Urban	Housewives	2 nd trimester	A1
28	27 yrs	Urban	Employed	2 nd trimester	A1
29	20 yrs	Urban	Housewives	3 rd trimester	A1
30	29 yrs	Urban	Housewives	2 nd trimester	A2
31	40 yrs	Rural	Employed	2 nd trimester	A1
32	25 yrs	Rural	Housewives	1 st trimester	A1
33	36 yrs	Rural	Housewives	1 st trimester	A1
34	25 yrs	Urban	Housewives	1 st trimester	A1
35	40 yrs	Rural	Housewives	2 nd trimester	A1
36	22 yrs	Urban	Housewives	1 st trimester	A2
37	25 yrs	Urban	Housewives	1 st trimester	A1
38	22 yrs	Urban	Housewives	2 nd trimester	A1
39	16 yrs	Urban	Employed	1 st trimester	A2
40	16 yrs	Urban	Housewives	2 nd trimester	A1
41	22 yrs	Rural	Housewives	1 st trimester	A1
42	16 yrs	Urban	Employed	2 nd trimester	A1
43	39 yrs	Rural	Housewives	1 st trimester	A2
44	20 yrs	Urban	Housewives	2 nd trimester	A2
45	22 yrs	Rural	Housewives	2 nd trimester	A1
46	30 yrs	Rural	Housewives	3 rd trimester	A1

47	26 yrs	Rural	Housewives	1 st trimester	A1
48	38 yrs	Urban	Employed	1 st trimester	A3
49	30 yrs	Urban	Employed	2 nd trimester	A1
50	30 yrs	Urban	Housewives	2 nd trimester	A4
51	28 yrs	Rural	Housewives	2 nd trimester	A2
52	43 yrs	Urban	Employed	1 st trimester	A4
53	35 yrs	Rural	Housewives	3 rd trimester	A3
54	38 yrs	Urban	Housewives	3 rd trimester	A1
55	37 yrs	Rural	Employed	2 nd trimester	A1
56	24 yrs	Urban	Housewives	1 st trimester	A1
57	30 yrs	Rural	Housewives	2 nd trimester	A1
58	19 yrs	Rural	Housewives	2 nd trimester	A1
59	29 yrs	Urban	Employed	3 rd trimester	A3
60	35 yrs	Urban	Employed	1 st trimester	A1
61	29 yrs	Urban	Housewives	2 nd trimester	A5
62	30 yrs	Rural	Housewives	1 st trimester	A3
63	30 yrs	Urban	Housewives	2 nd trimester	A1
64	38 yrs	Urban	Employed	2 nd trimester	A1
65	18 yrs	Urban	Employed	1 st trimester	A1
66	15 yrs	Urban	Housewives	1 st trimester	A1
67	19 yrs	Urban	Housewives	1 st trimester	A1
68	33 yrs	Rural	Employed	1 st trimester	A1
69	30 yrs	Urban	Employed	2 nd trimester	A3
70	17 yrs	Urban	Housewives	3 rd trimester	A1
71	24 yrs	Rural	Housewives	2 nd trimester	A1
72	18 yrs	Rural	Housewives	3 rd trimester	A1
73	30 yrs	Rural	Employed	1 st trimester	A1
74	23 yrs	Urban	Employed	2 nd trimester	A2
75	20 yrs	Urban	Housewives	1 st trimester	A1
76	19 yrs	Urban	Housewives	2 nd trimester	A1
77	32 yrs	Urban	Employed	1 st trimester	A3
78	28 yrs	Rural	Housewives	3 rd trimester	A3
79	18 yrs	Urban	Housewives	3 rd trimester	A2
80	33 yrs	Urban	Employed	2 nd trimester	A3
81	28 yrs	Urban	Housewives	1 st trimester	A3
82	30 yrs	Urban	Housewives	2 nd trimester	A1
83	25 yrs	Urban	Employed	3 rd trimester	A3
84	27 yrs	Urban	Housewives	2 nd trimester	A3
85	25 yrs	Rural	Housewives	1 st trimester	A1
86	30 yrs	Urban	Employed	1 st trimester	A1
87	18 yrs	Urban	Employed	3 rd trimester	A2
88	40 yrs	Rural	Housewives	1 st trimester	A1
89	33 yrs	Urban	Housewives	1 st trimester	A1
90	38 yrs	Urban	Employed	3 rd trimester	A1
91	25 yrs	Urban	Employed	1 st trimester	A1
92	20 yrs	Urban	Housewives	1 st trimester	A1
93	29 yrs	Urban	Housewives	1 st trimester	A3
94	22 yrs	Urban	Employed	1 st trimester	A1

95	20 yrs	Urban	Employed	1 st trimester	A1
96	37 yrs	Urban	Employed	1 st trimester	A2
97	20 yrs	Rural	Employed	2 nd trimester	A1
98	30 yrs	Urban	Employed	3 rd trimester	A5
99	24 yrs	Rural	Housewives	1 st trimester	A1
100	21 yrs	Urban	Employed	2 nd trimester	A1
101	29 yrs	Urban	Housewives	1 st trimester	A5
102	23 yrs	Urban	Employed	1 st trimester	A2
103	27 yrs	Urban	Housewives	3 rd trimester	A2
104	35 yrs	Urban	Employed	1 st trimester	A1
105	40 yrs	Urban	Housewives	3 rd trimester	A3
106	16 yrs	Rural	Employed	2 nd trimester	A1
107	25 yrs	Rural	Housewives	2 nd trimester	A1
108	19 yrs	Rural	Housewives	2 nd trimester	A1
109	36 yrs	Rural	Employed	1 st trimester	A2
110	26 yrs	Urban	Housewives	1 st trimester	A2
111	28 yrs	Urban	Employed	1 st trimester	A2
112	17 yrs	Urban	Housewives	2 nd trimester	A1
113	41 yrs	Urban	Housewives	2 nd trimester	A1
114	33 yrs	Urban	Employed	2 nd trimester	A1
115	30 yrs	Urban	Employed	1 st trimester	A1
116	22 yrs	Urban	Housewives	2 nd trimester	A1
117	39 yrs	Urban	Employed	1 st trimester	A3
118	20 yrs	Urban	Employed	1 st trimester	A1
119	22 yrs	Rural	Housewives	2 nd trimester	A1
120	30 yrs	Rural	Housewives	2 nd trimester	A1

A=Abortion Yrs=Years

Healthy women (control) group

No.	Age	Residence	Occupation	Pregnancy	Trimester of pregnancy	No. of abortions
1	25yrs	Urban	Housewife	-ve	-	-
2	41yrs	Rural	Housewife	-ve	-	-
3	23 yrs	Rural	Housewife	-ve	-	-
4	16 yrs	Urban	Housewife	-ve	-	-
5	30 yrs	Urban	Housewife	-ve	-	-
6	23 yrs	Rural	Housewife	+ve	2 nd trimester	-
7	40 yrs	Urban	Employed	-ve	-	-
8	35 yrs	Urban	Housewife	+ve	1 st trimester	-
9	18 yrs	Urban	Housewife	+ve	1 st trimester	-
10	37 yrs	Urban	Housewife	+ve	2 nd trimester	-
11	30 yrs	Urban	Employed	+ve	1 st trimester	-
12	20 yrs	Urban	Housewife	+ve	3 rd trimester	-
13	41yrs	Urban	Housewife	+ve	2 nd trimester	-
14	17 yrs	Urban	Housewife	+ve	3 rd trimester	-
15	37 yrs	Rural	Housewife	+ve	1 st trimester	-
16	25 yrs	Rural	Housewife	+ve	1 st trimester	-
17	19 yrs	Rural	Housewife	+ve	1 st trimester	-
18	23 yrs	Urban	Employed	+ve	1 st trimester	-
19	31 yrs	Urban	Employed	+ve	1 st trimester	-
20	22 yrs	Rural	Housewife	-ve	-	-

-ve=negative +ve=positive

We certify that this thesis was prepared under our supervision at the University of Babylon as partial Fulfillment of the requirements for the degree of M.Sc. in Medical Microbiology.

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Recommendations of The Head of Department of Microbiology:-

According to the available recommendations, we forward this thesis for debate by the examining committee.

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Date: / /2008

Decision of discussion committee

We, the examining committee, certify that we have read the thesis entitled (A study the role of Toxoplasmosis, Cytomegalovirus and anti-Phospholipids antibodies in cases of abortion among women in Hilla city) and have examined the student (Ghaida'a Jehadi Mohammed) in its contents, and that in our opinion; it is accepted as a thesis for the degree of Master of Science in Medical Microbiology.....

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Abbreviations

Symbol	Description
APP	Acute phase proteins
AIDS	Acquired immunodeficiency syndrome
AJCN	American journal of clinical nutrition
ApL	Anti-phospholipid antibody
CDC	Center of disease control and prevention
CNS	Central nervous system
CSF	Cerebral spinal fluid
CD	Cluster of differentiation
CFT	Complement fixation test
CRP	C-reactive protein
DT	Dye test
EM	Electron microscopy
EIA	Enzyme immuno assay
ELISA	Enzyme linked immunosorbent assay
d.f	Degree of freedom
DNA	Deoxy ribonucleic acid
gB,gH	Glycoprotein B, Glycoprotein H
HRP	Horseradish peroxidase
hr	hour
HCMV	Human cytomegalovirus virus
HIV	Human immuno deficiency virus
HCL	hydrochloric acid

H ₂ O ₂	Hydrogen peroxidase
IFA	Immunoflouresence assay
IFAT	Indirect fluorescent antibody technique
IgG	Immunoglobulin G
IgM	Immunoglobulin M
ISAGA	Immunosorbent agglutination assay
IHAT	Indirect hemagglutination test
IL	Interleukin
IU\ml	International unit per milliliter
LAT	Latex agglutination test
MCAF	Macrophage chemotactic and activating factors
MPL	IgM phospholipid units
mRNA	Messenger ribonucleic acid
O.D.	Optical density
PBS	Phosphate buffer solution
PCR	Polymerase chain reaction
P22,P30	Protein 22, Protein30
r.p.m	Round per minute
SLE	Systemic lupus erythematosus
TMB	Tetra methylbenzidine substrate solution
TORCH	<i>Toxoplasma</i> - Other- Rubella- Cytomegalovirus- Herpes simplex virus

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 *Toxoplasma* and CMV.

The occurrence of two pathogens in relation to the residence, age and occupation has been studied. There was a higher infection among women from rural areas than from urban areas and reaching peak in the age group (20-29) years old and higher in housewives in comparison to employees(P value <0.01).

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

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.

Out of 100 samples from aborted women, 34(34%) of the samples were positive for C - reactive protein by using latex test.

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.

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.

Conclusions & Recommendations

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.

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).

Both the human cytomegalovirus (CMV) and protozoon *T. gondii* are extremely common human pathogens. Depending on hygiene standards and habits, socioeconomic parameters and population demographic structure, 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). Here, the activation may lead to dissemination of CMV or *Toxoplasma* to various tissues, including those of the nervous system (Cinque *et al.*,1997).

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).

Antiphospholipid antibodies are heterogeneous group of antibodies with different characteristics and clinical associations (Parazzni *et al.*,1991).

Aims of study

The studies performed on the association of *Toxoplasma*, CMV and anti-phospholipids antibodies and their relationship to abortion are very little so, this study is aimed 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: Eimeriidae

Subfamily: Toxoplasmatinae (cyclosporidae)

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 8 μm 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 excreted 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, 10-200 μm 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).

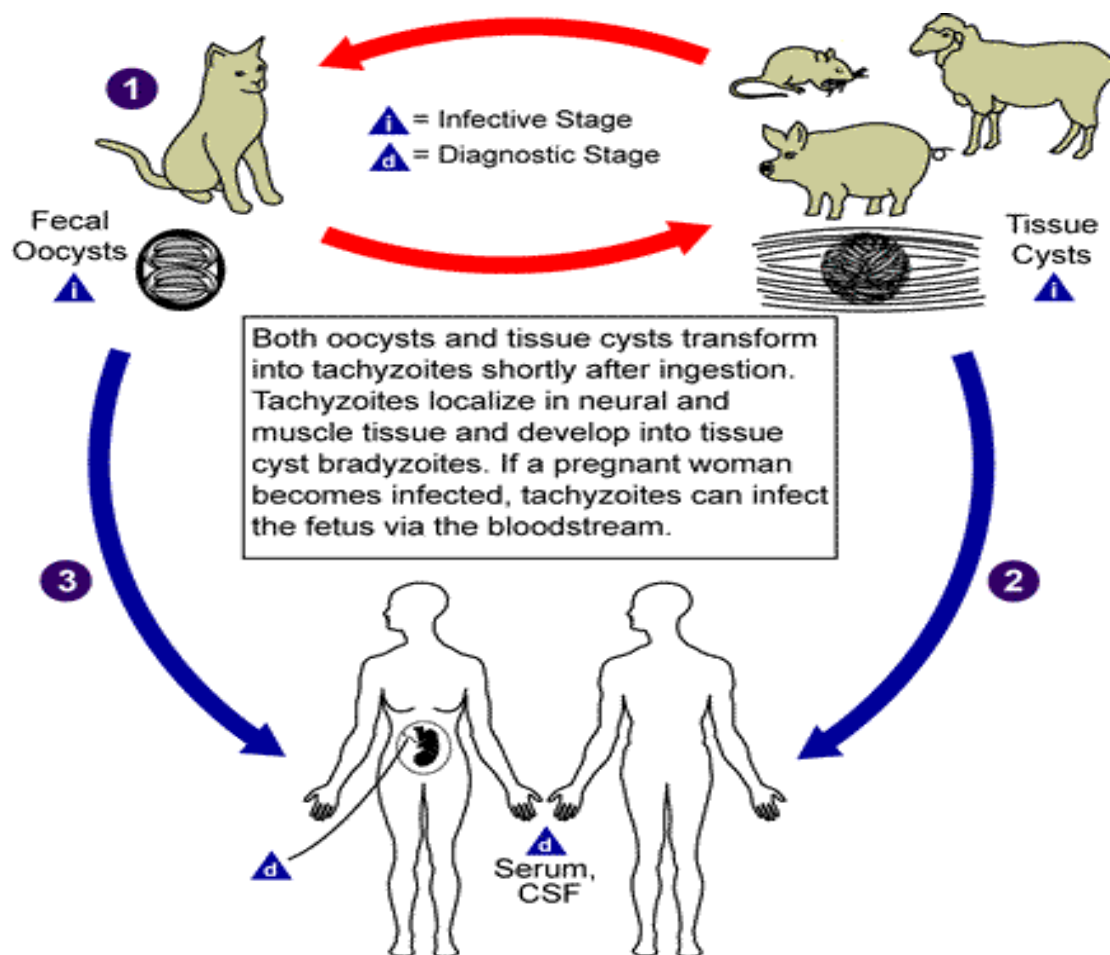


Figure (1-1): Life Cycle of *T.gondii* (Cohen *et al.*,2004;John and Petri,2006).

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 immunocompetent 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 chorioretinitis 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; Scharon-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 glycoprotein B. 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 162 capsomeres, 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 immunopathological 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 immunocompetent 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 trophoblast 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- α , 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).

Materials & Methods

2-1 Study design and study groups

2-1-1 Patients

In this study 120 aborted women were selected randomly to study the role of *T. gondii* and CMV in their abortions and the possible association between these two infections, and also to detect if auto antibodies for phospholipids have any relation that can be considered as other causative agent of abortion and its relation with anti-*Toxoplasma* and anti-CMV antibodies in aborted women with these antibodies. Patients were from visitors and in hospital patients of Maternity and Children Hospital and Hilla Teaching Hospital .The age of patients ranged between (15- 40) years old. The mean age was 27 years old. A blood sample was taken from each patient, while placental biopsies were taken from 50 patients only.

