Diagnostic study for some causes of abortion by Enzyme Linked Fluorescent Assay (ELFA) among women in Thi-Qar governorate

Fadil Abbas Al-Abady, Ali Naeem Salman & Zahraa Sadoon Hadi
Dept. of biology/College of Education for pure sciences/ Thi-Qar University
dr fadhil61@yahoo.com

SUMMARY

The present study aims to diagnosis and measure some causes of abortion by Enzyme Linked Fluorescent Assay (ELFA), included diagnosis *Toxoplasma gondii* and measuring anti cardiolipids antibodies, anti phospholipids antibodies as well as measuring blood parameters and phagocytosis. A total of 380 blood samples were collected from aborted women admitted to Thi Qar hospitals and private. The current study showed no the significant differences ($P > 0.05$) between infection rates in aborted women .The current study showed no significant differences between the values of IgM and there were significant differences ($P \leq 0.05$) between the values of IgG , while there were no significant differences at measuring both IgM and IgG.

The current results were recorded blood parameters applied on 40 blood samples for each of the *Toxoplasma gondii* parasite infected women and 10 blood samples for each of the women measured their cardio lipids and phospholipids compared with 10 blood samples a healthy sample. The current results showed the decrease significant for hemoglobin HB , blood volume compact PCV in infected women group compared with healthy group but increase significant for WBC in infected women group compared with healthy group.

In phagocytosis, the results showed a rise significantly in the coefficient of phagocytosis in infected women group compared with the healthy group.

Key words: Cardiolipid, Phospholipids , *Toxoplasma gondii*

INTRODUCTION

Abortion is incomplete pregnancy and lossing of the fetus during the first six months of pregnancy, which result in health problems for women (Ebadi *et al*., 2011; Kumar *et al* ., 2004), and is considered a major problem exposed a number of pregnant women have abortion due to the presence of congenital malformations rates ranging between 50% and 80% in the formation of the fetus, or the existence of problems in a woman's uterus such as fibroids, as well as the reasons for the hormonal or infectious diseases such as rubella and cytomegalovirus (CMV) and Toxoplasmosis, as well as psychological factors (Saeedi *et al* ., 2009; Sotoodeh,2007; Greenslade *et al*., 1992) .

Many parasitic infections are associated with significant maternal and fetal consequences if acquired during pregnancy. In general, perinatal infections have more severe fetal consequences when they occur early in gestation, because first-trimester infections may disrupt organogenesis. Second and third trimester infections can cause neurologic impairment or growth disturbances and abortion (Dohbit,2007; Finer and Henshaw ,2003).

*Toxoplasma gondii*is a single-cell protozoa that belongs to the family Coccidia. It is an obligatory intracellular protozoan with a heterogeneous life cycle in humans and other vertebrates (Fallah,2008). Human infection with *T.gondii*causes toxoplasmosis. Postnatal toxoplasmosis is usually an asymptomatic disease, but often takes a severe course in immune compremised hosts. Congenital toxoplasmosis is acquired through vertical transmission of *T.gondii*to the foetus by transplacental transfer from the mother usually following acute maternal infection. If congenital toxoplasmosis occurs early in pregnancy, it may lead to severe damage or abortion(Khalil,2009) .

Three different ways of *Toxoplasma* infection induction are: eating the cysts in not fully cooked contaminated meats, using water or food contaminated with oocytes excreted from the feces of cats

and transmission from mother, who has been contaminated by the previous ways, to fetus (Qublan,2002). The importance of *Toxoplasma* from the perspective of public health is mainly due to the risk of disease transmission during pregnancy (Gollub *et al* ., 2008). Although toxoplasmosis is often benign in the women, disease transmission through the placenta can lead to serious consequences such as abortion, still birth, different degrees of mental or physical retardation, hydrocephaly and blindness (Noorbakhsh,2002).

Anti phospholipid and Anti cardiolipin antibodies may play role as a cause in recurrent spontaneous abortion among women and obstetric complications. Anti phospholipid antibodies are a heterogenous group of autoantibodies directed against negatively charges phosphlpids and phosphlipid binding proteins. They are associated with aterial and venous thrombosis, thrombocytopenia, pregnancy complications and reproductive autoimmune failure (Houtkooper and Vaz.2008).

