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Project AGNI SHAKTI: Awakening Greatness & National Integration through Spiritual Heritage and Advanced Knowledge Transformation Initiative

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ABSTRACT

Background*: Toxoplasma gondii* is an obligate intracellular protozoan, that has the capacity to infect human and all warm blooded animals. Maternal infection with toxoplasmosis during gestation and its transmission to the fetus continue to be the cause of tragic yet preventable disease in offspring.
Objectives: The present study is designed to determine the infection rate of *T. gondii* in two groups of aborted women (non-pregnant and pregnant), to evaluate the most effective methods in detection of toxoplasmosis and to investigate the relationship between *T. gondii* infection and some of the demographic characteristics.
Methods: One hundred fifty women were included in this study to detect anti- Toxoplasma antibodies. The study included one hundred women divided in 2 groups; group 1: Include 58 sponteneous aborted women, group 2: Include 42 pregnant women with previous sponteneous abortion and 50 women with close ages who had no abortion or pregnancy were considered as a control group. All were attend to Al-Batool Teaching Hospital in Baqubah city/ Diyala province, during the period from 2016 till 2017. Their age ranged between (14 - 55) years. A questionnaire on personal information was prepared, asked them about different socio-demographic data. The presented study included serological and molecular examinations, toxoplasmosis diagnosed serologically by enzyme linked immunosorbent assay (ELISA) test for (IgM and IgG antibodies), IgG avidity-assay, in addition to the Real time polymerase chain reaction (RT-PCR) were used in an attempt to diagnose toxoplasmosis in the blood of abortive non pregnant and pregnant women.
Results: This study showed that 44(44%) were positive by ELISA (IgG and IgM) which considered as a confirmed toxoplasmosis cases, 4 (4%) of abortive women had IgM+, 38 (38%) had IgG+, and 2(2%) had both IgM+ and IgG+, whereas the control women were 0% for all types of antibodies. The highest results of these tests were observed in 25-34 years age, 70.5% of women with positive results were aborted at the first trimester, 47.7% of these women with single abortion and 56.8% haven't children.
The present study demonstrated a significant relation between non pregnant and pregnant women for IgG, the majority of IgG positive cases were observed in non-pregnant group. Also there was a relationship between increased the seropositive of aborted women for anti-*T. gondii* in housewives women and illiterate or in complete primary school, residence in rural area, and contact with animals.
Real-Time PCR test in blood of pregnant and non-pregnant women has advantages in detection of recent or active toxoplasmosis, 15/100 (15%) of aborted women were positive by RT-PCR for toxoplasmosis associated with low avidity IgG, 4 of abortive women with IgM+ were RT-PCR positive. In addition, 7 of abortive women with IgG+, 2 of abortive women with IgM+& IgG+ were RT-PCR positive. Although 2 cases of abortive women with IgM-& IgG- were RT-PCR positive. A point to be noted in this study, is the presence of 9 cases which were IgM negative by ELISA test but were positive by RT- PCR.
Conclusion: The results of the present study indicate that the most effective method in detection of the infection of *T. gondii* is RT- PCR in addition to avidity IgG method to give a real perception of the disease.

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Keywords- *Toxoplasma gondii*, aborted women, and molecular study.

I. INTRODUCTION

Toxoplasma gondii (*T. gondii*), a common protozoan parasite responsible for both severe congenital birth defect and fatal toxoplasmic encephalitis in immunocompromised people (1). During the infection, the parasite needs to transpose the intestinal barrier to spread throughout the body, which may be a trigger for an inflammatory reaction (2). Though most chronically infected patients are asymptomatic, In addition, congenital fetal toxoplasmosis may result in abortion, stillbirth, or severe mental retardation; infections in late pregnancy may be asymptomatic but present with retinal or neurological damage later in life (3). However, the most common pregnancy complication is fetal loss, occurring in 25-30% of recognized pregnancies. Recurrent pregnancy loss affects at least 1% and can be defined as two or more failed pregnancies (4).

Toxoplasmosis is the third most common cause of death from food-borne illness (5). In humans, the primary source of infection is through contact with the feces of infected animals, especially domestic cats, the definitive host in which the *T. gondii* protozoan completes its life cycle. Alternate sources of infection occur through contact and ingestion of infected meat, drinking raw milk (6), and maternal-fetal transmission (7).