The study was conducted from November 2006 to April 2007. The serum samples were separated from blood samples and stored at - 20°C in the laboratory of Public Health; where the ELISA test was done. Each biopsy specimen was used for histological examination with hematoxylin–eosin stain, which was done in Hilla Teaching Hospital.

2-1-2 Healthy women (control) group

Twenty healthy women were selected randomly as a control group. They were 13 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.

2-2 Materials

2-2-1 Equipments and tools

The following equipments and tools were used in this study:

Device	Company	Origin
Automatic Pipette 5,50,100,500µl	Slamed	Germany
Centrifuge	Heareus	Germany
ELISA Reader	Beckman coulter	Austria
Freezer	Liebher	Austria
Incubator	Heareus	Austria
Microscope	Olympus	Japan
Microtiter plate washer	Beckman coulter	Austria
Placental biopsies forceps	Olympus	Japan
Refrigerator	Liebher	Austria

2-2-2 Latex agglutination kit (LAT kit)

The using kits were Toxocell latex from Biokit co. (Spain).

2-2-2-1 The contents of latex agglutination kit:**1-Latex reagent:**

Suspension of polystyrene latex particles coated with *T.gondii* soluble antigen in a buffer containing bovine serum albumin. Contains <0.1 % sodium azide.

2- Positive control:

Diluted human serum containing rabbit IgG anti-*Toxoplasma*. Contains <0.1% sodium azide.

3- Negative control:

Non reactive diluted human serum. Contains <0.1% sodium azide.

4- Disposable slides: 18×6 units.**2-2-3 Cytomegalovirus (CMV) IgG and IgM ELISA kits:**

The ELISA Cytomegalovirus (IgG, IgM) kits were produced by BC (BioCheck) 323 Vintage Park Dr. Foster City, CA94404 (USA).

2-2-3-1 Contents of the test kit:*A) Cytomegalovirus IgG ELISA kit:*

- **Microtiter strips:** Strips with 8 breakable wells each coated with purified CMV antigen.
- **Negative calibrator**

- **Positive calibrator**
- **Cut-off calibrator:** CMV/IgG index= 1.0
- **Negative control**
- **Positive control**
- **Enzyme conjugate reagent:** Anti-human-IgG-HRP (rabbit), conjugated to protein-containing buffer solution.
- **Tetramethylbenzidin (TMB) substrate solution**
- **TMB stop solution:** 1N HCl.
- **Sample diluent:** PBS buffer.
- **Washing buffer concentrates (20x):** PBS+Tween 20, 20x concentrate.
- **Plastic foils:** 2 pieces to cover the microtiter strips during the incubation.
- **Plastic bag:** Resealable, for the dry storage of non-used strips.

B) Cytomegalovirus IgM ELISA kits:

- **Microtiter strips:** Strips with 8 breakable wells each coated with purified CMV antigen.
- **Cut-off calibrator:** CMV/IgM index = 1.0
- **Negative control:**
- **Positive control:**

- **Enzyme conjugate reagent:** Anti-human-IgM-HRP (rabbit), conjugated to protein-containing buffer solution.
- **Tetramethylbenzidin (TMB) substrate solution**
- **TMB stop solution:** 1N HCl
- **Sample diluent:** PBS buffer.
- **Washing buffer concentrates (20x):** PBS+Tween 20, 20x concentrate.
- **Plastic foils:** 2 pieces to cover the microtiter strips during the incubation.
- **Plastic bag:** Resealable, for the dry storage of non-used strips.

2-2-4 C - reactive protein (CRP) kit:

The used kit was CRP- latex slide agglutination provided from Spinreat, S.A.Ctra.santaColoma, 7E-17176 Sant Esteve De Bas (GI) Spain.

2-2-4-1 The contents of C - reactive protein Kit:

Reagents:

- **Latex:** Latex Particles coated with goat IgG anti-human CRP, PH, 8.2. Sodium azide 0.95 g/L.
- **Positive control (red cap):** Human serum with a CRP concentration > 20 mg/L. Sodium azide 0.95 g/L.

- **Negative control (blue cap):** Animal serum. Sodium azide 0.95 g/L.
- **Disposable slides:** 18×6 units.

2-2-5 Anti-Phospholipids antibody kit:

The ELISA anti-phospholipids antibody kit was Aeskulisa antiphospholipid-GM REF 7204 provided by Aesku. Diagnostics Gmbh Mikroforum Ring 2 55 234 Wendelsheim (Germany).

2-2-5-1 The contents of anti-Phospholipid antibody Kit:-

- **5x Sample buffer**
- **50x Wash buffer**

Ready to use:-

- **Negative control**
- **Positive control**
- **Cut-off control**
- **Calibrators**
- **Conjugates:-**Containing anti-human immunoglobulins conjugated to horseradish peroxidase and thimerosal 0.01% (Preservative).
- **TMB substrate:-** Containing stabilized TMB/H₂O₂
- **Stop solution:** -Containing 1M hydrochloric acid
- **Microtiterplate:** - 12x8 well strips with breakaway microwells.

2-3 Methods

2-3-1 Collection of Blood samples

Five ml blood samples were obtained by vein puncture from all studied women after cleaning the skin with 70% alcohol. Blood samples were stored in plastic tubes and left to clot undisturbed for about 1hr at room temperature. Then they were centrifuged for about 5 min at 3000 r.p.m. and then the serum was transferred into other tubes(Voler and Bidwell,1985;Cremer,1985;Starr and Friedman, 1985). After the serum samples were collected, they were stored at – 20°C until they were tested by Latex agglutination for anti *T. gondii* antibody and C-reactive protein and by ELISA for quantitative determination of anti Cytomegalovirus IgG and IgM antibodies, and anti-phospholipids IgM antibody.

2-3-2 obtaining of biopsies

Biopsies were taken from the placenta of aborted women, 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).

2-3-3 Detection of anti-Toxoplasma (IgG) antibody:-

2-3-3-1 Latex agglutination test:

Toxocell latex is a one step rapid latex particles agglutination test on slide for qualitative and semi quantitative determination of *Toxoplasma* antibodies in serum.

2-3-3-1-A Procedure (qualitative test)

Procedure of the test was performed according to the manufacturer's instructions (Gray *et al.*,1990; Dunford and Johnson ,1991)as follows:

- 1- Allowing the reagent and sera to reach room temperature (20° to 30°C).
- 2- Shacking the reagent vial to disperse and suspend the latex particles in the buffer solution.
- 3- Placing 50 µl of the serum on one section of the disposable slide.
- 4- Adding one drop of reagent next to the drop of serum.
- 5- Mixing both drops with a stirrer covering the whole surface of the slide section.
- 6- Gently rotating the slide for 5 minutes manually.
- 7- Looking for the presence or absence of agglutination.

2-3-3-1-B Interpretation of the results

The results interpreted according to (Gray *et al.*,1990; Dunford and Johnson,1991)

A homogenous appearance (negative reaction) should be interpreted as the absence of *Toxoplasma* antibodies, the titer lower than 10-15 IU/ml.

A clear agglutination (positive reaction) should be interpreted as presence of *Toxoplasma* antibodies, which may reflect either a past infection or an evolving *Toxoplasma* infection.

◆ Positive reactions

3+ Large clumps with clear background.

2+ Moderate clumps with fluid slightly opaque in background.

1+ Small clumps with opaque fluid in background.

◆ Negative reactions

No visible clumping, uniform suspension.

2-3-3-2 Semiquantitative technique:

The approximate titer corresponds to the highest serum dilution that still presents a clearly visible agglutination. Results were read after 5 minutes at well lightened room by naked eye or using magnifying lens.

The diluent used was normal saline 0.9%. The titer 1/32 dilution, if it was still strongly positive more dilutions can be made by using

another slide starting from dilution 1/32 then 1/64 and soon. Results were obtained after rotating the slides for five minutes (Gray *et al.*,1990; Dunford and Johnson,1991).

2-3-4 Detection of anti-CMV (IgG & IgM) antibody:-

2-3-4-1 ELISA procedure

BioCheck instructions in ELISA of IgG (Catalog Number: BC-1089), and IgM (Catalog Number: BC-1091) (Cremer,1985;Voler and Bidwell,1985; Starr and Friedman,1985) were followed:

2-3-4-1-A -Preparation of samples and reagents

1) Samples

Before use, the patients' serum samples were diluted 1:40 with buffer serum diluent (the best chequre-board titration) (5µl of sample + 200µl buffer).

2) Wash buffer

The wash buffer was diluted with distilled water 1 to 20 (10 ml concentrate + 190 ml distilled water).

2-3-4-1-B Assay procedure

1. A sufficient amount of microtiter wells was prepared for the standard controls and samples.
2. 1:40 dilutions of test samples, Negative Control, Positive Control, and Calibrator by adding 5µl of the sample to 200µl of sample diluent were prepared.

3. 100µl of diluted sera, Calibrator, and Controls were dispensed into the appropriate wells of the strip.
4. The plates were covered with the enclosed foil and incubated at 37°C for 30 minutes.
5. At the end of incubation period, liquid was removed from all wells. The microtiter were rinsed and flicked 4 times with diluted wash buffer (1x) and then once with distilled water.
6. 100µl of Enzyme conjugate was added to each well in sequence.
7. The plates were covered with enclosed foil and incubated for 30 minutes at 37°C.
8. We rinsed and flicked the microtiter wells 4 times with diluted wash buffer (1x) and then one time with distilled water.
9. 100 µl of TMB reagent was added into each well.
10. The plates were covered with enclosed foil and incubated for 15 minute at 37°C.
11. The reaction was stopped by adding 100 µl of TMB stop solution to each well.
12. The microtitter strips were shaken gently and the reading was taken at 450 nm within 15 minutes.

2-3-4-2 Calculation of results

The results calculated according to (Cremer, 1985; Voler and Bidwell, 1985; Starr and Friedman, 1985).

- 1- Cut-off calibrator value x_c was calculated.

- 2- Positive control (x_p), negative control (x_n) and patient samples (x_s) were calculated.
- 3- The CMV IgG and IgM Index of each determination by dividing the values of each sample (x) by calibrator value (x_c) were calculated.

2-3-4-3 Quantitative determination of CMV IgG

For a quantitative determination of anti-CMV IgG levels of positive specimens in IU/ml, the O.D. of cut-off, negative, and positive calibrators are plotted on the Y-axis of a graph against their corresponding anti-CMV IgG concentrations of 0, 1.2, 6, and 18 IU/ml on the X-axis. The estimates of levels in patient sera are read off the graph using their individual O.D. values (Cremer, 1985; Voler and Bidwell, 1985; Starr and Friedman, 1985).

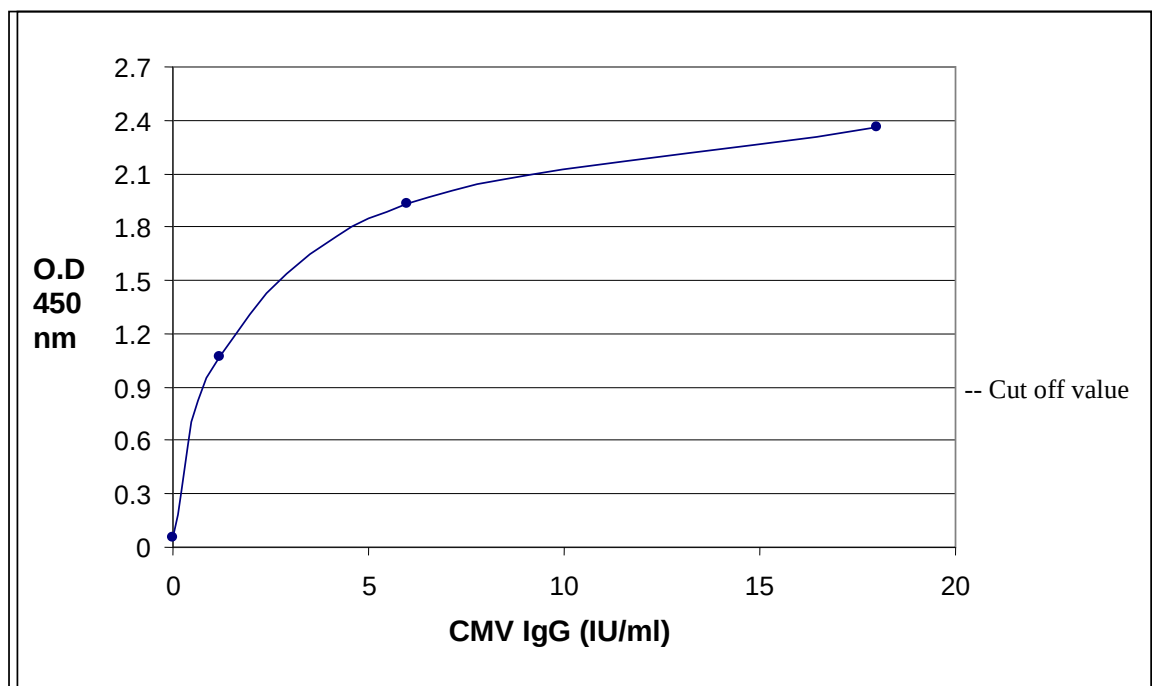


Figure (2-1): Standard Curve of anti- CMV IgG (positive ≥ 1 and negative < 0.9).

2-3-4-4 Interpretation of the results

The results of each sample were assessed according to (Voler and Bidwell,1985;Cremer,1985;Starr and Friedman,1985) as follows:

1. Negative: CMV IgG and IgM Index less than 0.90 are seronegative for IgG and IgM antibody to CMV (<1.2 IU/ml).
2. Equivocal: CMV IgG and IgM Index between 0.91-0.99 are equivocal. Sample should be retested.
3. Positive: CMV IgG and IgM Index of 1.00 or greater, or IU value greater than seropositive (>1.2 IU/ml).

2-3-5.C - reactive protein (CRP-latex) slide agglutination:-

The CRP –latex is a slide agglutination test for the qualitative and semi-quantitative detection of C-reactive protein (CRP) in human serum. Latex particles coated with goat IgG anti-human CRP are agglutinated when mixed with samples containing CRP.

2-3-5-1 Procedure (qualitative method)

The procedure was performed according to manufacture instructions (Chetana,1996 and Lars-Olof *et al.*,1997) as follows:

1. Allowing the reagents and samples to reach room temperature.
2. Placing 50µl of the sample and one drop of each positive and negative control into separate circles on the slide test.

3. Swirling the CRP-latex reagent gently before using and added one drop (50µl) next to the sample to be tested.
4. Mixing the drops with a stirrer, spreading them over the entire surface of the circle .Using different stirrer for each sample.
5. Rotating the slides for 2 minutes (False positive can appear if the test is read later than two minutes).

2-3-5-2 Reading and interpretation

The presence or absence of visible agglutination immediately after rotating the slide was examined macroscopically. The presence of agglutination indicates a CRP concentration equal or greater than 6 mg/L (1-The strength of agglutination is not indicative of the CRP concentration in the samples tested; 2- Clinical diagnosis should not be made on findings of a single test result, but should integrate both clinical and laboratory data)(Chetana,1996 and Lars-Olof *et al.*,1997).

2-3-6 Detection of anti-Phospholipids antibody:-

Aeskulisa kit instructions in ELISA of IgM (Catalog Number: REF-7204) (Wohrle *et al.*,2000) were followed:

2-3-6-1- Preparation of samples and reagents

1) Reagents

- The concentrated sample buffer was diluted 1:5 with distilled water (20 ml sample buffer plus 80 ml distilled water).

- The concentrated wash buffer was diluted 1:50 with distilled water (20 ml wash buffer plus 980 ml distilled water).

2) Samples

Before use, the patient's serum samples were diluted 1:101 with sample buffer diluent (the best chequre-board titration) (10µl of sample + 1000µl buffer).