Leukocytes are the basic elements of the immune response, involved in defending the body against pathogens and toxic compounds, consisting of white blood cells from five types, but arise from a single cell that located in the bone marrow called Haemopoietic stem cell and a process called Haemopoiesis (Lafleur– Brooks, 2008) . White blood cells are classified depending on the presence or lack of granules in the cytoplasm into granulocytes and agranulocytes(Gratner and Hiatt , 2007; Lydyard and Grossi ,1998).

Such Neutrophile is important in the innate immune response in the process of phagocytosis assisted by the ability of migration from the bloodstream to sites of infection and help also possess Adhesion Molecules on the surface of the cell and present granules cytoplasmic containing lethal protein of microorganisms, the neutrophils to defence against microbial infections (Peakman and Vergan , 1997).

Phagocytosis represent the second line of defence of the body as well as they get rid the body of solids and immune

complexes formed in the body and prevent deposition within tissues, which may result in the survival of the tissue to an imbalance in the function of the member who happened where deposition of complexes immune (Klebanoff , 2005; Hyde , 2000).

MATERIALS AND METHODS

The current study conducted on women suffering from abortion who admitted to Hussein Teaching Hospital, Shatra Hospital , Rifai Hospital and private laboratories after refer them by a specialist doctor . 380 serum samples were collected from aborted women and doubtful of contracting disease toxoplasmosis and where withdrawing(5 ml) of blood venous which divided into:

1. Mode (2 ml) of blood in (EDTA) tube for the purpose of examining the blood parameters include the percentage of blood hemoglobin Hb, blood volume compact PCV , differential count of leukocytes and the process of phagocytosis.

2. Mode (3 ml) of blood in a test tube that left to clot for half an hour then it separated using a centrifuge (3000 r / and for 15 minutes) so as to obtain serum which used for the diagnosis of Toxoplasmosis by used ELFA test for estimation the level of IgM , IgG, anti cardiolipids antibodies and anti phospholipids antibodies.

3.Healthy group

Collected (10) blood sample from healthy pregnant women that does not suffer from abortion and revisions to care of pregnant women department in the Hussein Teaching Hospital.***Enzyme Linked Fluorescent Assay(ELFA)**

In the current study Minividas device was used to detect IgM and IgG by several kit (VIDAS Toxo IgM, France) , (VIDAS Toxo IgG, France) , (VIDAS cardiolipid IgM, France), (VIDAS cadriolipid IgG, France) , (VIDAS phospholipid IgG, France) and (VIDAS phospholipid IgM, France).

The method includes Sandwich two steps enzyme and the basis for action depends on the interaction between antibodies in patient serum with antigens that line the pot SPR the end

of the steps to measure the amount of enzyme linked fluorescent commensurate with the concentration of antibodies in the sample.

***Hemoglobin level determination:**

By using Micro haematocrite been measuring the level of hemoglobin in a blood Capillary tubes container material heparin and blocked one of extremes then blood separation by centrifugation (6500 r / min for 5 minutes) that depending on the size of blood cells using a private ruler (Baker and Silverton,1976).

***Total and differential count of leukocytes**

1.Total count of leukocytes:

Haemocytometer or called counting chamber was used in the calculation of white blood cells, where a dilution solution, which is a 2% glecial acetic acid and taking (0.02 ml) of blood using micropipet and then added a solution of dilution to be arrived size to (0 .04 ml) and then shake the solution for a minute and left for a period of (10) minutes to break down red blood cells and stained the nuclei of white blood cells.

One drop was taken and puton a counting chamber with a slide cover for calculation according to the equation:(Beck,1987)

Rate cells for four boxes x 200 = (cell / mm^3)

2. Differential count of leukocytes:

Worked (Blood smear) on a clean slide clean then left to dry and colored with Lishman stain for (2) minutes then flooded slide Baffer dye for (10) minutes and washed with running water and left to dry then examined slides using magnification power oil (100x) and calculation according to the following equation: (Schaechter *et al*., 1999).

$$\text{The total number of white blood cells} = \frac{\text{the total number of white blood cells} \times \text{the percentage of celltype number of cells (cell / mm}^3\text{)}}{100}$$

3.**Phagocytosis**

Macrophage percentage studied to phagocytic yeast cells slain according stated in (Met-Calf *et al*., 1986) and in peripheral blood. 0.025 ml of peripheral blood mixed with 0.05 ml of stuck yeast and 0.025 ml of solution Hank saline balanced (HBSS) in a sterile test tube and then incubated in (37) C˚ for half an hour.