The ubiquity of the infection source and the differential exposure of the individuals to it, due to cultural and hygienic habits, may explain why the prevalence of toxoplasmosis is extremely variable between countries (8). *T. gondii* infection stimulates both cell mediated immunity (CMI) in addition to humeral immune response as antibody production, which includes IgM and IgG antibodies (9). The IgG, IgM, IgA and IgE antibodies are increased in patients with acute and chronic toxoplasmosis depending on the strain and the stage of the parasite (10). It is therefore essential to estimate the time of infection as precisely as possible to properly manage the risk to the patients. Primary infection is suspected if the *Toxoplasma* IgG antibody level is high, IgM is positive and the IgG avidity is low. Latent infection is suspected by IgG positivity, high IgG avidity and IgM negativity. Detection of the parasite by molecular methods like Real time Polymerase chain reaction (RT-PCR) is crucial for diagnosing the disease. Real time- PCR can be used as an additional diagnostic tool for the rapid detection of *T. gondii* in various clinical materials (11). The present study was conducted to study the infection rate of *T. gondii* among aborted women (pregnancy and non-pregnancy) to detect the possible association between infection with *T. gondii* and abortion in Baqubah, investigate the relationship between *Toxoplasma* infection and some of the demographic characteristics, and provide knowledge of the natural history of the *Toxoplasma* specific antibodies response special emphasis on the IgM response, developmental IgG avidity, and detection amplification of DNA of *T. gondii* by using RT-PCR technique.

II. MATERIALS AND METHODS

Patients and control

One hundred fifty women were included in this study to detect anti- *Toxoplasma* antibodies. The study included one hundred women divided in 2 groups; group 1: Include 58 aborted women, group 2: Include 42 pregnant women with previous abortion and 50 women with close ages who had no abortion or pregnancy were considered as a control group. All were attend to Al-Batool Teaching Hospital in Baqubah city/ Diyala province, during the period from 2016 till 2017. Their age ranged between (14 - 55) years. A questionnaire on personal information was prepared, asked them about different socio-demographic data. Approximately 8 milliliters (ml) of venous blood sample was collected from each women under sterile condition, 2 ml put in EDTA tube and stored at -80 ºC until be used to detect *T. gondii* DNA in the blood specimen, 6ml put in gel tube and left at room temperature then undergone centrifugation at 1000 rpm for 15 minutes. The serum was collected in plain tubes and stored at -20 ºC until be used to determine the IgM, the IgG, avidity IgG.

A- Immunological study by Enzyme linked immunosorbent assay (ELISA**)**

The *T. gondii* antibodies detection by ELISA kits **(**Toxo IgM μ-capture. Germany, catalog No. 51119, Toxo IgG Germany, catalog No. 51209 and *T. gondii* IgG – avidity test NovaLisa Germany, catalog No. 0483), are a qualitative determination of the parasite antibodies in blood samples. These test were performed according to the manufacturer's specifications**.**

B-Molecular study

- The DNA was extracted from the whole blood samples by using *ExiPrep*TM Plus Genomic DNA Kit from BioNeer catalog No. K-4214. The extraction was performed according to the manufacturer's specifications.
- Amplification and detection of *T.gondii* DNA by Real-Time Polymerase chain reaction by using AccuPower® TG Real -Time PCR Kit from BioNeer catalog No. TGN-1111. The test was performed according to the manufacturer's specifications.

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Statistical analysis

The data were presented in terms of observed numbers and percentage frequencies, and then analyzed by using Chi- square ($\chi 2$) test. P value ≤ 0.05 was considered statistically significant

Odd ratio and confidence intervals were used to assess the risk effect of studied factor between groups (SAS, 2012) (12).

III. RESULTS

Immunological study: *Toxoplasma gondii* infection

The seroprevalence of IgG and IgM anti-*Toxoplasma* antibodies in non-pregnant women were positive in 22/58 cases (37.9%) and 3/58 cases (5.2%) respectively, while the seroprevalence of IgG and IgM anti-*Toxoplasma* antibodies in pregnant women were positive in 16/42 cases (38.1%) and 1/42 cases (2.4%) respectively, mixed seropositive for IgG and IgM 2/100, one for each non-pregnant and pregnant women were recorded in this study. However the Overall Seroprevalence of anti- *T. gondii* antibodies in aborted women was 44%, whereas the healthy women were 0% for all types of antibodies, as show in Table 1.