2-3-6-2 Assay procedure

The procedure was performed according to manufacture instructions (Wohrle *et al.*,2000) as follows:

1. A sufficient amount of microtiter wells was prepared for the standard controls and samples.
2. Allowing all components to reach room temperature (20-26 °C/64-78.8 °F) before use, mixed well.
3. Pipetting 100µl of each patient's diluted serum into the designated microwells.
4. Pipetting 100µl calibrators or cut-off control, negative and positive controls into the designated wells.
5. Incubating for 30 minutes at room temperature (20-26°C/64-78, 8°F).
6. Washing 3 times with 300 µl washing buffer (diluted 1:50).
7. Pipetting 100µl conjugate into each well.
8. Incubating for 15 minutes at room temperature (20-26°C/64-78.8 °F).

9. Washing 3 times with 300µl washing buffer (diluted 1:50).
10. Pipetting 100 µl TMB substrate into each well.
11. Incubating it for 15 minutes at room temperature (20-26°C/64-78.8 °F), in the dark.
12. Pipetting 100µl stop solution into each well, using the same order as pipetting the substrate.
13. Incubating it for 5 minutes minimum.
14. Agitating plate carefully for 5sec.
15. Reading absorbance at 450 nm (optionally 450/620nm) within 30 minutes.

2-3-6-3 Quantitative and qualitative interpretation:

The results were interpreted according to (Wohrle *et al.*,2000). For quantitative interpretation, the standard curve by plotting the optical density (O.D.) of each Calibrator (Y-axis) with respect to the corresponding concentration values in MPL/ml (x-axis) was established. From the O.D. of each sample, the corresponding antibody concentrations expressed in MPL/ml was read.

Normal Range	Positive Results
≤15 MPL/ml	>15 MPL/ml

For **qualitative interpretation** the optical density of the cut-off control and the patient samples were read. Compared patient's O.D. with the O.D. of the cut-off control, all samples which are higher than cut-off are considered positive.

2-3-7 Procedure of histopathological changes assessment:-

The slides of biopsy were prepared according to the procedure given by Qais *et al.*(1990).

2-3-7-1 Schedule for automatic tissue processor: -

The specimens were processed as follows:-

- | | |
|-----------------------------|---------------|
| 1. (2 hrs) formalin | |
| 2. (1 hr) 70% alcohol | |
| 3. (1 hr) 90% alcohol | |
| 4. (2 hrs) absolute alcohol | First change |
| 5. (2 hrs) absolute alcohol | Second change |
| 6. (2 hrs) absolute alcohol | Third change |
| 7. (1 hr)Xylol | First change |
| 8. (2 hrs)Xylol | Second change |
| 9. (2 hrs)Xylol | Third change |
| 10. (2 hrs) Paraffin wax | First change |
| 11. (2 hrs) Paraffin wax | Second change |
| 12. (2 hrs) Paraffin wax | Third change |

2-3-7-2 Scheme for rapid method (Urgent tissue): -

Thin slices of tissue (3-5mm thick) were used.

1. It was heated in formalin saline (just of the boil) 10-15 min.
2. It was dehydrated in acetone at 56°C in wax oven, changed several times in 2 hours.
3. It was cleared in Xylol at 56 °C (2 changes) 20-25 minutes.
4. It was impregnated with wax (several changes) 1.5-2 hours.

Preferably vacuum embed for 1-2 hours, blocked out and cut.

2-3-7-3 Hematoxylin and eosin staining method:-

1. Sections were dewaxed in Xylol, then treated with graded alcohol and placed in water.
2. It was stained in hematoxylin for 3-10 minutes.
3. It was washed well in running tap water.
4. It was removed excess stain by differentiating in 1% acid alcohol (1% HCl in 70% alcohol) for 5 to 10 minutes.
5. It was washed well in tap water until sections regained blue color, 5 minutes.
6. It was stained in 1% eosin for 2-5 minutes.
7. It was dehydrated slowly through gradated alcohol to Xylol.
8. It was mounted in Canada Balasm.

Two pathologists examined the prepared slides, in order to read the tissue changes.

2-3-8 Statistical analysis

The chi-square (X^2) test was used as a test of significance and regression was also used (to determine whether there was an association). 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 (Hopkins, 2002).

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).

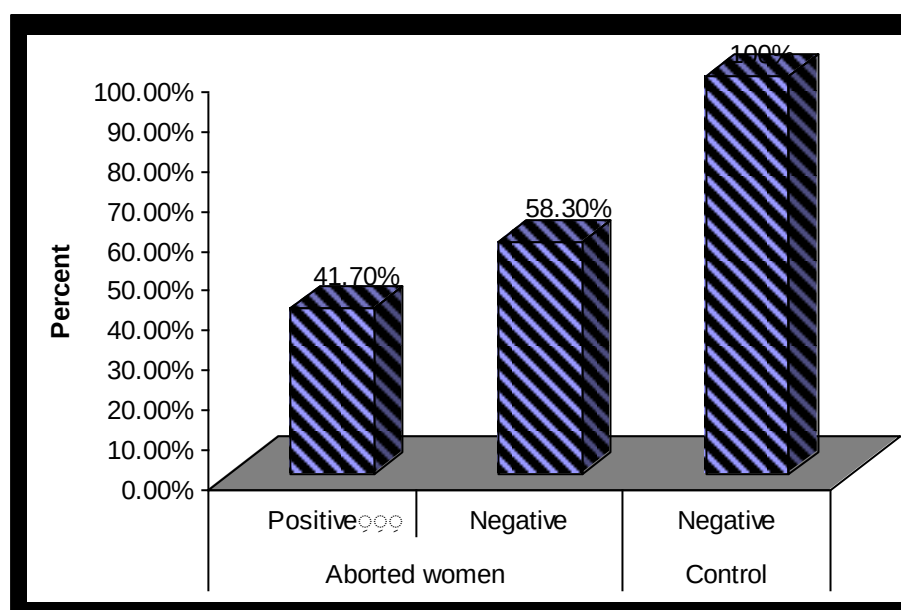


Figure (3-1): Distribution of the samples subjected to *Toxoplasma Latex agglutination test*.

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). This similarity may belong to availability of the same appropriate conditions including temperature and humidity that allow to the longer infectivity and viability of the oocysts which considered as a main source for infection spread in these cities. But it is lower than that reported in Mosul,(69.2%) by Al-Khaffaf(2001) and (49.85%) by Al-Wattari(2005), in Duhok,(46.9%) by Al-Doski(2000) 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-Qurashi(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).

3.1.1. Distribution of anti-Toxoplasma (IgG) antibodies among the different age groups:

Table (3-1) illustrates the distribution of anti-*Toxoplasma* (IgG) antibodies titer by taking the total number of patients in each age group; which shows the highest number of patients (15) was in the age groups (20-24) and (25-29) years old, while the lowest number (1) was in the age group ≥ 40 years old and as cleared the titer of these positive cases range from 20 to 640 IU/ml.

All titers that were in 20 and 40 may represent previous exposure to *T.gondii* and not possibly related to active infection. However the titer category in the 80 and 160 may be related to exposure and chronic infection. While the 320 and 640 may represent activation of previous infection or acute infection that is possibly resulted in abortion as mentioned by Jones *et al.*(1996).

Table (3-1):Anti-*Toxoplasma* antibodies titer by Latex agglutination test according to age groups.

Age groups (Years)	No. examined	Positive No.	IgG Titer(IU/ml)					
			20	40	80	160	320	640
<20	16	5	1	1	2	-	1	-
20-24	32	15	3	4	1	1	2	4
25-29	29	15	2	7	1	-	-	5
30-34	21	8	2	4	2	-	-	0
35-39	16	6	1	2	-	-	-	3
≥40	6	1	1	-	-	-	-	-
Total	120	50	10	18	6	1	3	12

Tabulated $\chi^2=15.08$, Calculated $\chi^2=19.11$, d.f=5, P value<0.01

All women entered in this study were investigated just after abortion in the hospital. Most of the aborted women were in the age groups (20-29) years old, this finding may be due to that this age group represents optimum period for fertility. Thus this critical period of women's life has higher chances for activation of latent infection of *T.gondii* that can be transmitted vertically to the fetus which is considered as one cause of abortion as mentioned by Remington *et al.* (2001) and as clarified in the above table, the highest number of patients from (15) positive cases in this age group (20-29) years old recorded have a titer of (40 IU/ml); this titer may explain previous

latent infection or patients under treatment that lead to reduced neutralizing antibodies. However, the parasite is an opportunistic organism which remains in the body in a latent form, and reactivates during depression of immunity such as that occurs during pregnancy where there are certain physiological changes in the body and weakness which lead to activation of latent parasite thus during this period higher titer can be determined as a marker for acute infection or reactivation of latent infection which has a role in abortion.

According to the previous data in the above table, it has been concluded that *Toxoplasma* infection is associated with age and this is verified statistically (P value <0.01) and this result is consistent with almost all other studies, as the study of Al-Doski(2000) and Al-Ani(2004) in Iraq, Tabbara *et al.*(1999) in Saudi Arabia, Coelho *et al.*(2003) in Brazil.

3.1.2. The residence distribution for positive samples by Toxoplasma Latex agglutination test:

The role of residency in influencing the prevalence of *Toxoplasma* infection has been studied. There were a higher number of patients in urban areas (29) than in the rural ones (21). The titer of the positive cases in rural and urban areas ranged from 20 to 640 IU/ml as presented in table (3-2).

Table (3-2):Anti-*Toxoplasma* antibodies titer by Latex agglutination test according to residency.

Residency	NO. examined	Positive NO.	IgG Titer(IU/ml)					
			20	40	80	160	320	640
Urban	77	29	4	10	4	1	2	8
Rural	43	21	3	4	1	2	5	6
Total	120	50	7	14	5	3	7	14

Tabulated $\chi^2=6.63$, Calculated=1.28, d.f=1, P value >0.01

As shown from these data in the above table, the association between the residency and prevalence of *Toxoplasma* antibodies is not significant (P value >0.01). In spite of the number of women patients from urban areas was higher but it represents only 37.6% from the total urban women. However, 48.8% of the rural women were with positive anti *Toxoplasma* antibody titer and this finding may belong to the fact that many women in the rural regions live in a poor sanitary, low personal hygiene, in adequate proper treatment and low educational level about risk factors that lead to transmission of *T. gondii*. The role of health education was considered by James and Daniel(2000) as an important factor in decreasing the incidence of this infection. The urban areas in Hilla city is a mixture of different

socioeconomic classes. So, many women in urban regions live in a poor sanitary and low socioeconomic state as or even less than many of rural areas included in the present study that have resulted in non significant difference in the number of infected aborted women. From urban women there were 14 out of 29 positive cases had a titer of 20 and 40 which might be refer to previous exposure in these women or may be on treatment. However, the rest 15 positive cases recorded have a titer ranging between 80 to 640 that may be refer to previous (latent) infection in titer 80 and 160 or may be reactivation of latent infection or acute infection in titer 320 and 640. Whereas from rural women there were 7 from 21 positive cases had a titer of 20 to 40 and the rest 14 positive cases recorded have a titer ranging from 80 to 640. This result is consistent with Ageel(2003) in Tikrit, who found that the seropositivity was higher in rural areas (36.36%) than urban ones (32.05%) and Othman(2004) in Kirkuk who found that the seropositivity was higher among pregnant women from rural areas (50.0%) than those in urban areas (33.5%). This may be attributed to the presence of indoor cats, or even the stray one in the rural areas more than in the urban ones, which is considered as a final host for *T.gondii* and a main source for spread the infectious oocyst in the soil, vegetables, fruits and water that used for daily consuming. But it is not consistent with a study in Ninewah by Ahmed(1995), who

found a higher prevalence among the urban resident (38%) than the rural one (29%), also in Osorno (Chile) by Zamaorano *et al.*(1999) who found a slight higher prevalence in the urban areas than the rural ones.

3.1.3. The distribution of *Toxoplasma* seropositivity according to occupation of aborted women (employees and housewives):

Regarding the distribution of *Toxoplasma* seropositivity according to occupation, it was found that 37(49.3%) of housewives carry specific anti-*T.gondii* antibody with titer ranging between 20 to 640IU/ml; while only 13(29%) of employed women were with specific anti-*T.gondii* antibody recorded have a titer of 40 and 640 IU/ml. These findings are clarified more in table (3-3).

Table(3-3):Anti-*Toxoplasma* antibodies titer by Latex agglutination test according to occupation.

Occupation	No. examined	Positive No.	IgG Titer(IU/ml)					
			20	40	80	160	320	640
Housewives	75	37	6	12	5	2	3	9
Employees	45	13	-	8	-	-	-	5
Total	120	50	6	20	5	2	3	14

Tabulated $\chi^2=6.63$, Calculated $\chi^2=11.52$, P value<0.01, d.f=1

From housewives 18 out of 37 positive cases recorded have a titer of (20-40) that refers to previous exposure in these women or may be on treatment .However, the rest 19 positive cases show a titer ranging between (80-640) that might be refer to previous infection in titer 80 and 160 or activation of previous infection or acute infection in titer 320 and 640. While there were 8 employed women having a titer of (40) and 5 a titer of (640) from 13 positive cases.

According to these data in the above table, there is a significant association with housewives (P value <0.01), where the housewives are exposed to infection more than employees. This finding might be due to the fact that they were likely to be of low education about the routes of transmission, also being in direct contact with infection through handling and preparing of food (contaminated meat and vegetables) in addition to cleaning of house garden contaminated with cat feces containing oocyst. This result is in accordance with Al-Waely (1998), who found that the *Toxoplasma* seropositivity among housewives in Baghdad province was (49.5%); this similar results may belong to the highest number of housewives in comparing to employees who included in these studies. But it is not in accordance with a result reported by Al-Hamdani and Mahdi(1996) in Basra who did not find relationship between *Toxoplasma* infection and occupations; this dissimilarity may belong to that housewives and employees in this study may be have the same chance for exposure to infection sources.

3.1.4. The distribution of Positive samples by *Toxoplasma Latex* agglutination test according to trimester of pregnancy:

Figure (3-2) demonstrates the rate of infections according to trimester of pregnancy. It is observed that there is a slight increase in the second trimester, where there are 25(50%) women as compared to 19(38%) and 6(12%) women patients in the first and third trimesters respectively.

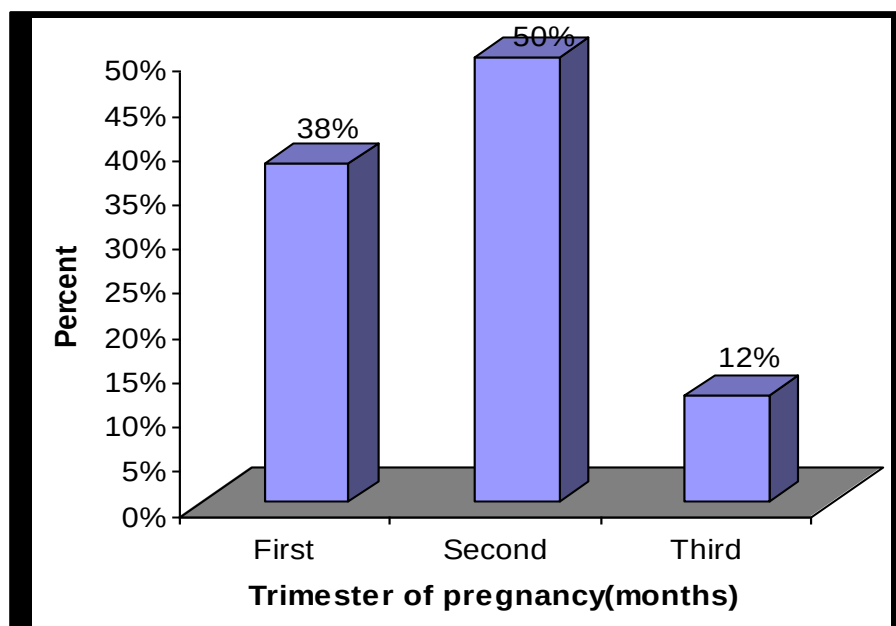


Figure (3-2): Distribution of *Toxoplasma* seropositivity according to trimester of pregnancy.