Attended Blood smear on a glass slide , left to dry colored ,with (Wright's stain) and used the ready produced by CRESCENT in Saudi Arabia, left for (2) minutes and then flooded slide Buffer dye for (10 - 20) minutes then washed slides with running water and left to dry and examined using optical microscopy on the power zoom (100 x) and by phagocytosis coefficient according to the following equation:

$$\text{Phagocytosis coefficient (\%)} = \frac{\text{Number Almthblama cells}}{\text{The total number of phagocytic cells}} \times 100$$

* The results of the current study were analyzed by one sample test and Least Square Difference (LSD) and below the level of probability 0.05 using SPSS.

RESULTS

The current study was conducted on 380 blood samples from aborted women and doubtful infected their when application hospitals in the province of Thi Qar and private laboratories and divided into 4 groups:
Group 1: diagnosis of infected women with toxoplasmosis
Group 2: measuring anti cardiolipids antibodies in aborted women
Group 3: measuring anti phospholipids antibodies in aborted women
Group 4: a healthy sample of non infected women

Table No. (1) The current study showed no significant differences (P> 0.05) between infection rates among aborted women that reached the highest rate in the Group 1 (26.84%) and lowest in Group 2 (3.42 %). Also the current study showed no significant differences between the values of IgM the highest value of IgM in group 2 (23.09%) and the lowest value IgM in group 1 (5.88%),while showed significant differences (P≤ 0.05) between the values of IgG the highest value of IgG in group 1 (92.16%) and the lowest value IgM in group 2 (76.92%), while no significant differences at measuring both IgM and IgG.

Table (1) Frequency of immunoglobulins levels instudied groupsusing ELFA

****IgM+ IgG %	***IgG %	**IgM %	*Infected samples %	Examined samples	studied groups
1.96	**92.16**	**5.88**	**26.84**	**380**	**Group1 (Toxplasmosis)**
0	**76.92**	**23.09**	**3.42**	**380**	**Group2 Cardiolipid**
0	**88.89**	**11.11**	**4.74**	**380**	**Group3 Phospholipid**

*t (2) = 1.54 , P = 0.26 , α = 0.05

**t (2) = 2.62 , P = 0.12 , α = 0.05

***t (2) = 18.56 , P = 0..003, α = 0.05

****t (2) = 1 , P = 0.42, α = 0.05

Table No. (2) blood parameters showed a significant reduction in the concentration of HB and PCV in groups 1 When the comparison with groups 2 and 3, as well as when compared with Group 4 (healthy sample) at a probability level $P \leq 0.05$. the current results for the high founded in WBC both groups 1 when compared with both groups 2 and 3, as well as when compared with group 4(healthy sample) at a probability level $P \leq 0.05$.

The present study did not show significant differences in the preparation of PCV and WBC in groups 2and 3 when compared the healthy group while showed significant differences in the preparation of Hb in groups 2and 3 when compared the healthy group

Table (2) Frequency of some blood parameters among studied groups in the province of Thi Qar

Groups	NO.	Hb	PCV	WBC
1	**40**	**b**11.33 ±0.71	**b**0.34 ±0.021	**a**9.14 ±1.61
2	**10**	**b**11.96 ±0.47	**a**0.36 ±0.013	**b**7.77 ±1.40
3	**10**	**b**12.21 ±0.66	**a**0.38 ±0.020	**b**7.34 ±2.14
4 healthy	**10**	**a**12.15 ±0.72	**a**0.37 ±0.021	**b**6.55 ±1.42
LSD*		**0.62**	**0.018**	**1.36**

a, b:Means of each variable with different letter differ significantly at 5%.

*LSD=Revised Least Square Difference.

Table No. (3) differential count of leukocytes showed significant reduction in the proportion of preparing neutrophil in first three groups when compared with Group 4 (healthy) at the possibility $P \leq 0.05$. The current of results showed no significant differences between the four groups in the proportion of lymphocytes at a probability level $P > 0.05$.

On the other hand the results showed a rise significantly in the proportion of monocyte in groups 1when compared with the healthy group, as well as when compared with group 2 and 3 with the healthy group . Also explained the results of counting differential rise significantly in the proportion of eosinophiles in group 1 and a non significant increase in groups 2 and 3 when compared with the control group. Results did not show significant differences between the four groups in the proportion of basiophiles at a probability level $P > 0.05$.