Table (1): Overall Seroprevalence of anti- *Toxoplasma gondii* antibodies in patients and control groups.

Study groups		No.	IgG +ve		IgM +ve		IgG + IgM +ve		Total +ve	
			No	%	No	%	No	%	No	%
Aborted women	**non pregnant**	58	22	37.9	3	5.2	1	1.7	26	26
	Pregnant	42	16	38.1	1	2.4	1	2.4	18	18
Total		100	38	38.0	4	4.0	2	2.0	44	44
Control		50	0	0	0	0	0	0	0	0

Table (2) shows there was a significant relation between non pregnant and pregnant women for IgG (P≤0.05), the majority of IgG positive cases were observed in non-pregnant who were 0.99 times more likely to have *Toxoplasma* (95% CI: 0.44-2.25). While no significant relation for IgM (P=0.482) and IgG+IgM (P=0.816) between non pregnant and pregnant women groups. Statistical analysis showed that there were significant differences (P≤0.05) between types of *Toxoplasma* antibodies for each non-pregnant and pregnant groups**.**

Table (2): Seroprevalence of anti- *Toxoplasma gondii* antibodies in patients women.

Groups	**Aborted women**				**P- value**	**OR CI**
Parameter	Non pregnant		Pregnant			
	No. 58	100%	No. 42	100%		
+ve IgM	3	5.2	1	2.4	0.482 NS	2.24 0.22-22.28
+ve IgG	22	37.9	16	38.1	0.040 *	0.99 0.44-2.25
+ve IgM+IgG	1	1.7	1	2.4	0.816 NS	0.72 0.04-11.84
P- value	P<0.005*		P<0.005*			

OR: Odds Ratio; **CI:** Confidence interval, **NS:** Not significant, *=Significant P≤ 0.05

Demographic characteristics of women with toxoplasmosis

The age distribution among the total *T. gondii* positive women ranged between <25 to above 45 years. The highest infection rate was 36.4% in 25-34 years age group with 16 cases, followed by 34.1% (15/44) in 35-44 age group, 18.2% (8/44) in < 25 age group, and 11.4% (5/44) in 45 years and older group.

The data analysis showed that 31/44 patients were aborted at first trimester, while 13 aborted at second trimester. According to questioner patients with single abortion found to have the highest toxoplasmosis were found 21(47.7%), while the lowest infection found in patients with double abortion 7 (15.9%).

The present results indicate that greatest number of aborted women infected with toxoplasmosis haven't children, the level of anti-*Toxoplasma* antibodies was higher in aborted patients without children (Secondary Abortion) than with

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children who have higher percentage (56.8%). The higher level of infections was shown in aborted women without treatment (61.4%). Also the study showed that 18(40.9%) of toxoplasmosis women found to be pregnant, 9 of them in first trimester and the other 9 in second trimester of pregnancy. The data analysis showed that 14/18 patients were with different clinical sigh of abortion as shown in Table (3).

Table (3): Variable factors of *Toxoplasma gondii* infection according to questionnaire obtained from 44 patients with abortion.

Factors		*Toxoplasma gondii*	
		Count	%
Age Groups	<25 years	8	18.2
	25-34 years	16	36.4
	35-44 years	15	34.1
	45+ years	5	11.4
Time of abortion	1st trimester	31	70.5
	2nd trimester	13	29.5
	3rd trimester	0	0.0
No. of abortion	Single	21	47.7
	Double	7	15.9
	Multiple	16	36.4
Drugs Yes		17	38.6
No		27	61.4
No. of children No children		25	56.8
With children		19	43.2
Pregnancy	Yes	18	40.9
	No	26	59.1
Pregnancy time	1st trimester	9	50.0
	2nd trimester	9	50.0
	3rd trimester	0	0.0
Clinical signs*	Present	14	77.7
	Absent	4	22.3

* mild to severe back pain, heavy spotting, vaginal bleeding, expulsion of tissue with clots from vagina, and cramps or severe abdominal pain.

Distribution of Toxoplasmosis in aborted women according to types of avidity

In this study, avidity was categorized into two groups: low avidity, lower than 40 and high avidity, above 40. The results of the analysis of 44 serum samples from pregnant during the first and second trimester of pregnancy and non-pregnant women are categorized into three groups. Group IgG+& IgM+, 1 and 1; group IgG+& IgM-, 16 and 22 and group IgG-&, IgM+1 and 3 respectively.