These findings might be due to the fact that mostly aborted women were in the starting of the second trimester and the end of first trimester; which is considered as a critical period of gestation where

the fetus is not well established in the uterus. Studies suggest that when mothers are infected in the first trimester, 14% of fetuses become infected; as compared to 29 % in the second and 59 % in the third trimester (Roizen *et al.*,1995), where there is an increase in the possibility rate of fetal infection with progress pregnancy trimester that may cause fetal death or severe malformation incompatible with fetal viability that is considered as one mechanism leading to abortion as mentioned by Brent and Beckman (1994) and by Tabbara (1992) who referred to that early transmission is associated with severe effects that may result in stillbirth or abortion.

3.1.5. The distribution of Positive samples by Toxoplasma Latex agglutination test according to number of abortion:

Figure (3-3) demonstrates the number of repeated abortion to the studied samples. Out of the 50 positive cases 24(48%) were with single abortion, 13(26%) with two abortions, 7(14%) with three abortions, 4(8%) with four abortions and 2(4%) with five repeated abortions.

This finding may be due to the fact that almost women with single abortion were gravid for the first time, where the gestation being more risky and susceptible for every factor different from the common; hormonal, physiological, infections such as infection with

T.gondii that can pass the placenta and infect the fetus that is considered as one cause of abortion occurrence as mentioned by Jones *et al.*(2001) or may belong to that there is no previous screening for toxoplasmosis that may help in precaution from reactivation latent infection and following the needful treatment that can play a role in protection the gestation. But in comparison to women with repeated abortions, there is possibility to the presence of other causes lead to repeated abortions rather than toxoplasmosis as being chromosomal abnormalities, Physical problem such as distortion of the uterine cavity, Endocrine or hormone disorders or acquired through sexually transmitted disease and presence of abnormal antibodies in the blood that cause formation of blood clots such as anti-phoispholipids antibodies (Rosen and Barkin,1998).

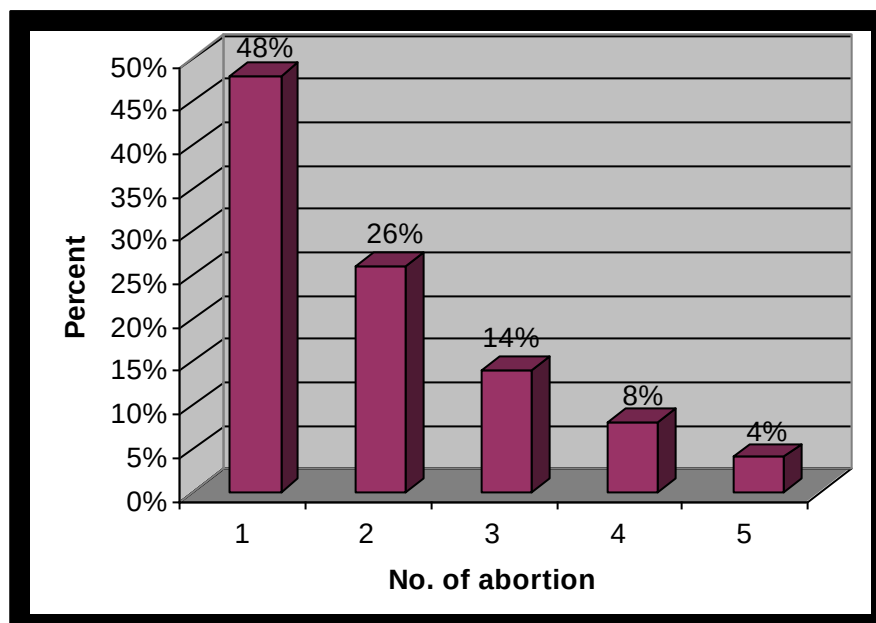


Figure (3-3): Distribution of *Toxoplasma* seropositivity according to the number of abortion.

These results were in accordance with Othman (2004) in Kirkuk who found the high percentage of seropositivity, (55.17%) was among pregnant women with single abortion. But it is not accordance with Al-Hamdani and Mahdi(1996) in Basra who found that the women with five or more abortions had the highest prevalence rate of *T. gondii* antibodies and also it is not accordance with Al-Maqdisy (2000) in Ninewah, who found that women with two abortions had higher percent of seropositivity,(34.14%). These findings may belong to those women included in these studies aborted from another cause rather than *Toxoplasma* in addition to the presence of previous infections with *Toxoplasma* that detect by the presence of specific antibodies and not necessity refer to that it's the main cause for abortion.

The presence of anti-*T.gondii* (IgG) antibodies in the sample of serum is sufficient to establish the fact that the patient has been infected (Li *et al.*,2000), while the absence of IgG early or before pregnancy allows identification of women at risk for acquiring the infection(Montoya and Liesenfeld,2004). However, IgG class of antibodies is generally not helpful in the diagnosis of acutely presenting illnesses because these antibodies take 1-9 weeks to develop (Jones *et al.*,2003), and persists for months or years. It has been observed that a significantly greater incidence of abortion

occurred in patients with high antibody titers, this finding is supported by Jones *et al.*(1996).

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; Starr and Friedman, 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).

Table (3-6) shows the differences in mean values of IgG and IgM of CMV between aborted women and healthy women (control) which were significant at 0.01 level.

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(3-4):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 (3-5): The concentration of anti-CMV (IgG & IgM) antibodies in healthy women (control) detected by ELISA technique.

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

Table (3-6): Means and Standard deviation of anti-CMV IgG and IgM antibodies concentrations in aborted women and healthy control women.

Treat	Total Number	Mean of concentration	±Std. Deviation	±Std. Error	P Value
IgG	P	44	2.125	0.9426	<0.01*
	C	6	0.500	0.5754	
IgM	P	44	0.668	0.5754	<0.01*
	C	6	0.367	0.2658	

*Significant

P =Patient

C=Control

3-2.1. Distribution of the tested sera for anti-CMV antibody according to age groups of aborted women:

The age of patients ranged between (15-40) years (Mean $\pm$ SD= 25.66 $\pm$ 6.42). Table (3-7) shows the number of patients, who are considered positive for anti-CMV (IgG & IgM) antibodies with range of concentration for patients in each age group. As shown in this table anti CMV IgG antibody was positive in 35(79.5%) aborted women, there were 3(75%), 12(70.5%), 11(84.6%), 3(100%), 4(80%) and 2(100%) from aborted women in age groups (<20), (20-24), (25-29),

(30-34), (35-39) and (≥ 40) years old respectively. The concentration of antibodies in these patients ranged between (1.8-3.5 IU/ml).

Table (3-7): Anti-CMV (IgG & IgM) antibodies in aborted women according to age groups.

Age groups (Years)	Total No.	Positive No.		Concentration range (IU/ml)	
		IgG	IgM	IgG	IgM
<20	4	3	1	2.0-3.0	1.0
20-24	17	12	3	1.8-3.0	1.0
25-29	13	11	1	2.3-3.0	1.0-3.0
30-34	3	3	1	1.8-2.9	1.0
35-39	5	4	2	1.9-3.5	1.0-3.0
≥ 40	2	2	-	1.9-3.5	-
Total	44	35	8	1.8-3.5	1.0-3.0

Tabulated $\chi^2=16.750$, Calculated $\chi^2=17.05$, d.f=5, P value<0.01

Tabulated $\chi^2=13.277$, Calculated $\chi^2=21.1$, d.f=4, P value<0.01

The highest numbers (12 & 11) in the age groups (20-24) and (25-29) years old respectively may be due to the same cause as mentioned for aborted women with Toxoplasmosis, where these age groups represent optimum period for gestation and delivery as compared to women above 40 years old. The presence of anti-CMV IgG antibody may be refer to previous latent infection acquired either

in childhood, adolescence, or in the childbearing period. These findings are in accordance with the results of studies carried by Abdul-Kariem(1989)in Baghdad and Ali(1992) in Mosul, who have found that the majority of women of childbearing age are seropositive for anti-CMV antibodies and that they contract the infection either through prenatal or postnatal transmission or during early childhood. Also it is in accordance with Vancikova and Dvorak(2001) who found that almost all people have had CMV infection during their life from early childhood and become persistent. These findings indicate that CMV has a worldwide distribution and infects humans of all ages, with possible reactivation of latent CMV infection with no seasonal or epidemic patterns of transmission as reported by Murray *et al.*(2003).

Anti CMV IgM was positive only in 8(18.18%) of aborted women, there were 1(25%), 3(17.6%),1(7.6%),1(33.3%)and 2(40%) in (≤ 20), (20-24), (25-29), (30-34) and (35-39)years age groups respectively. The concentration of antibodies for these positive cases ranged between (1.0-3.0 IU/ml).

The presence of anti-CMV IgM antibody represents the acute state of CMV (primary infection) or reactivations of latent infection, where those patients were also positive for anti-CMV (IgG) antibody in the same time which represents previous infection and this is found to be in accordance with Vancikova and Dvorak(2001) and Foti *et al.*(2002) who revealed that primary infection with CMV is

followed by persistence of the virus in a latent form. During life, the virus can be reactivated due to many factors including stress, disease, physiological or hormonal disturbance resulting in renewed shedding of the CMV or development of disease, which results in an abortion. According to these results it has been concluded that CMV infection is associated with age (P value <0.01).

3.2.2. Distribution of the tested sera for anti-CMV antibody according to residence of aborted women:

The residential distribution of aborted women is studied and the results are shown in table (3-8). The rural residents' patients were seventeen while urban residents' patients were twenty seven. From these patients, 14 and 21 cases were positive for anti-CMV IgG antibody from rural and urban resident's respectively which represents chronic state of infection.

Table (3-8): Anti-CMV (IgG & IgM) antibodies in rural and urban aborted women.

Residency	Total No.	Positive No.		Concentration range (IU/ml)	
		IgG	IgM	IgG	IgM
Rural	17	14	5	1.8-3.5	1.0
Urban	27	21	3	1.9-3.5	1.0-3.0
Total	44	35	8	1.8-3.5	1.0-3.0

Tabulated $\chi^2=6.635$, Calculated=20.9 (IgG), d.f=1, P <0.01

Tabulated $\chi^2=6.635$, Calculated=0.5 (IgM), d.f=1, P >0.01

The concentration of these positive cases ranged between (1.8-3.5 IU/ml). In spite the fact that the number of women patients from urban areas was higher but it represents 77.7% from the total urban women. However, 82.3% of the rural women were positive for anti-CMV IgG antibody and this finding may indicate that many women in the rural regions live in a minimal personal hygiene and low educational level about the disease transmission and ways of prevention of the disease and as mentioned by Brooks *et al.*(2004), where the prevalence of infection varies with socioeconomic status, living conditions and hygienic practice.

There were 5(29.4%) and 3(11.11%) positive cases for anti-CMV IgM antibody with concentration ranging between (1.0-3.0 IU/ml) from rural and urban areas respectively, where patients with acute state of CMV infections from rural areas were more than from urban areas. This finding may be due to the same causes as mentioned for cases with chronic state of CMV infection. The presence of anti-CMV IgM antibody may point to either primary infection or reactivation of latent infection where these patients at the same time were positive for anti-CMV IgG antibody. CMV remains latent within the host, reactivating and shedding when the host's immune system is compromised by many personal or environmental factors (Gregory and Taylor,2003).It is usually difficult to distinguish asymptomatic

primary maternal infection from reactivation or to determine precisely when infection occurs as mentioned by Gilbert(2002).

3.2.3. The distribution of the tested sera for anti-CMV antibody according to occupation of aborted women (employees and housewives):

The distribution of aborted women according to occupation is shown in Table (3-9). Employed women were 8; while housewives were 36. From these patients only 3(37.5%) from employed women and 32(88.8%) from housewives were positive for anti-CMV IgG antibody with concentration ranging between (1.8-3.5 IU/ml). It has been found that there was a significant association with housewives (P value <0.01). This result may be due to the fact that housewives being somewhat less learning than employees in addition to that may be exposed to infection sources more than employed women such as the over time exposure to the excretion that may be contain CMV (e.g. saliva and urine) from their children or seminal fluids through sexual intercourse (Gautheret *et al.*,1997; Kashden *et al.*,1998 and Tierney *et al.*,1999).Thus there is more chances for housewives to acquired infection in compared to employed women who expended most the time in their work thus being less exposure to the important routes for transmit CMV.

Table (3-9): Anti-CMV (IgG & IgM) antibodies in aborted women according to occupation.

Occupation	Total No.	Positive No.	Concentration range (IU/ml)
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		IgG	IgM	IgG	IgM
Employees	8	3	2	1.9-3.0	1.0
Housewives	36	32	6	1.8-3.5	1.0-3.0
Total	44	35	8	1.8-3.5	1.0-3.0

Tabulated $\chi^2=6.6$, Calculated=24 (IgG), d.f=1, P value <0.01
 Tabulated $\chi^2=6.6$, Calculated=21.3 (IgM), d.f=1, P value <0.01

Anti-CMV IgM antibody was positive in 2(25%) cases from employed women and 6(16.6%) cases from housewives and the concentrations of these positive cases ranged from 1.0 to 3.0 IU/ml, these cases were positive for anti-CMV IgG antibody too. This result refers to primary infection or reactivation of latent infection also it may be refer to recurrent infection. However, this may be result in fetal infection that is considered as one mechanism that can lead to abortion occurrence. According to these data in the above table, it has been found that there is also a significant association with housewives (P value <0.01). This finding may be due to the same causes as patients with anti-CMV IgG antibody.

3.2.4. Distribution of the tested sera for anti-CMV antibody according to trimester of pregnancy:

The distribution of aborted women according to trimester is shown in table (3-10). There were 17(73.9%), 16(88.9%) and

2(66.7%) positive cases for anti-CMV IgG antibody from women in the first, second and third trimester respectively and the concentration of them ranged from (1.8-3.5 IU/ml).

Table (3-10) Anti-CMV (IgG & IgM) antibodies in aborted women according to trimester.

Trimester of pregnancy	Total No.	Positive No.		Concentration range (IU/ml)	
		IgG	IgM	IgG	IgM
First trimester	23	17	5	1.8-3.0	1.0
Second Trimester	18	16	2	1.9-3.5	1.0-3.0
Third trimester	3	2	1	2.0-3.0	3.0
Total	44	35	8	1.8-3.5	1.0-3.0

Tabulated $\chi^2=9.21$, calculated=12.01 (IgG), d.f=2, P <0.01

Tabulated $\chi^2=9.21$, calculated=3.15 (IgM), d.f=2, P >0.01

As noticed in the above table, the number of aborted women who carry specific anti-CMV IgG antibody in the first trimester was more than aborted women in the second and third trimester; there is a

significant association with trimester (P value <0.01). These findings may be due to the fact that the first trimester of pregnancy is considered as a critical period regarding the fetus is not well established in the uterus in beginning the gestation and is threatened for abortion whenever mother exposed to any risky factors such as

reactivation of latent infection as CMV that results from immunosuppression concomitant with pregnancy which can lead to placental infection and next placental insufficiency, with subsequent fetal death as a mechanism play a role in abortion occurrence or infection with CMV may induce a genetically and anatomically altered embryo or fetus which is also considered as a cause leading to miscarriage as mentioned by Brent and Beckman (1994).