As for the process of phagocytosis, the results showed a rise significantly in the coefficient of phagocytosis in groups 1 when compared with the healthy group at the possibility $P \leq 0.05$.

Table (3) differential count of leukocytes and phagocytosis of the samples studied in the province of Thi Qar

Groups	No.	N	L	M	E	B	P
1	**40**	**b** 59.55 ±5.15	31.17 ±3.91	**a**10.00 ±2.46	**a**6.15 ±1.99	0.82 ±0.78	**a**58.87 ±8.79
2	**10**	**c**53.70 ±4.11	34.20 ±3.42	**b**3.00 ±1.92	**b**2.30 ±0.67	0.50 ±0.52	**ab** 54.40 ±5.05
3	**10**	**b**59.30 ±5.45	31.60 ±3.92	**b**6.40 ±2.36	**b**2.20 ±0.42	0.60 ±0.51	**b**52.50 ±3.86
4	**10**	**a** 65.20 ±4.82	35.30 ±4.43	**c**4.00 ±3.07	**b**2.00 ±0.66	0.70 ±0.82	**b**49.80 ±1.75
LSD		**5.5**	**NS**	**1.0**	**3.84**	**NS**	**6.36**

a, b:Means of each variable with different letter differ significantly at 5%.

*LSD=Revised Least Square Difference

DISCUSSION

Enzyme Linked Fluorescent Assay (ELFA) is a new technology that was introduced recently in the diagnosis of pathogens either parasite, Viruses or bacteria and measuring various immunoglobulines to many of the tests. The use of a Minividas a quantum leap in the field of scientific and medical research where there is efficient diagnostic with privacy and high sensitivity in addition to a shortcut time and effort with the accuracy of the results (Gorman & Donnenberg,2008) . The results of the current study showed spread of the *Toxoplasma* parasite infection largely in the province of Thi Qar, from abortion common causing addition to recording infection increase anti cardiolipid and anti phosphiolipid antibodies, , which are also the causes of abortion .

The study showed the current rate of infection *Toxoplasma* reached 26.84% and studies that recorded ratios approach the study Rajendra*et al*.,(2006) in India recorded 27.27% as recorded Elamine *et al*., (2012) in Sudan ratio infection 20.7%.

The results show that immunoglobulins *Toxoplasma* get high incidence of chronic IgG reached 92.16% while the record IgM 5.88% either for both IgG and IgM was incidence of 1.96%, from Note diagnostic results, we find that the majority of infections chronic (found IgG in women) and this confirms that the parasite at the stage of phase slow Bradyzoite which hide inside host cells to get rid of the effectiveness of the immune system, where it is known that this phase accompanied with injuries chronic (Dubey, 2002)but when it goes down immunity body by ingestion of immune suppres sive properties or AIDS, these cystsexplodeandturnintotheactivephaserapidTachyzoitecausingseveresymptoms again, causing pathological relapsing(Jonson , 1999) .this is consistent with the study Aladlan, (2007) at Thi Qar and study AL-Harthi *et al*.,(2006) in Saudi Arabia, where log ratio injury IgM 5.6% and IgG 29% and study Khuran *et al*., (2010) in India, which found the proportion injury IgM 3% while IgG 15.33%, is IgG is an antibody only one capable of crossing

placenta will move from an infected mother to her fetus was due to rise in blood of women to the accumulation levels in the pregnant mother because a defect in the transfer process placental or due to lack of access blood mother to her fetus to cause immune pathogens related diseases tissues placenta (Abbas, 2005).

Results recorded cardiolipids less percentage compared with aggregates other amounted to 3.42% and the ratio of IgM 23.09% while the percentage of IgG 76.92% was recorded, both IgG and IgM not recorded infection, from studies on this topic study by Hasan *et al*.,(2010) in Diyala, which found that the percentage of pregnant women for antigen immune anti cardiolipids attack was 30% among women who have experienced abortion and 3.3% in women who did not get their abortion earlier and another study conducted by Ali, (2010) on a sample of aborted women previously found that the incidence of 24.4% antigen-positive immune attack of cardiolipids In a study conducted in India Sheth,(2001), which showed 26.4% woman carrying both (IgG and IgM) or one with a history of abortion. Also recorded phospholipids ratio infection amounted to 4.74% and the ratio of IgM 11.11% while the record IgG 88.89% and none recorded infection for both(IgG and IgM) . In a study conducted by Yassin, (2007) in Baghdad, a group of women with a history of abortion ages of 35-45 years the proportion of women antigen-positive immune phospholipids at 60.86%.