The results of the avidity tests were analyzed according to these groups (Table 4). In the first and third groups the avidity was low, while in the second group in pregnant and non-pregnant patients, where all had negative *Toxoplasma*-IgM, the most of avidity was high, pointing to the inactivity of the infection.

Table (4): Anti-*Toxoplasma* IgG, IgM and IgG avidity in the sera of pregnant and non –pregnant women.

ELISA		IgG avidity				Total	Diagnosis
		High		Low			
		Count	%	Count	%		
Pregnant	IgG + & IgM +	0	0	1	2.3	1	AI
	IgG + & IgM –	11	25.0	5	11.4	16	CI
	IgG - & IgM +	0	0	1	2.3	1	AI
Non pregnant	IgG + & IgM +	0	0	1	2.3	1	AI
	IgG + & IgM –	20	45.5	2	4.5	22	CI
	IgG - & IgM +	0	0	3	6.8	3	AI
	Total	31	70.5	13	29.5	44	

AI: Acute Infection, **CI:** Chronic Infection

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IV. MOLECULAR STUDY OF *TOXOPLASMA GONDII*

***Toxoplasma gondii* infections**

The PCR amplification were performed using RT- PCR BionerCycler. There was no evidence of inhibition of the amplification in any of the samples, with the No Template Control (NTC), Positive Control (PC) and Internal Positive Control (IPC) of the RT-PCR samples as shown in Figure (1).

Table (5) shows that positive *T. gondii* DNA was detected in 15/100, 1/50 of aborted and control women, respectively by RT-PCR.

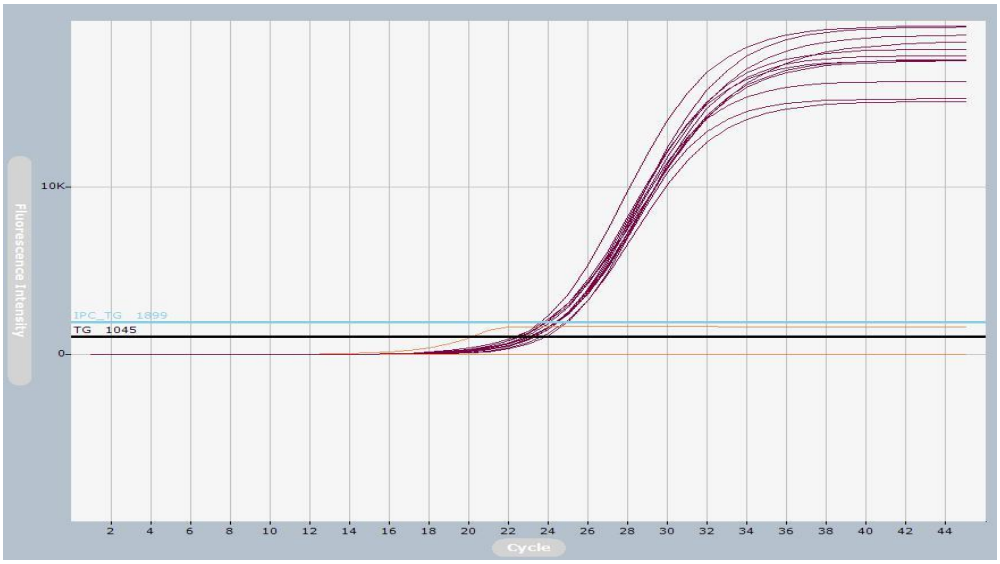

Figure (1): *Toxoplasma gondii* RT- PCR results; positive sample (red), negative sample (yellow), no template control (blue) positive control (black), each one with internal positive control.

Table (5): The *Toxoplasma gondii* identified from patients and control groups by Real-time polymerase chain reaction.

Method			Aborted groups			Total
			Non pregnant	Pregnant	Control	
RT-PCR	Positive	Count	9	6	1	16
		%	15.5%	14.3%	3.3%	10.7%
	Negative	Count	49	36	49	134
		%	84.5%	85.7%	96.7%	89.3%
Total		Count	58	42	50	150
		%	100.0%	100.0%	100.0%	100.0%

Comparation between serological and molecular methods for detection of Toxoplasma gondii in patients study

ELISA test detected 40 of IgG and 6 IgM anti-*Toxoplasma* antibodies in aborted women. However, RT-PCR test detected 15 cases of *T. gondii* infection among 100 women with abortion. A point to be noted in this study, is the presence of 9 cases which were IgM negative by ELISA test but were positive by RT- PCR. Statistically, the most differences were significant between IgM and IgG anti-*Toxoplasma* antibodies in detection of this protozoan (Table 6).