Anti-CMV IgM was seen in 5(21.7%), 2(11.11%) and 1(33.3%) cases from aborted women in the first, second and third trimesters, the range of concentration for these positive cases was from 1.0 to 3.0 IU/ml. In addition to that these positive cases were positive for anti-CMV IgG antibody at the same time. The number of patients in the first trimester was more than patients in second and third trimester. These findings may belong to the same cause as mentioned for cases with anti-CMV IgG antibody, where primary or reactivation of latent infection of pregnant woman with CMV specially in the first trimester of pregnancy, frequently results in severe damage to the nervous system of the fetus or abortion as mentioned by Britt(1996).

3.2.5. Distribution of the tested sera for anti-CMV antibody according to number of abortion:

The distribution of aborted women according to the number of abortion is shown in table (3-11). Womens' numbers were 30, 10, 2

and 2 with 1, 2, 3 and 4 abortions respectively. However, there were no women with ≥ 5 abortions.

Table (3-11): Anti-CMV (IgG & IgM) antibodies concentration in aborted women according to number of abortion.

No. of abortion	Total No.	Positive No.		Concentration range (IU/ml)	
		IgG	IgM	IgG	IgM
1	30	25	6	1.8-3.5	1.0-3.0
2	10	7	1	2.1-3.5	3.0
3	2	1	1	2.7	1.0
4	2	2	-	3.0	-
Total	44	35	8	1.8-3.5	1.0-3.0

Tabulated $\chi^2=11.34$, calculated=42.45 (IgG), d.f=3, $P < 0.01$

Tabulated $\chi^2=9.2$, calculated=6.17 (IgM), d.f=2, $P > 0.01$

The number of patients with anti-CMV IgG antibody (latent infection) was 25(83.3%) from women with single abortion, 7(70%) from women with two abortions, 1(50%) from women with three abortions and 2(100%) from women with four abortions. The concentration of antibodies in these positive cases ranged between (1.8-3.5 IU/ml). From these data it has been found that the number of positive cases from aborted women with single abortion was more than women with repeated abortions, wherein there is a significant association with single abortion (P value < 0.01). This may refer to that CMV is associated with random miscarriage but not recurrent miscarriage as mentioned by Liesnard *et al.*(2000) where the woman has had CMV previously but reactivates her own virus or is infected with a new virus variant, her immune defence reduces the

transmission risk substantially although it is not completely eliminated.

The results for anti-CMV IgM antibody were 6(20%), 1(50%) and 1(50%) positive cases from women with 1, 2 and 3 abortions respectively with concentration ranged from 1.0 to 3.0 IU/ml and these cases were also positive for anti-CMV IgG antibody at the same time. These results can be considered as evidence for primary infection acquired before pregnancy as CMV IgM antibodies may persist for months after a primary infection as well as may be refer to women with reactivation or recurrent infections, if they were simultaneously positive for anti-CMV IgG antibody. The number of positive cases was also found to be higher in women with single abortion than women with repeated abortion, these findings might be due to the same cause as mentioned for aborted women with anti-CMV IgG antibody, wherein CMV infection may be cause spontaneous abortion and intrauterine foetal death, but habitual

abortion, on the other hand, has not been associated with CMV infection. Also it may be due to the same causes as mentioned for aborted women with Toxoplasmosis in which most women with single abortion were gravid for the first time, wherein the gestation is risky and susceptible for every factor different from the common; hormonal, physiological, infections such as infection with *T.gondii* and CMV that can pass the placenta and infect the fetus that is

considered as one cause of abortion occurrence as pointed to it (Brent and Beckman,1994).

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 (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

Concentration of aPL IgM antibody(MpL\ml)

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

Toxoplasma or CMV infection). These selected cases which were tested for anti-phospholipids antibody by ELISA technique are more clarified in table (3-12).

Table (3-12): Shows the number of abortion cases tested for anti-phospholipid (IgM) antibody.

Table (3-13): The concentration of anti-phospholipid (IgM) antibody in aborted women detected by ELISA technique.

No.	Patients with Toxoplasmosis or CMV	Control(Abortion with no <i>Toxoplasma</i> 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	

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 (3-13), 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 (Harris *et al.*,1983;Gastineau *et al.*,1985; Asherton and Harris,1986 and 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: Distribution of the examined sera for C - reactive protein by Latex agglutination test:

The presence of C-Reactive protein (CRP) in sera of women patients was also carried in this study, by taking 120 samples including 100 samples from aborted women and 20 samples from healthy women (control). Out of these samples, 34 samples from aborted women were positive for CRP. However, the rest and all control group were negative. These findings are presented in figure (3-4).

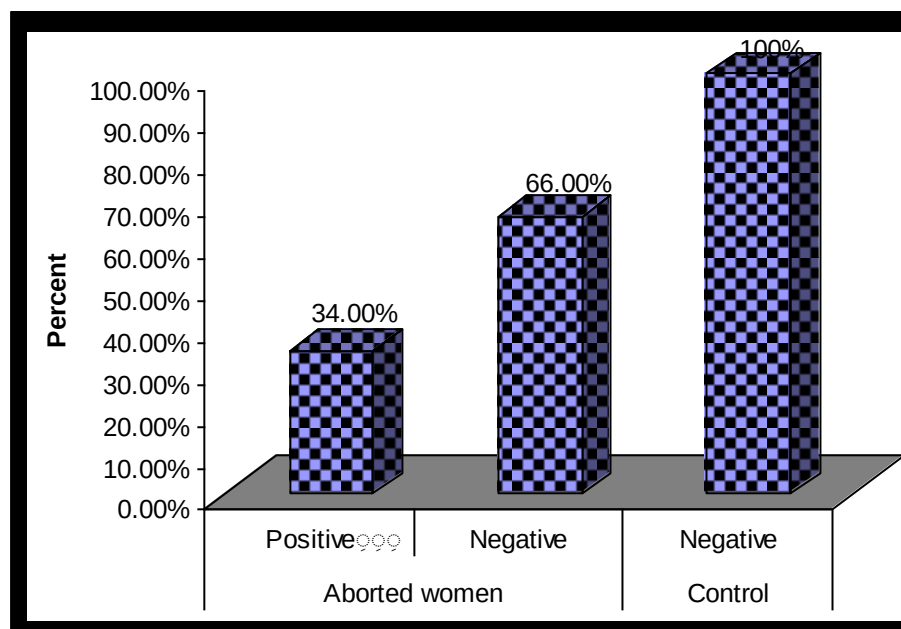


Figure (3-4): Distribution of the samples subjected to C - reactive protein latex test.

From these 34 positive cases, 17(50%) cases were positive for CRP and anti-*Toxoplasma* antibody, while the other 17(50%) cases were positive for CRP only. The presence of CRP in sera of aborted women with anti-*Toxoplasma* antibody indicates the presence of

inflammation or tissue injuries that result from *T.gondii* infection. Whereas the presence of CRP only can be used as a marker for the presence of another cause rather than *Toxoplasma* infection (e.g. Viral, Bacterial....etc). These findings are supported by Lars-Olof *et al.*(1997),who pointed to significant increase of CRP level after most forms of tissue injuries, inflammation, bacterial and virus infections. In addition to that the presence of CRP in sera of aborted women with no *T.gondii* or CMV infections may be due to the fact that maternal CRP levels are raised as early as 4 weeks gestation and thus the maternal inflammatory response is established during the earliest phases of implantation. Sacks *et al.*(2004), hypothesized that an abnormal response (either exaggerated or absent) can cause some cases of miscarriage.

3-5 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

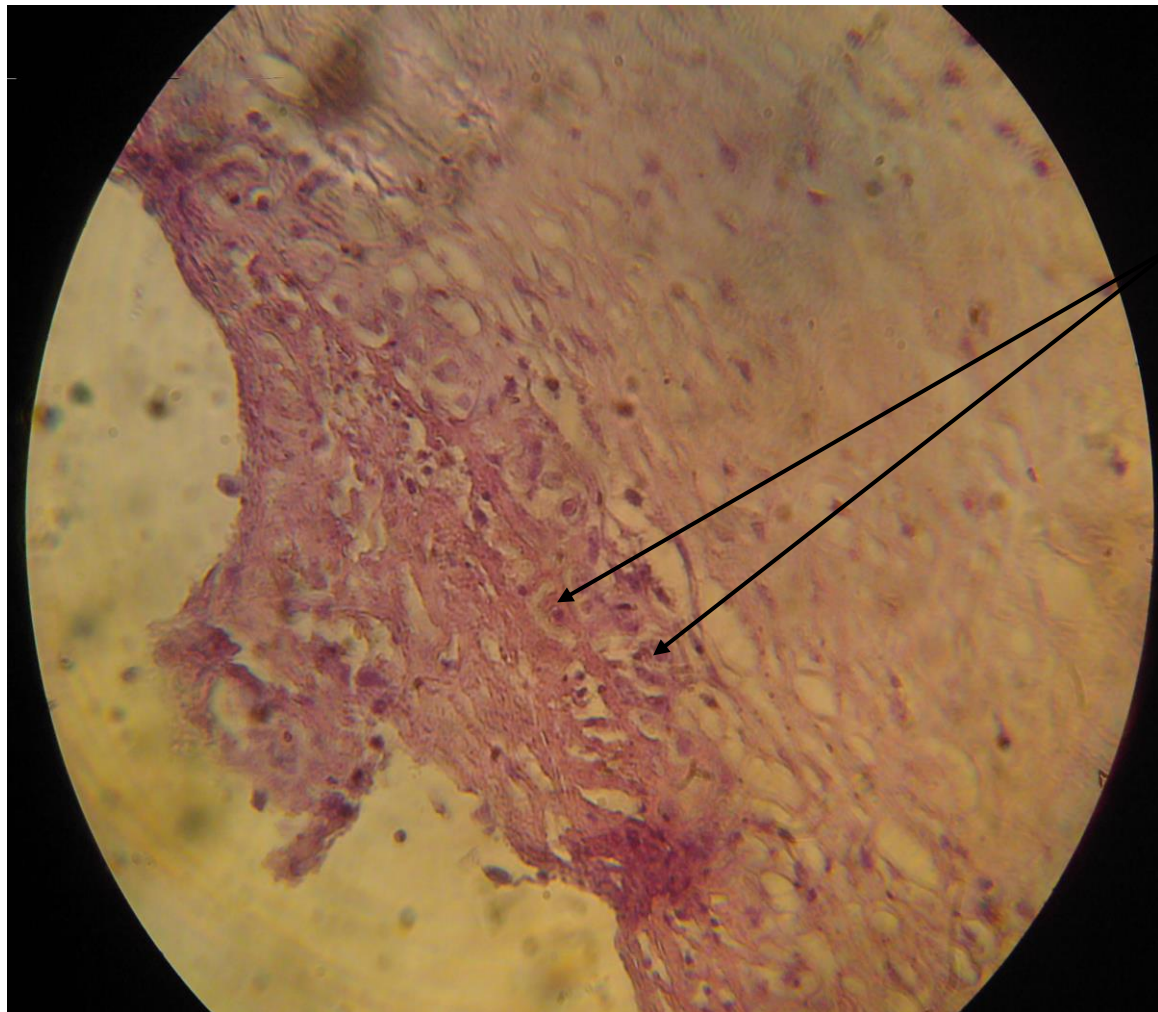
considered as a characteristic of CMV infection in section of placenta biopsy stained by H&E stain, inflammatory cells spread very little through the section when examined by light microscope. Figure (3-7) shows the giant cell formation and Owl's eye in placenta section taken from aborted women infected with CMV.

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 trophoblasts, provides the environment for placental vascular development and contains lymphocyte cells that act as a secondary barrier against fetal infection (Burstein *et al.*,1973; 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.



Cyst of
T. gondii

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

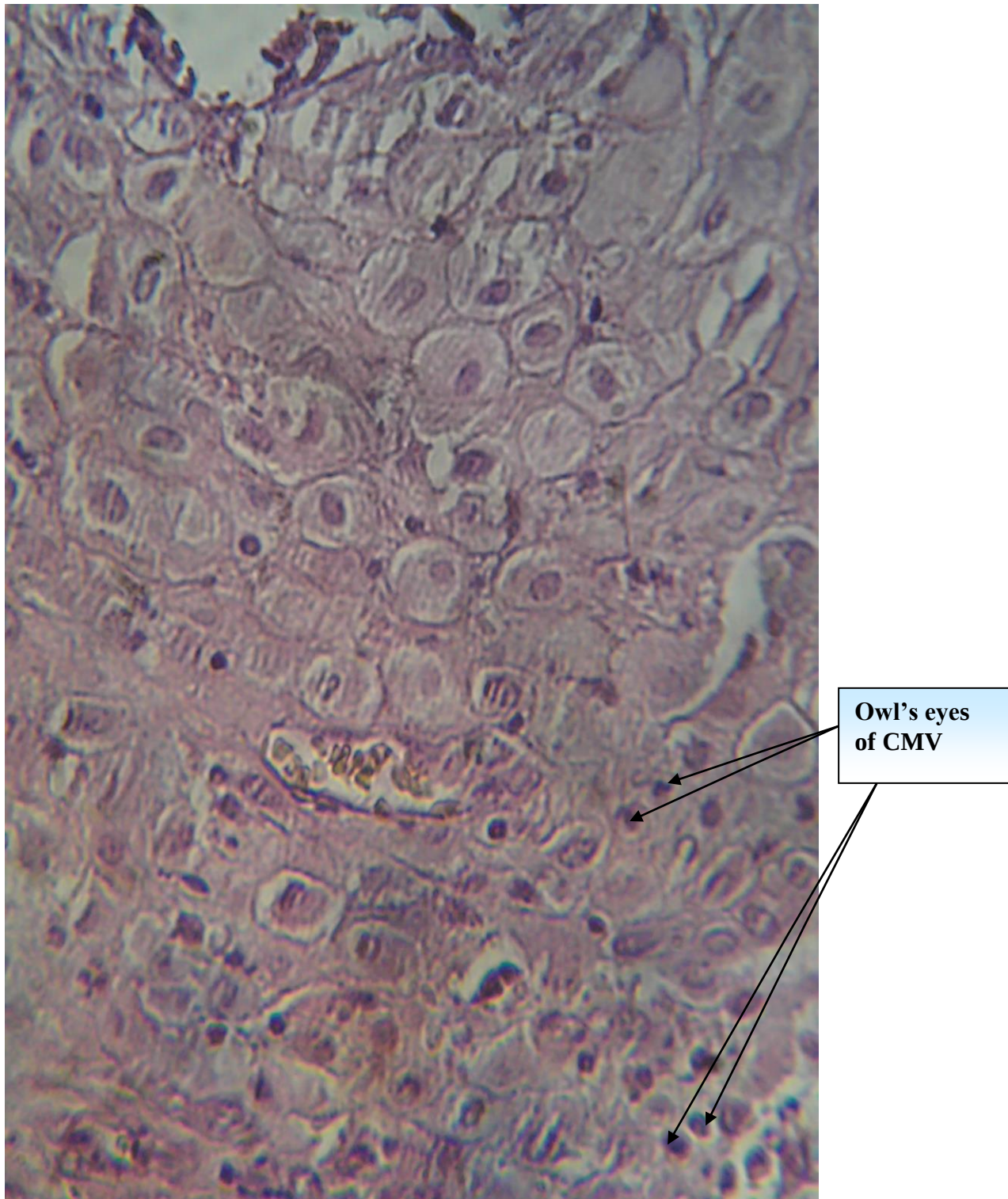


Figure (3-6): Light microscopic appearance of placenta from infected women with CMV showing Owl's eye formation (H&E X 100).