As results showed blood parameter for a decrease in HB and PCV compared with the control group due to decrease into cases with repeated abortions suffered by most examinated women addition to the injury pathological private parasitic cause consumption vitamins such as (C, B6, B12) and the lack of absorption food such as fats, vitamins and folic acid(Brabin *et al* ., 2001).

The results also showed high significant in the WBC in groups 1 compared with the control group and this is normal where increasing numbers of white cells in reaction of the immune system which works to direct

the immune response inherent and acquired for resistors infection parasitic (Ajioka *et al*., 2002) as This increase is consistent with the increase in the rate of phagocytosis, which was recorded in groups 1 where increases egg cells with increased rate of phagocytosis.The results showed an increase in the types of blood cells such as Eosinophiles and this is linked to the type of immune response in stress resistance which corresponds to the body, the high proportion of Eosinophiles in Group 1 infected Toxoplasmosis is normal because it is characterized by resistance to parasitic infection where increasing (Lydyard and Grossi,1998) and this agrees with the study Al-Quraishi(2009) who recorded high in WBC and Eosinophiles due to parasitic infections.

The Phagocytosis measure the ability of macrophages to kill cellular Pathogens, exotic particles and toxic .The result showed high rate of phagocytosis in groups 1 compared with the control group and this is normal because the parasitic infection caused activate macrophages and increase their phagocytic to resist infection in reaction to the device the host's immune (Hideyuki, 2002) is also the process of phagocytosis complement the work of opposites immune against infection parasitic ,addition to cells phagocytosis produce nitric oxide after stimulated by interferon gamma (IFN- ɣ), which is an important factor in resistance infection acute and chronic (Khan *et al.*, 1999) In a study conducted in the province of Thi Qar by Hussein *et al*., (2013), which found a high rate of phagocytosis because of parasitic infection , In another study in the province of Baghdad by Al-Sheikh, (2004) didn"t find significant differences between the coefficient phagocytosis and parasitic infection.

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دراسة تشخيصية لبعض مسببات الاجهاض لدى النساء بطريقة (ELFA) في محافظة ذي قار

فاضل عباس العبادي علي نعيم سلمان زهراء سعدون هادي
قسم علوم الحياة / كلية التربية للعلوم الصرفة / جامعة ذي قار

الخلاصة

تهدف الدراسة الحالية لتشخيص وقياس بعض مسببات الاجهاض بأستخدام لاختبارالتالقي للانزيم المرتبط (Enzyme Linked Fluorescent Assay (ELFA ، شملت تشخيص طفيلي مقوسة كوندي *Toxoplasma gondii*وقياس نسبة الدهون القلبية Cardiolipid والدهون الفوسفاتية phospholipids بالاضافة لقياس المعايير الدموية والبلعمة . جمعت 380 عينة دم من النساء المجهضات عند مراجعتهن مستشفيات محافظة ذي قار والمختبرات الخاصة.اظهرت نتائج الدراسة الحالية عدم وجود فروق معنوية في نسبة الاصابة للنساء المجهضات وكذلك في قيمة IgMواظهرت النتائج الحالية وجود فروق معنوية في قيمة IgG بينما لا توجد فروق معنوية بين قيمة IgM و IgG.

سجلت النتائج الحالية المعايير الدموية التي اجريت على 40 عينة دم لكل من المصابات بطفيلي *Toxoplasma gondii* و10 عينة دم لكل من النساء المقاس لهن نسبة الدهون القلبية والفوسفاتية بالمقارنة مع 10 عينة دم عينة سيطرة. بينت النتائج الحالية نقص لنسبة خضاب الدم HB وحجم الدم المضغوط P.C.V في مجموعة النساء المصابات بالطفيلي بالمقارنة مع مجموعة السيطرة لكن زيادة للعدد الكلي لكريات الدم البيضWBC في النساء المصابات بالطفيلي بالمقارنة مع مجموعة السيطرة . اظهرت نتائج الدراسة الحالية ارتفاع لمعامل البلعمة في المجموعة الاولى بالمقارنة بمجموعة السيطرة.