Table (6): Comparation analysis between ELISA and RT-PCR methods for detection of toxoplasmosis in study patient groups.

Methods	Type	No. (%)	Relation	X^2	P value
ELISA	**IgM**	6 (6.0%)	IgM & IgG	2.55	0.005*
	IgG	40 (40.0%)	IgG & PCR	0.563	0.453NS
RT-PCR		15 (15.0%)	IgM & PCR	23.61	0.110NS

X^2: Chi square, **P:** Probability, **NS:** Not significant.*= significant

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Anti-Toxoplasma IgG, IgM, and IgG avidity with RT-PCR in the toxoplasmosis women

In this study, avidity was categorized into two groups: low avidity, lower than 40 and high avidity, above 40. The results of the RT-PCR analysis of 15 blood samples from aborted women are categorized into four groups. Group IgG+& IgM+, 2; group IgG+& IgM-, 7; Group IgG-& IgM+4, and group IgG-& IgM-, 2.

The results of the avidity tests were analyzed according to these groups (Table 7). In the first , third and fourth groups the avidity was low. While in the second group, where all had negative anti-*Toxoplasma* IgM the avidity was high and only 7 detected by RT-PCR cases have low avidity.

Table (7): Anti-*Toxoplasma* IgG, IgM, with RT-PCR and IgG avidity in the pregnant and non –pregnant women.

Patients groups	RT-PCR	IgG avidity		Diagnosis
		High	Low	
	Count	Count	Count	
IgG + & IgM +	2	0	2	AI
IgG + & IgM –	7	31	7	CI and RA
IgG - & IgM +	4	0	4	AI
IgG - & IgM -	2	-	2	RI

AI: Acute Infection, **CI:** Chronic Infection, **RA:** Reactive infection, **RI:** Recent Infection.

V. DISCUSSION

Immunological study of Toxoplasma gondii

Maternal infection by *T. gondii* during pregnancy may have serious consequences for the fetus, ranging from miscarriage or spontenous abortion, central nervous system involvement, retino choroiditis, or subclinical infection at birth with a risk of late onset of ocular diseases (13).

The ELISA technique for the detection of IgM and IgG anti-*Toxoplasma* antibodies was chosen in this study, due to the fact that in acute infections, IgM antibodies levels generally elevated within one to two weeks of infection (14). The presence of increased levels of *T. gondii* specific IgG antibodies indicates that toxoplasmosis has occurred but does not distinguish between recent infection and infection acquired in the distant past. Pregnant women are sometimes seropositive only for IgM, and IgG may not be detected during the serological follow-up. Timing the onset of the infection is crucial in pregnant women, especially post-conceptional acquisition represents a risk for the fetus. For this purpose, other diagnostic tools can be utilized, such as IgG avidity detection (15).

In current study out of 100 samples, the overall seroprevalence of anti- *T. gondii* antibodies in aborted women was 44%. The seroprevalence of IgG and IgM anti-*Toxoplasma* antibodies in non-pregnant women were positive in 22/58 cases (37.9%) and 3/58 cases (5.2%), respectively. While the seroprevalence of IgG and IgM anti-*Toxoplasma* antibodies in pregnant women were positive in 16/42 cases (38.1%) and 1/42 cases (2.4%), respectively, mixed seropositive for IgG and IgM 2/100, one for each non-pregnant and pregnant women were recorded. a significant relation between non pregnant and pregnant women for IgG ($P<0.05$), as well as there were significant differences ($P<0.05$) between types of *Toxoplasma* antibodies for each non-pregnant and pregnant groups.

However, seroprevalence of toxoplasmosis in the present study was similar to many studies around the world and some Arabic countries in women with Bad Obstetric History (BOH), such as 44.8% in Libya (16), 35.1% in Qatar (17), 39.4% in Saudi Arabia (18), and 49.52% in India (19).