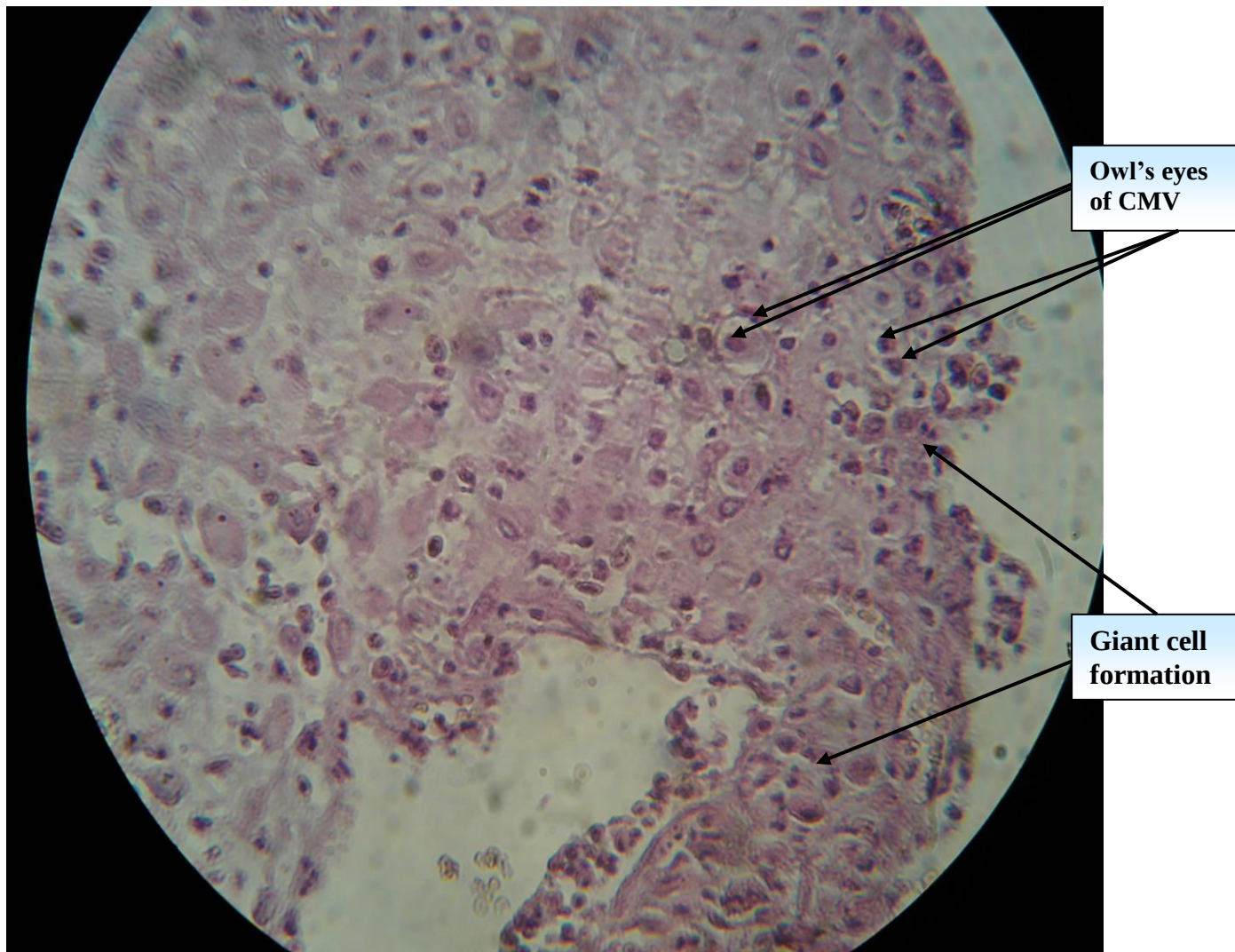


Figure (3-7): *Light microscopic appearance of placenta from infected women with CMV showing Owl's eye and Giant cell formation (H&E X 100).*

References

- Abbas, M.M.A.(2002). Seroepidemiological study with a history of abortion. M.Sc. Thesis, Nahrain Med. Coll., Nahrain Univ.
- Abdul-Kariem, E. A. *et al.*(1989). Seroepidemiology of cytomegalovirus infection among a healthy population in Baghdad. Journal of community medicine, 2:19-27.
- Ageel, N. F.(2003).Serological and biochemical study of toxoplasmosis in Tikrit teaching Hospital. M.Sc. Thesis, College of Medicine, Tikrit University.
- Ahmed, N. I.(1995). Zoonotic disease in Ninewah 1990-1994. Thesis for professional Diploma degree in Community Medicine. College of Medicine, Mosul Univeristy.
- Al-Ani, S. K.(2004).Epidemiological and immunological study of toxoplasmosis among aborted women in Ramadi City. M.Sc. thesis. College of Medicine. Al-Anbar Univeristy.
- AL-Doski, B. D. A.(2000).Seroepidemiological study of toxoplasmosis among different groups of population in Duhok city by using Latex agglutination test and indirect hemagglutination test. M.Sc. Thesis, College of Medicine, Duhok University.
- Al-Hamdani, M. M. and Mahdi, N. K.(1996).Toxoplasmosis among women with habitual abortion. Eastern. Med. Health. J.,3(2):310-315.

- Ali, H.Y.M.; Yaseen, S. A. and Najem, S. N.(1992). Prevalence of Cytomegalovirus infection in childbearing age women in Mosul. Jordan medical journal, **26**:53-58.
- Al-Khaffaf, F. H. M.(2001). Isolation and seroepidemiological study of toxoplasmosis in childbearing age women in Mosul province. M.Sc. Thesis, College of Science, Mosul University.
- Al-Maqdisy, M. Kh.(2000). A survey study of the aborted cases in sheep and human caused by *Toxoplasma gondii* in Ninewah Governorate. M.Sc. Thesis, College of Veterinary Medicine, Mosul University (In Arabic).
- Al-Nakib, W.; Ibrahim, M. E. ;Hathout, H. ;Moussa, M. A. ;Deverajan, L. V. ;Thorburn, H. and Yousof, A. M. (1983). Seroepidemiology of viral and *Toxoplasma* infections during pregnancy among Arab women of childbearing age in Kuwait. In: J. Epidemiol., **12**:220-223.
- Al-Qurashi, A. R. ;Ghandour, A. M. ;Oobeid, O. E.; Al-Mulhim, A. and Makki, S. M.(2001). Seroepidemiological study of *Toxoplasma gondii* infection in the human population in the eastern region. Saudi Med. J., **22**:13-18.
- Al-Shaway, A. A.(1994). A seroepidemiological study of *Toxoplasma* among patients with habitual and single

abortion. M.Sc. thesis. College of Medicine. Baghdad University.

Al-Sorchee, S. M. A.(2005). Immunological Study on Women Infected with Toxoplasmosis with a History of Abortion. College of Education (Ibn Al-Haitham), Baghdad University.

AL-Waely, R. M. H.(1998).The prevalence of seropositive *Toxoplasma gondii* antibodies among Iraqi mothers and their new born babies in Baghdad. The Diploma in Primary Health Care. Dissertation. College of Medicine, Tikrit Univ.

Al-Wattari, I. T. A.(2005).Seroprevalence of *Toxoplasma gondii* antibodies among unmarried women of childbearing age in Mosul. College of Medicine, Mosul University.

American Journal of Clinical Nutrition. (2005).C- Reactive Protein and Pregnancy, **82**(5):1052-1058.

Araujo, F. G.; Hunter, C. A. and Remington, J. S. (1997). Treatment with IL-12 in combination with atovaquone or clindamycin significantly increases survival of mice with acute toxoplasmosis. Antimicrob. Agents Chemother., **41**:188-190.

- Arribas, J.R.; Storch, G.A.; Clifford, D.B. and Tselis, A.C.(1996). Cytomegalovirus encephalitis. *Ann. Intern. Med.*,**125**: 577-587.
- Asherton, R. A. and Harris, E. N.(1986).Anti-phospholipid antibody. *Post. grad. Med. J.*, **62**: 1081-1087.
- Ashrafunnessa; Khatum, S.; Islam, M. N. and Huq, T.(1998). Seroprevalence of *Toxoplasma* antibodies among the antenatal population in Bangladesh. *J. Obstet. Gynaecol. Res.*,**24**: 115-119.
- Banatvala, J. E. *et al.*(2004). Rubella. *Lancet*, **363**:1127. In: Ana, M.; Wayne, X. and Shandera, M. D. *Infectious Diseases: Viral & Rickettsial*.
- Barbara, A. B.; Normant, B. and Stephen, H. G.(2000).Latex agglutination test (LAT).*Infectious disease*. 2nd ed. Cited by Abbas, (2002).
- Barros, J. S.; Goncalves, C. A. and Bairos, V. A.(2003). Elastic fibers in human placenta. *Arch. Gynecol. Obstet.*, **267**: 208-212.
- Baumann, H. and Gauldie, J. (1994).The acute phase response. *Immunol.*, **15**:74-80.
- Beckwith, D. G.; Halstead, D. C.; Alpaugh, K.; Schweder, A.; Blount-Fronefield, D. A. and Toth, K.(1985).Comparison of a latex agglutination test with five other method for

- determining the presence of antibody against cytomegalovirus. *J. Clin. Microbiol.*, **21**:328-331.
- Bogges, K. A. ;Lieff, S. ; Murtha, A. P. ; Moss, K. ; Jared, H.; Beck, J. and Offenbacher, S.(2005). *Am. J. Perinatol.*, **22**(6):299-304.
- Brent, R. L. and Beckman, D. A.(1994).The contribution of environmental teratogens to embryonic and fetal loss. *Clin. Obstet. Gynecol.*, **37**(3): 646-670.
- Brezin, A.; Kasner, L. and Thullies, P. *et al.*(1994). Ocular toxoplasmosis in the fetus immunohistochemistry analysis and DNA amplification .*Retina.*,**19**: 2120-2124.
- Britt, W. J. and Alford, C. A.(1996).Cytomegalovirus. In: Fields, B.; Knipe, D. and Howley, P.(eds.). *Fields Virology*. 3rded. Philadelphia: Lippincott-Raven Publishers., **3**: 2493-2523.
- Britt, W. J.(1996). Human cytomegalovirus overview: The virus and its pathogenic mechanisms. *Baillieres Clin. Infect. Dis.*, **3**:307-325.
- Brooks, G. F.; Butel, J. S. and Morse, S. A.(2004). Herpes viruses. In: Jawetz, Melnick and Adelberg's(eds.).*Medical Microbiology*. 23rd ed. McGraw-Hill Company, **33**:440-446.

- Brooks, G. F.; Butel, J. S. and Morse, S. A.(2004). Herpes viruses.
In: Jawetz, Melnick and Adelberg's. Medical Microbiology. 23rd ed. McGraw-Hill Company., **43**:416.
- Brown, Z. A.; Selke, S. and Zeh, J. *et al.*(1997). The acquisition of herpes simplex virus during pregnancy. N. Engl. J. Med., Pp:337-509.
- Buffolano, W.; Lappalainen, M.; Hedman, L.; Ciccimarra, F.; Del Pezzo, M.; Rescaldani, R.; Gargano, N. and Hedman, K. (2004). The measurement of *T. gondii* IgG avidity in the postnatal of congenital toxoplasmosis is usefulness. European Journal of Clinical Microbiology and Infectious Diseases, **23**(11):825-830.
- Burstein, R.; Frankel, S. and Soule, S. D.(1973).Aging of the placenta: Autoimmune theory of senescence. Am. J. Obstet. Gynecol.,**116**:271-274.
- Cannon, M. J. and Pellet, P. E.(2005).Risk of congenital cytomegalovirus infection. CID.,**40**:1701-1702.
- Carl, Z.(2006).The Loom. A Nation of Neurotics? Blame the Puppet Masters?
- Carter, A. O. and Frank, J. W.(1986). Congenital toxoplasmosis: Epidemiologic features and control. CMAJ.,**135**:618-623.

- Chen, C. P. and Aplin, D. G.(2003). Placental extracellular matrix: Gene expression, deposition by placental fibroblast and the effect of oxygen, 24: 316-325.
- Chetana, V.(1996).C-Reactive protein. Immunology and Infectious Diseases, 6:139-144.
- Cinque, p.; Scarpellini, P. and Vago, L. *et al.*(1997). Diagnosis of central nervous system complications in HIV-infected patients: Cerebrospinal fluid analysis by the polymerase chain reaction. AIDS., 11: 1-17.
- Cinque, P.; Marenzi, R. and Ceresa, D.(1997). Cytomegalovirus infections of the nervous system. Intervirology,40:85-97.
- Coelho, R. A. ;Kobayashi, M. and Carvalho, L. B.(2003). Prevalence of IgG antibodies specific to *Toxoplasma gondii* among blood donors in Recife. Northeast Brazil. Rev. Inst. Med. trop. S. Paulo., 45(4):229-231.
- Cohen; Jonathan; Powderly and William, G.(2004). Life cycle of *Toxoplasma gondii*. Infectious Diseases. 2nd ed. Mosby.
- Collier, L.; Balows, A. and Sussman, M.(1998). In: Mahy, B.W. and Collier, L. Topley&Wilson's: Microbiology and Microbial Infections. Virology, 9th ed. Co- Published in the USA by Oxford Univ. Press, Inc., New York., 1(19):339-343.

- Cremer, N. E.(1985).Antibodies in serodiagnosis of viral infection.
In: Lennett, H. Laboratory diagnosis of viral infection.
Mercel Dekker, Inc., New York. Pp: 73.
- Crumpacker, C. S.(2000). Cytomegalovirus. In: Mandell, J. E.;
Bennet, R. and Dolin. Principles and practice of Infectious
Diseases. 5th ed. NY: Churchill Livingstone.Pp:1586-
1596.
- Cunningham, F.G. ;MacDonald, P.C. ;Gant, N.F. ;Leveno, K.J.;
Gilstrap, L.C. ;Hankins, G.D. and Clark, S.L. (2001).
Congenital toxoplasmosis. In: William Obstetrics, 21sted.
USA., Pp:530-548.
- Da-Cunha, S.; Ferreira, E.; Ramos, I.; Martins, R.; De, F. L.;
Borges, J. L.; Corte-Real, R. ;Mota, A.; Melico-Silvestre,
A. and Furtado, A. L.(1994). Cerebral toxoplasmosis after
renal transplantation. Case report and review. Acta. Med.
Port., 7:S61-66.
- David, A.(2003).Guardian Unlimited. Can a parasite carried by
cats change your personality?
- Dehner, L.P. and Askin, F.B.(1975).Cytomegalovirus endometritis:
Report of a case associated with spontaneous abortion.
Obstetrics and gynecology, 45:211-214.
- Deibel, R.; Smith, R. and Clarke, L. M.(1974). Cytomegalovirus
infections in New York State: Laboratory studies of

- patients and healthy individuals. NY. State. J. Med., 74:785.
- Demmler, G.(1991). Summary of a workshop on surveillance for congenital cytomegalovirus disease. Rev. Infect. Dis., 13:315-329.
- Demmler, G. J.(1996). Congenital cytomegalovirus infection and disease. Adv. Pediatr. Infect. Dis., 11:135–162.
- Drury, R. A. B. and Wallington, E. A.(1980). Carleton's histological technique .5th ed. Oxford University Press, Oxford.
- Dubey, J. ;Kotula, A.W. and sharer, A.(1990).Effect of high temperature obinfectivity of *T.gondii* tissue cysts in pork. J. Parasitol.,79:201-204.
- Dubey, J.(1998).Advances in the life cycle of *Toxoplasma*. Int. Parasitol., 28:1019-1024.
- Dunford, P. and Johnson, J.(1991). Detection of *Toxoplasma*-specific immunoglobulin-G: Assessment of a slide agglutination test .Med. Lab. Sci., 48:137-141.
- Dunn, D.; Wallon, M.; Peyron, F.; Petersen, E.; Peckham, C. and Gilbert, R.(1999). Mother-to-child transmission of toxoplasmosis: Risk estimates for clinical counselling. Lancet,353:1829-1833.

- Epps, R. E.; Pittelkow, M. R. and Su, W. P.(1995).TORCH syndrome. Semin. Dermatol., **115**:680.
- Fadul, C.E.; Chanon, J.Y. and Kasper, L.H.(1995).Survived of immunoglobulin Gopsonized *Toxoplasma gondii* in non adherent human monocyte. Infe. Immunol.,**63**: 4290-4294.
- Fatoohi, F. (1985). Screening of toxoplasmosis in different groups of population in Mosul city by using IHAT and CFT. M.Sc. thesis, College of Medicine, University of Mosul.
- Fatoohi, A.F.; Cozon, J.N. and Greenland, T. *et al.*(2002). Cellular immune responses to recombinant antigens in pregnant women chronically infected with *Toxoplasma gondii*. Am. Soc. Microbiol. Clin. Diag. Immunol., **9**(3):704-707.
- Feldman, H. A.(1996). Laboratory methods in current use for the study of toxoplasmosis. In: Streiff, E. B., Advances in Ophthalmology. Lausanne Bibliotheca. Ophthalmologic Fasc., **3**(39):1-11.
- Foti, G.; Hyeraci, M.; Kunkar, A.; Ieropoli, G.; Sofu, D.; Lorenzo, S.D. and Carpentieri, M.S.(2002). Cytomegalovirus infection in the adult. Minerva Med., **93**(2):109-117.
- Fowler, K.B.; Stagno, S. and Pass, R.F.(2003). Maternal immunity and prevention of congenital cytomegalovirus infection. JAMA., **289**(8):1008–1011.

- Frenkel, J. K.(1989).Toxoplasmosis. In: Goldsmith, R. and Hynemann, D.(eds.). Tropical medicine and parasitology, 1st ed. Prentice-hall international editions, USA.,Pp:332-342.
- Garcia, L.S. ;Fritsche, T. R. ;Grady, K. K. ;Healy, G. R. ;McAuley, J.; Rocha, A. ;Wilson, M. and Wong, J.(2004).Clinical use and interpretation of serologic tests for *Toxoplasma gondii*. NCCLS global consensus guideline, **24**(6):1-21.
- Gastineau, D.A.; Kazmier, F.J.; Nichols, W.L. and Bowie, E.J. (1985). Lupus anticoagulant: analysis of the clinical and laboratory features of 219 cases. Am. J. hematol., **19**:265-267.
- Gautheret, A. D.; Aubin, J. T. and Poirel, L. *et al.*(1997). Detection of human Betaherpesvirinae in saliva and urine from immunocompromised and immunocompetent subjects. Journal of Clinical Microbiology, **35**:1600-1603.
- Gaytant, M. A.; Steegers, E. A. and Semmekrot, B. A. *et al.*(2002). Congenital Cytomegalovirus infection: Review of the epidemiology and outcome. Obstet. Gynecol. Surv., **57**:245–256.
- Gerald, D.; Schmidt and Larry, S. R.(1996). Morphology of *Toxoplasma*. Foundations of Parasitology. 6th ed. McGraw- Hill Companies, Inc. USA., Pp: 129-132.

- Gershon, A.; Gold, E. and Nankervis, G.(1997). Viral Infections of Humans(Cytomegalovirus). Plenum. Medical, 4th ed. New York, NY., Pp:229-251.
- Gilbert, G. L.(2002). Infections in pregnant women. In: Grayson, M. L. and Steven, L. W.(eds.) M.J.A. Practice Essentials-Infectious Diseases, **176** (5): 229-236.
- Girardi, G.; Berman, J. and Redecha, P. *et al.*(2003).Complement C5a receptors and neutrophils mediate fetal injury in the anti-phospholipid syndrome. J.Clin.Invest.,**112**;1644 1654.
- Godson, D. L.; Campos, M. and Attah-Poku, S. K. *et al.*(1996). Serum haptoglobin as an indicator of the acute phase response in bovine respiratory disease. Vet. Immunol. Immunopathol., **51**:277–292.
- Gray, J. J. ;Balfour, A. H. and Wreighitt, T. G.(1990).Evaluation of a commercial latex agglutination test for detecting antibodies to *Toxoplasma gondii*. Serodiag. Immunoth. Infect. Dis., **4**:335-340.
- Greco, P. ;Vimercati, A. ;Angelici; M.C. ;Carbonara, S. ;Doria,G. ; Nappi, L. ;Angarano, G. and Selvaggi, L.(2003). Toxoplasmosis in pregnancy is still an open subject. J. Perinat. Med., **31**: 36–40.
- Green Wood, D. ;Slack, R. C. and Pentherer, J. F.(2002). Herpes viruses. Medical Microbiology: A guide to microbial

- infections, pathogenesis, immunity, laboratory diagnosis and control. 16thed. Churchill living stone. Pp: 503.
- Gregory, H. and Tylor, M.D.(2003). Cytomegalovirus infections. *Am. Fam. Physician*, **67**:(3):519-524,526.
- Griffiths, P. and Emerym, V.(1997). Cytomegalovirus. In: *Clinical Virology*. New York, NY. :Churchill Livingstone, Pp: 445-470.
- Grundy, J.E. and Shanley, J.D.(1987). Is Cytomegalovirus interstitial pneumonitis in transplant recipients an immunopathological condition? *Lancet*, **2**:996-999.
- Gutman, L. T.(1998). Syphilis. In: Feigin, R. D. and Cherry, J. D.(eds.). *Textbook of Pediatric Infectious Diseases*, 4th ed., W.B. Saunders, Philadelphia, Pp:1543.
- Hamadto, H.A. ;Rashid, S.M. ;El-Fakahany, A.F. and Lashin, A.H. (1997). Seroepidemiological studies for Toxoplasmosis among out and in patients in Benha University Hospitals, Qalyobia Governorate. *J. Egypt. Soc. parasitology*, **27**(1): 223-231.
- Harris, E.N.; Gharavi, A.E. and Boey, M.L. *et al.*(1983). Anti phospholipids antibodies: Detection by radioimmunoassay and association with thrombosis in systemic lupus erythematosus. *Lancet*, **26**:1211-1214.

- Hassony, H.J.; Ali, A.H. and Mahmood, M.I.A.(1987). Prevalence of *Toxoplasma* antibodies in blood donors and outpatients attending Basra university teaching hospitals. The MJBU.,6(2):36-43. Cited by Al-Doski, B.D.(2000). Seroepidemiological study of toxoplasmosis among different groups of population in Duhok city by using latex agglutination test and IHAT. Thesis of M.Sc. Medical Parsitology. College of Veterinary Medicine, Mosul University.
- Henshaw; Stanley, K.; Singh; Susheela and Haas, T.(1999). The incidence of abortion worldwide. International family planning perspectives, 25(Supplement):30 –38.
- Hirsch, M.S.(1994). Cytomegalovirus Infection. Harrison's Principles of Internal Medicine, 13th ed. McGraw Hill Inc., St. Louis, Pp:794-797.
- Ho, M.(1991).Cytomegalovirus. Biology and Infection. 2nd ed. New York Plenum., Pp: 440.
- Hopkns, W. G.(2002).A new view of statistics. www.sportsci.org.
- Horadagoda, N.U.;Knox, K.M. and Gibbs, H.A.*et al.*(1999). Acute phases proteins in cattle: Discrimination between acute and chronic inflammation. Vet. Rec., 144:437–441.

- Hursh, D.A. ; Abbot, A.D. ;Sun, R. ; Iltis, J.P. ; Rice, D.H. and Gleaves, C.A.(1989).Evaluation of a latex particle agglutination assay for the detection of cytomegalovirus antibody in patient serum. J. Clin. Microbiol., **27**:2878-2879.
- Hynemann, D.(1982). Parasitic diseases. In: Stites, D.P.; Stobo, J. D. ; Fudenberg, H.H. and Wells, J.V.(eds.). Basic and clinical immunology, 4thed. Lange medical publications, California, Pp:678.
- James, D.; Steer, Ph. J.; Weiner, C. P. and Gonik, B.(1999).Anti-phospholipid (APL) antibodies. High Risk pregnancy. 2nd ed. W.B. Saunders, Pp: 95-96.
- James, M.H. and Daniel, G.C.(2000). Preventing congenital toxoplasmosis. CDC., **49**:57-75.
- Jawetz, E.; Melnick, J. L. and Adelberg, E. A.(1987).Herpes viruses. Medical Microbiology. 17thed. Appelton and Lange,California,**34**:416.
- Jaye, D. L. and Waites, K. B.(1997).Clinical applications of C-reactive protein in pediatrics. Pediatr. Infect. Dis. J., **16**:735-744.
- John; David, T.; Petri and William, A.(2006).Life cycle of *T.gondii*. In: Markell and Voge's. Medical Parasitology. 9th ed. St. Louis: Elsevier Inc.

- Johnson, L.L. and Sayles, P.C.(2002). Deficient humoral responses underlie susceptibility to *Toxoplasma gondii* in CD4-deficient mice. Infect. Immun., **70**(1):185-191.
- Jones, M. H. *et al.*(1996). Toxoplasmosis and abortion. American journal of obstetrics and gynecology, **104**(6):919-920.
- Jones, J.L. ;Lopez, A.; Wilson, M.; Schulkin, J. and Gibbs, R. (2001).Congenital toxoplasmosis: Review. Obstet. Gynecol. Surv.,**56**(5):296–305.
- Jones, J.L.; Kruszon-Moran, D.; Wilson, M.; McQuillan, G.; Navin, T. and McAuley, J.B.(2001). *Toxoplasma gondii* infection in the United States: Seroprevalence and risk factors. Am. J. Epidemiol., **154**:357-365.
- Jones, J.L.; Kruszon-Moran, D. and Wilson, M.(2003). *Toxoplasma gondii* infection in the United States, 1999–2000. www.cdc.gov.
- Jones, J.; Lopez, A. and Wilson, M.(2003).Congenital Toxoplasmosis. Journal of the American Academy of Family Physicians, www.aafp.org.
- Jones, J.; Lopez, A. and Wilson, M.(2003).Congenital toxoplasmosis. Am. Fam. Phys., **67**:2131–2138.
- Jorge, M. and Quiñonez.(2004). Screening of Congenital Toxoplasmosis and Congenital Cytomegalovirus Infection. Hospital Physician Board Review Manual.

- Pediatric Medicine. By Turner White Communications Inc., Wayne, PA., 2(2):1-12.
- Jumaian, N. F.(2005).Seroprevalence and risk factors for *Toxoplasma* infection in pregnant women in Jordan. Eastren Mediterr Health J.,11:45-51.
- Kashden, J.; Frison, S. and Fowler, K. *et al.*(1998). Intellectual assessment of children with asymptomatic congenital Cytomegalovirus infection. Journal of Developmental and Behavioral Pediatrics,19:254-259.
- Katlama, C.(1993). Cytomegalovirus infection in acquired immune-deficiency syndrome. J. Med. Virol. Suppl., 1:128-133.
- Katz, S. L.; Gershon, A. A. and Hotez, P. J.(1998). Toxoplasmosis. In: Roberts, F. ;Boyer, K. and McLeod, R.(eds). Infectious disease of children.10th ed. St. Louis,USA: Mosby;Pp:539.
- Kiwi, R.(2006).Anti-phospholipid antibody syndrome. Cleveland Clinic Journal of Medicine, 73(10):916.
- Klein, J. O. and Remington, J. S.(1995).Current Concepts of Infections of the Fetus and Newborn Infant. In: Klein, J.O. and Remington, J.S.(eds.).Infectious Diseases of the Fetus and Newborn Infant, 4thed, W.B. Saunders, Philadelphia, Pp: 2.

- Kravetz, J.D. and Federman, D.G.(2005). Toxoplasmosis in pregnancy. *Am. J. Med.*, **118**(3):212–216.
- Lars-Olof, H. *et al.*(1997).C-Reactive protein. *Current Opinion in infectious diseases*, **10**:196-201.
- Leblebicioglu, H. (2004). Toxoplasmosis. **e-medicine INC . www.e- medicine.com**
- Levine, N. D.(1977). Taxonomy of *Toxoplasma gondii*. *J. Protozool.*, **24**: 36-41.
- Liesnard, C.; Donner, C. and Brancart, F. *et al.*(2000). Prenatal diagnosis of congenital Cytomegalovirus infection. *Obstetrics & Gynecology*, **95**(6):881-888.
- Li, S.; Maine, G.;Suzuki, Y.;Araujo, F. G.;Galvan, G.; Remington, J.S. and Parmley, S.(2000). Serodiagnosis of recently acquired *Toxoplasma gondii* infection with a recombinant antigen. *J. Clin. Microb.*, **38**(1):179-184.
- Lopez, A.; Dietz, V.J.; Wilson, M.; Navin, T.R. and Jones, J.L. (2000). Preventing congenital toxoplasmosis. *MMWR. Recomm. Rep.*, **49**(RR-2):59–68.
- Machin, S. J.(1996).Platelets and anti-phospholipid antibodies. *Lupus*, **5**(5):386-387.
- Miesbach, W. ;Gokpinar, B. ;Gilzinger, A. ;Claus, D. and Scharrer, I.(2005).Predictive role of C-reactive protein in patients

with anti-phospholipid syndrome. Immunobiology, **210**:755-760.

Montoya, J. G. and Remington, J. S.(2000). *Toxoplasma gondii*. In: Mandell, G.L.; Douglas, R.G.; Bennett, J.E. and Dolin, R.(eds.). Principles and Practice of infectious diseases. 5thed. Philadelphia: Churchill Livingstone, Pp:2858-2888.

Montoya, J. and Liesenfeld, O.(2004).Toxoplasmosis. Lancet; **363**:1965-1976.

Murray, R.P. ;Ellen, J.B.; James, H.J. ;Michael, A. P. and Robert, H.Y.(2003). *Toxoplasma*. Manual of Clinical Microbiology; 8th edition, ASM press. Washington. **2**(131):1970-1980.

Murray, R.P.; Ellen, J.B.; James, H.J.; Michael, A.P. and Robert, H.Y.(2003). Cytomegalovirus. Manual of Clinical Microbiology; 8th edition, ASM press. Washington, **2**(83): 1304-1318.

Nelson, C.T. and Demmler, G.J.(1997).Cytomegalovirus infection in the pregnant mother, fetus, and newborn infant. Clin. Perinatol., **24**:151–160.

Niazi, A.; Omer, R.; Al-Hadithi, T. S. and Aswad, A.(1988). Prevalence of *Toxoplasma* antibodies in pregnant women in Baghdad. J. Fac. Med. Baghdad, **30**(3): 7.