Although, the results of present study was agreement with other studies in Iraq, that deal with a seropositivity by using ELISA were 37.5% for IgG in Erbil (20), and in Samara city recorded a rate of positive *T. gondii* IgG and IgM antibodies in sera of aborted women (38.15%) (21). While, in Diyala province found high infection were 37 (46.26%) women out of eighty were infected with toxoplasmosis (22). Although, low result obtained by Mohammad (2012) in Al-Ramadi who showed that the prevalence of toxoplasmosis was 30.7% among women (23).

The present study revealed low anti- IgM (2.4%) in pregnant women, this finding is similar to the results of a study in Salah- Adden among pregnant women, the acute infection was 7(3.1%) of cases (24) , While another study done by Aziz and Majida, at 2011 among pregnant women showed a higher results, 25(59.5%) women were positive for IgG, and 17(40.5%) women were positive for IgM, while 9(17.6%) women were positive for both (25).

The variation in the prevalence rates of *T. gondii* among studies can be attributed to the differences in the study populations and study areas, as well as the different diagnostic methods used for blood examination. Also, the differences in toxoplasmosis rates can be interpreted on the basis of similarity or differences of climatic conditions of location and that the various ages of patients study, nutritional and immune status, hygienic habits, sanitary supplies and socioeconomic conditions in study's region. In general the prevalence of protozoans was strongly associated with a variety of risky factors including host, sociodemograph environmental and zoonotic transmission (26).

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This relatively high rate of toxoplasmosis in present study may be related to differences factors including the sample size which was only 100 and the types of patients were selected; first group who had abortion and the second group who had previous abortion with suspension of toxoplasmosis during pregnancy and physical screening. This indicates that is considerable number of females in this society harboring the parasite, transmitter to other people and it is representing a real problem that should not be neglected and must receive attention from health authorities.

Demographic characteristics of aborted women with toxoplasmosis

The highest infection rate was shown in age groups 25-34 years (36.4%) with 16 cases. This result was agreement with a study that showed the most frequent age group for abortive women and abnormal pregnancy was among those of 26 to 30 years and it represents 35.7%, this could be due to the fact that the seroprevalence of *T. gondii* infection was high in women of child-bearing age (27). While other study that give an indication that there is no association between age of pregnant women and related abortion factors (28).

The present study observed high incidence rates of toxoplasmosis in women suffering from abortion during the first trimester of pregnancy. This results were agreed with a study done by Al-Abudy (2014) found the highest rate of abortion was in the first trimester (29). While the results of the present study disagreement with the study conducted the highest proportion of cases recorded in the second trimester of pregnancy (30).

The highest rate of toxoplasmosis were found in women with single abortion (47.7%) which is deal with the study of Hadi *et al.* (2016) in women of Al-Qadisiyah province that showed a near result from the current study of single previous abortion (43.6%) (31). It has been proposed that during pregnancy, systemic maternal immune response is biased in favor of a Th2 cytokines, and also in women with first abortion. Moreover, Th2 cytokines pattern of with the pregnancy induces the susceptibility to toxoplasmosis infection, together risk of placental infection and congenital transmission (32).

The present study findings about relationship between infection with *Toxoplasma* and the ability to have child. However, the results of present study were indicated that women with children have the lowest rate of injury by parasite.

While the distribution of toxoplasmosis in present study was higher in women without treatment (61.4%). This agree with the result that revealed that the *T. gondii*-specific IgG was significantly delayed in pregnant women receiving therapy compared to non-pregnant, untreated controls (33).

The study showed that 18(40.9%) of toxoplasmosis women found to be pregnant. However, there are many studies on the prevalence of anti-*T. gondii* antibody among Iranian aborted women. Seropositivity of *T. gondii* is 22%-37% in south (34). While the seroprevalence of the infection in pregnant women in other study was reported as 17.9% (35). Which is deal with present study in the results that detected the prevalence of toxoplamosis in aborted non-pregnant women was higher than in pregnant women.

In the current study the rate of toxoplasmosis during pregnancy was in the first and second trimesters equal and higher than the rate in the third trimester. This agree with the result of a study among pregnant women that found equal results in the first and second trimesters and higher than the third trimester, in which the seroprevalence in the first, second and third trimesters were 30.4%, 30.6% and 26.1%, respectively (36). While the result of present study disagreement with a study that revealed the third trimester of pregnancy as a high risk for infection (42.69%) (37).