- Nigro, G.L.; Torre, R.; Anceschi, M.M. ;Mazzocco, M. and Cosmi, E.V.(1999). Hyper immunoglobulin therapy for a twin fetus with cytomegalovirus infection and growth restriction. Am. J. Obstet. Gynecol., **180**(5):1222-1226.
- Novotná, M. ;Hanusova, J. ;Klose, J. ;Preiss, M. ;Havlicek, J. ;Roubalová, K. and Flegr, J.(2005).Probable neuroimmunological link between *Toxoplasma* and Cytomegalovirus infections and personality changes in the human host. BMC Infectious Diseases. BioMed Central Ltd.,**5**:54.
- Nussenblatt, R. B.; Mittal, K. K. and Fuhrman, S. *et al.*(1998). Lymphocyte proliferative responses of patients with ocular toxoplasmosis to parasite and retinal antigens. AJO.,**107**:632-641.
- Olle, P.; Bessieres, M. H.; Malecaze, F. and Seguela, J. P.(1996). The evolution of ocular toxoplasmosis in anti-interferon gamma treated mice. Curr. Eye Res., **15**:701-707.
- Othman, N. F.(2004). Seroprevalence study of *Toxoplasma gondii* among pregnant women in Kirkuk City. M.Sc. Thesis, College of Medicine, Tikrit University.
- Out, H. J.; Bruinse, H. W. and Derksen, R. H.(1991).Anti-phospholipids antibodies and pregnancy loss. Human Reproduction, **6**:889-897. In: James, D.; Steer, PH. J.;

- Weiner, C. P. and Gonik, B.(1999).Anti-phospholipid antibodies. High Risk pregnancy .2nd edition, W.B. Saunders, Pp: 95-96.
- Paniker, C.J.(2002). Textbook of medical parasitology. 5th ed, Jaypee brothers medical publishers Ltd, Newdelhi, Pp:89-95.
- Parazzni, F.; Acaia, B. and Faden, D.(1991): Antiphospholipid antibodies and recurrent miscarriage. Obstetrics Gynecology ,77: 854-858.
- Parslow, T.G.; Stites, D.P.; Terr, A.I. and Imboden, J.B. (2001). Cytomegalovirus. Medical immunology.10th ed., Mc Graw-Hill. Pp:649.
- Pass, R. F. and Kinne, J. S.(1985). Child care workers and children with congenital cytomegalovirus infection. Pediatrics,75(5): 971-973.
- Pass, R.F. and Hutto, C.(1986). Group day care and cytomegaloviral infections of mothers and children. Rev. Infect. Dis.,8(4):599– 605.
- Pass, R. F.(1990). Day care centers and transmission of cytomegalovirus: New insight into an old problem. Seminars in Pediatric Infectious Diseases,1(2):245–251.

- Pass, R.F.; Hutto, C.; Lyon, M.D. and Cloud, G.(1990). Increased rate of cytomegalovirus infection among day care center workers. *Pediatr. Infect. Dis. J.*, **9**(7):465–470.
- Pattison, N. S.; Chamley, L. W. and Mckay, E. J. *et al.*(1993). Antiphospholipid antibodies in pregnancy: Prevalence and clinical associations. *British Journal of obstetrics and Gynecology*,**100**:909-913. In:James, D.; Steer, PH. J.; Weiner, C. P. and Gonik, B.(1999). Anti-phospholipid (APL) antibodies. *High Risk pregnancy* .2nd edition, Pp: 95-96.
- Pierro, E.; Cirino, G.; Bucci, M.R. *et al.*(1999). Antiphospholipid antibodies inhibit prostaglandin release by decidual cells of early pregnancy: possible involvement of extracellular secretory phospholipase A2. *Fertil. Steril.*, **71**(2): 342-346.
- Piona, A. ;La Rosa, L. and Tincani, A. *et al.*(1995). Placental thrombosis and fetal loss after passive transfer of mouse lupus monoclonal or human polyclonal anti-cardiolipin antibodies in pregnant naive BALB/c mice. *Scand. J. Immunol.*, **41**(5):427-432.
- Pitiphat, W. ;Kaumudi, J. ;Joshi pura and Janet, W. *et al.*(2005). C-Reactive protein during pregnancy. *Journal of Periodontology*, 77(5):821-825.

- Population and public health branch.(2001). Infectious agent office of laboratory security, PPHB.,Pp: 3.
- Qais, E. S. ;Al-Zahwi, H. M. ;Al-Senjaqli, N. B. F. ;Aziez, F. H. and Odasi, N.S.(1990). Booklet of Laboratory Methods for Pathology and Cytology in Health Laboratory in Iraq, Pp:5-8.
- Rai, R.; Regan, L. and Clifford. *et al.*(1995). Antiphospholipid antibodies and β 2 glycoprotein I in 500 women with recurrent miscarriage: result of a comprehensive screening approach. Human Reproduction,**10**:101-105.
- Rai, R.; Regan, L. and Clifford, K. *et al.*(1995).Antiphospholipid antibodies and beta 2 glycoprotein-1 in 500 women with recurrent miscarriage: results of a comprehensive screening approach. Human Reproduction, **10**:2001-2005.
- Rai, R. and Regan, L.(1996). Antiphospholipid antibodies and adverse pregnancy outcome. In: Stud, J. Progress in Obstetrics and Gynecology. Edinburgh: Churchill Livingstone, **12**:135-152.
- Rayan, K. J. and Ray, C. G.(2004). Cytomegalovirus. Sherris Medical Microbiology, 4th ed., McGraw Hill, **556**:566–569.
- Reindollar, R. H.(2000).Contemporary issues for spontaneous abortion. Does recurrent abortion exist? Obstet. Gynecol. Clin. North Am., **27**:541-554.

- Remington, J. S. ;McLeod, R. and Desmonts, G.(1995).
Toxoplasmosis. In: Remington, J. S. and Klein, J.O.(eds).
Infectious diseases of the fetus and newborn infants. 4th ed.
Philadelphia: WB Saunders, Pp: 140–267.
- Remington, J.S.; McLeod, R.; Thulliez, R. and Desmonts,G.
(2001). Toxoplasmosis In: Remington, J.S. and Klein, J. O.
(eds) Infectious diseases of the fetus and newborn infants.
5th ed. Philadelphia: WB Saunders, Pp: 205–346.
- Roizen, N. ;Swisher, C. W. and Stein, M. A.(1995). Neurologic
and developmental outcome untreated congenital
toxoplasmosis, **95**: 11-20.
- Rosen, P. and Barkin, R.(1998). Emergency Medicine: Concepts
and Clinical Management. 4th ed. St. Louis, Mo: Mosby-
Year Book.
- Ross, D. S.; Jones, J. L. and Lynch, M. F.(2006).Maternal
Toxoplasmosis, Cytomegalovirus, Listeriosis, and
Preconception Care. Child Health J., **10**(Suppl 7):189–193.
- Rote, N. S.; Walter, A. and Lyden, T. W.(1992).Antiphospholipid
antibodies losters or red herrings. American Journal of
reproductive Immunology **28**:31-37. In: James, D.; Steer,
PH. J.; Weiner, C. P. and Gonik, B.(1999). Anti-
phospholipid antibodies. High Risk pregnancy .2nd edition.
W.B. Saunders, Pp: 95-96.

- Rousseau, T.; Douvier, S. and Reynaud, I. *et al.*(2000). Severe fetal cytomegalic inclusion disease after documented maternal reactivation of cytomegalovirus infection during pregnancy. *Prenat. Diagn.*, **20**: 333-336.
- Sabin, A.B. and Feldman, H.A.(1948).Dyes as micro chemical indicators of a new immunity phenomenon affecting a protozoan parasite.*Science*,**108**:660-663.
- Sacks, G.P.; Seyani, L.; Lavery, S. and Trew, G.(2004). Maternal C-reactive protein levels are raised at 4 weeks gestation. *European Society of Human Reproduction and Embryology*, **19**(4):1025-1030.
- Sambrook, J. and Russel, D.W.(2001). *Molecular Cloning: A Laboratory Manual*, 3rd ed., Cold Spring Harbor, N.Y.: Cold Spring Harbor Laboratory Press. ISBN 0-87969-576-5. Chapter 8: In vitro Amplification of DNA by the Polymerase Chain Reaction.
- Sarikaputi, M.; Morimatsu, M.; Syuto, B.; Saito, M. and Naiki, M. A.(1991). New purification procedure for bovine C-reactive protein and serum amyloid P component. *Int. J. Biochem.*, **23**:1137–1142.
- Savva, D.; Morris, J.C.; Johnson, J.D. and Holliman, A.F.(1990). Polymerase chain reaction for the detection of *Toxoplasma gondii*. *J. Med. Microbiol.*, **32**: 25-31.

- Scharton-Kersten, T.; Caspar, P.; Sher, A. and Denkers, E. Y. (1996). *Toxoplasma gondii*: Evidence for IL-12-dependent and independent pathways of interferon-gamma production induced by an attenuated parasite strain Exp. Parasitol., **84**:102-114.
- Schopfer, K.; Lawber, E. and Krech, U.(1978). Congenital cytomegalovirus infection in newborn infants of mothers infected before pregnancy. Archives of disease in childhood, **53**:536-539.
- Sheridan, E.(2002).Congenital rubella syndrome: A risk in immigrant populations. Lancet, **359**:675.
- Solano, C. I. ;Munoz, A. ;Gutierrez, A. ;Farga, F. ;Prosper, J. ;Garcia-Conde, D. ;Navarro and Gimeno, C.(2001).Qualitative plasma PCR assay(AMPLICOR CMV test) versus pp65 antigenemia assay for monitoring cytomegalovirus viremia and guiding preemptive ganciclovir therapy in allogenic stem cell transplantation. J. Clin. Microbiol., **39**:3938-3941.
- Stagno, S.; Dworsky, M.E.; Torres, J.; Mesa, T. and Hirsh, T. (1982).Prevalence and importance of congenital cytomegalovirus infection in three different populations. J. Pediatr. Dec., **101**(6):897– 900.

- Stagno, S. *et al.*(1982).Congenital cytomegalovirus infection: The relative importance of primary and recurrent maternal infection. New England journal of medicine, **306**:945-949.
- Stagno, S. ;Pass, R.F. ;Cloud, G. ;Britt, W.J. ;Henderson, R. E. ;Walton, P.D. ;Veren, D.A. ;Page, F. and Alford, C. A. (1986).Primary cytomegalovirus infection in pregnancy, incidence, transmission to fetus, and clinical outcome. JAMA.,**256**(14):1904–1908.
- Stagno, S.(1990).Cytomegalovirus. In Remington, J. S. and Klein, J. O.(eds.) Infectious diseases of the fetus and newborn infant. 3rd ed. Philadelphia, PA: W.B. Saunders, Pp: 242-281.
- Stagno, S.(2001). Cytomegalovirus. In: Remington, J. S. and Klein, J. O.(eds.) Infectious diseases of the fetus and newborn infant. Philadelphia: W.B. Saunders Company, Pp: 389–424.
- Staras, S. A. S. ;Dollard, S. C. and Radford, K. W. *et al.*(2006). "Seroprevalence of cytomegalovirus infection in the United States, 1988–1994". Clin. Infect. Dis., **43**:1143–1151.
- Starr, S. E. and Friedman, H. M.(1985). Human CMV. In lennett, E. H. *et al.*(eds.) Manual of Clin. Microbiol. 4thed., Am. Soc. Microbiol.,**65**:771-779.

- Suzuki, Y.; Yang, Q. and Yang, S. *et al.*(1996). IL-4 is protective against development of toxoplasmic encephalitis. *J. Immunol.*,**157**:2564-2569.
- Tabbara, K. F.(1992). Early transmission of Toxoplasmosis is associated with severe effects that may result in stillbirth or abortion. In Tasman, W. and Jaeger, E.A.(eds.) *Duane's Clinical Ophthalmology*, Philadelphia, J.B. Lippincott., Pp: 1-23.
- Tabbara, K. F.; Al-Omar, O. M.; Tawfic, A. and Al-Shammary, F. (1999). Toxoplasmosis in Saudi Arabia. *Saudi Med. J.*, **20**(1): 46.
- Taber, L.H.; Frank, A.L.; Yow, M.D. and Bagley, A.(1985). Acquisition of cytomegalovirus infections in families with young children: A serological study. *J. Infect. Dis.*, **151**(5):948–952.
- Tenter, A.M. ;Barta, J.R. ;Ian, B. ;Duszynski, D.W. ;Mehlhorn, H. ;Morrison, D.A. ;Thompson, R.C. and Conrad, P.A. (2002). The Conceptual basis for a new Classification of the Coccidia . *Inter. J. Parasitol.*, **32**:595 – 616.
- Tierney, E. L.; McPhee, S. J. and Papadakis, M. A.(1999). Cytomegalovirus also found in blood, semen, vaginal secretions, milk, and stool. *Current Medical Diagnosis and*

- Treatment, 38th ed. Appleton and Lange, Stamford CT., Pp: 1263-1264.
- Udvarnoki, K. ;László, C. ;Uray, K. ;Hudecz, F. ;Kacskovics, I. and Spallek, R. *et al.*(2007).C-reactive protein. Clinical and Vaccine Immunology,**14**(4):335-341.
- Vallochi, L. ;Nakamura, M. V. ;Schlesinger, D. ;Martins, M. C. ;Silveira, C. ;Belfort, R. J. and Rizzo, L. V.(2002). Ocular toxoplasmosis. Scandnavian Journal of Immunology, **55**(4):324.
- Vancikova, Z. and Dvorak, P.(2001). Cytomegalovirus Infection in Immunocompetent and Immunocompromised Individuals- a review. Curr. Drug Targets Immune Endocr. Disord., **1**(2): 179-187.
- Van-Waeman, B. K. and Schurs.(1971). Immuno assay using antigen- enzyme conjugates. FEBS. Lett., **15**: 232-236.
- Vogt, E. ;Ng, A. K. and Rote, N. S.(1996). A model for the antiphospholipid antibody syndrome: monoclonal anti-phosphatidylserine antibody induces intrauterine growth restriction in mice. Am. J. Obstet. Gynecol., **174**(2):700-707.
- Voler, A. and Bidwell, J.E. *et al.*(1985).In: Rose,N. and Friedman, H.(eds.). Cytomegalovirus. Manual of clinical immunology. Am. Soc. Microbiol., **69**: 506.

- Walter, E. A.; Greenberg, P. D. and Gilbert, M. J.(1995). Reconstitution of cellular immunity against cytomegalovirus in recipients of allogeneic bone marrow by transfer of T-cell clones from the donor. *N. Engl. J. Med.*, **333**(16):1038-1044.
- Wilson, C.B.; Remington, J.S.; Stagno, S. and Reynolds, D.W. (1980). Development of adverse sequelae in children born with subclinical congenital *Toxoplasma* infection. *Pediatrics*, **66**:767-774.
- Wilson, M.; Ware, D.A. and Jurank, D.(1990).Serologic aspect of toxoplasmosis .*JAMA.*, **196** (2):277-281.
- Wilson, M.; Remington, J. S.; Clavet, C.; Varney, G.; Press, C. and Ware, D.(1997). Evaluation of Six Commercial Kits for Detection of Human Immunoglobulin M Antibodies to *Toxoplasma gondii*. *J. Clin. Microb.*, **35**(12):3112–3115.
- Wilson, M. and McAuley, J. M.(1999). *Toxoplasma*. In: Murray, P. R. (ed.). *Manual of clinical microbiology*. 7thed. Washington, D.C: American Society for Microbiology, Pp:1374-1382.
- Wohrle, R. ;Mtthias, T. ;Von, L.P. ;Oppermann, M. ;Helmke, K. and Forrger, F.(2000).Clinical relevance of antibodies against different phospholipids. *Journal of Autoimmunity* **15**: 60.
- Zamaorano,C.G. ;Contreas,M.C. ;Villalobos,S. ;Sandoval,L. and Salinas, P.(1999). Seroepidemiological survey of human

- toxoplasmosis in Osorno, Region X ,Bol. Chil. Parasitol., **45**(1-2):33-36.
- Zhang, L. J.; Hanpf, P.; Rutherford, C.; Churchill, W. H. and Crumpacker, C. S.(1995). Detection of human Cytomegalovirus DNA, RNA, and antibody in normal donor blood. J. Infect. Dis., **171**: 1279-1289.
- Zhu, J. ;Quyyumi, A. A. ;Norman, J. E. ;Csako, G. and Epstein, S. E.(1999).Cytomegalovirus in the pathogenesis of atherosclerosis: The role of inflammation as reflected by elevated C-reactive protein levels. JACC.,**34**:1738-1743.
- Ziefert, P.; Leary, M. and Rinaldi, J.(2002).AIDS and the Impact of Cognitive Impairment. UCSF AIDS Health Plan. **www.ucsf.ahp.org.**
- Zuckerman, A. J.; Banatvala, J. E. and pattison, J. R.(2000). Enzyme Immuno assay (EIA) of Cytomegalovirus. Principle and Practice of Clin. Virology.4th ed., Jhon Wiley and Sony, LTD.,**2**: 99.