The data analysis of toxoplasmosis pregnant women (77.7%) showed clinical characteristics most of them have lower abdominal pain, back pain and cramps. This finding is similar to the result found different clinical signs of abortion in toxoplasmosis pregnant women (38).

Molecular study of Toxoplasma gondii

Several polymerase chain reaction based techniques have been developed for the detection of toxoplasmosis. The advent of innovative qualitative and quantitative RT- PCR techniques has proven useful in various applications, including pathogen detection and gene expression investigations (39). RT-PCR can be used diagnostic tool for the rapid detection of *T. gondii* in various clinical materials.

The current study reveal that 15/100 of aborted women were positive by RT-PCR for toxoplasmosis. This result was agreement with other studies in Egypt that showed (12.5% and 11.8%) of toxoplasmosis in aborted Egyptian women were obtained by PCR (40).

Several studies have documented that RT- PCR can actually detect *T. gondii* in blood specimens of women before or during pregnancy (41,42). Other study have reported that, PCR could detect parasitaemia a few weeks prior to the appearance of any clinical signs or symptoms. Based on these studies, it has been accepted that the gold-standard examination for identifying fetal infection is PCR, which has high sensitivity and specificity (42).

Comparation between serological and molecular methods for detection of Toxoplasma gondii in patients study

The results of the present study indicate that molecular diagnoses are highly sensitive, strong and specific methods to detect *T. gondii* infection, while the serological methods do not give a real perception of the disease (43).

Remarkably, in present study 4 of abortive women with IgM+ were RT-PCR positive. They represent acute infection. In addition, 7 of abortive women with IgG+, 2 of abortive women with IgM+& IgG+ were RT-PCR positive. They represent active infections due to reactivate of latent toxoplasmosis. Although 2 cases of abortive women with IgM-& IgG- were RT-PCR positive. They represent recent infection. A point to be noted in this study, is the presence of 9 cases which were IgM negative by ELISA test but were positive by RT- PCR, this could be explained depending on the fact that

IgM might be below the detection level of the kit used (very early in the disease or during of switching from IgM to IgG) or those cases might be false negative by ELISA method. However, the presence of *Toxoplasma* DNA in the whole blood probably indicates a recent infection or apparent parasitemia or active toxoplasmosis, which is likely to be clinically significant (44).

Anti-Toxoplasma IgG, IgM, and IgG avidity with RT-PCR in the toxoplasmosis women

The IgG avidity test is the laboratory resource accepted for the diagnosis of the approximate time when the initial infection occurred. This measures the affinity of IgG for binding to the antigens of *T. gondii*, and it tends to increase with the length of time that has elapsed since the initial infection (45). The current study indicates results (25%) of high IgG avidity and (11.5%) of low IgG avidity in pregnant women, According to Al-Saidi *et al*. (2015) that reported the infection rate 75(93.75%) case of the pregnant women had positive IgG and negative IgM and all had high avidity test results which is higher than results of the presented study (61.36%), and at the same time, the study showed a lower results of low avidity test (6.25%) than present results (46).

On the other hand, the result of the present study are higher than other study done by Pour Abolghasem *et al*. (2011) that found seventy percent of the pregnant women had positive IgG and IgM among which 7.1% had low avidity which revealed an active infection in the pregnant women (45). In the current study eleven of pregnant women had positive IgG and negative IgM, all of which had high avidity, which is an indication that in present patients the level of toxoplasmosis infection is high and most women had contacts with this parasite before pregnancy.

The overall results of avidity IgG and RT- PCR were the best methods in detection the recent, acute and reactive infection by *T. gondii*. This result has agreement with the other study that showed the importance of the specific IgG avidity test and PCR for toxoplasmosis with their significance and importance as auxiliary methods for determining the most likely time for the initial infection by this coccidian and for defining the therapeutic strategy (44)

VI. CONCLUSIONS

There is association between infection with T. gondii and occurrence of abortion, as well as the presence of relationship between increased the seropositive of aborted women for anti-T.gondii and illiterate or in complete primary school, residence in rural area, housewives women, and contact with animals, which may be consider these demographic characteristics as risk factor for toxoplasmosis. The use of ELISA IgG and IgM followed by an IgG-avidity assay was a step forward. As well as, the IgG avidity and RT- PCR methods in aborted women were the best method in detection the recent and reactive infection by T. gondii.

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