

Isolation and Diagnosis of *Toxoplasma gondii* among Abortive Women Attending Salah al-Din Hospital and Emergency Hospital in Iraq

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Abstract

Toxoplasmosis is caused by *Toxoplasma gondii* parasite .this parasite is one of the main causes of miscarriage in married women in Salah al-Din Governorate. This study is aimed to diagnose the parasite by isolating it from the tissues of the uterus and placenta for aborted women, in Salah al-Din Hospital and Emergency Hospital . Also, the study was interested in highlighting some important data about abortions and its relationship to the proportion of infection such as profession, age, type of housing, contact with cats and some blood picture tests, in addition to some other important data.

The study was conducted in Salah Al-Din General Hospital and the Emergency Hospital (from July 2019 to January 2020), the study included 100 aborted women aged between (20 - 45 years), and to obtain the required information a special questionnaire was using for each aborted woman.

The latex test (LAT) and ELISA-IgM agglutination tests were used to compare the sensitivity of these tests in detecting infection. The results showed that the isolation was performed in 6 samples from a total of 58 samples with a rate of 10.34%, and the study also showed that the percentage of infection was 42% among abortions using the latex test and 7% in the ELISA test.

The results of the study showed that the highest incidence of infection was during the first three months of pregnancy and that the most frequent cases of miscarriage were within the twice abortion. It was found that the proportion of infection in the 20-30 age groups was higher than the other age groups, as was the higher rate for uneducated women living in rural areas.

Key words: *Toxoplasma gondii* , Toxoplasmosis , Aborted Women , Infection .

Introduction:

Toxoplasmosis is a common human-animal disease spread around the world caused by the parasite *Toxoplasma gondii*. It is one of the protozoan parasitic that live inside cells (Obligate) and can infect all warm-blooded animals. Most mammals and birds are considered as hosts ^[1] ^[2].

Cats infected with the parasite pose a threat to public health because they release ovarian cysts that are resistant to external environmental conditions in their feces ^[3] ^[4] . The protozoan *Toxoplasma gondii* infects 2 billion people worldwide and has been linked to behavioral alterations in humans and other vertebrates ^[5] .

The parasite infects a wide range of warm-blooded vertebrates, birds and mammals, including humans, which are intermediate hosts, but the definitive host, the main source of infection, is domestic, wild and other cats of *Felidae* species ^[6] .

Toxoplasmosis is a global disease, with 30-60% of the world's population believed to be infected, and about one-third of the world's population has antibodies, which means they are exposed to infection at some point in their lives ^[7] . Toxoplasmosis is considered as one of the most prevalent human parasitic infections that can be transmitted from mother to the fetus. The toxoplasmosis has clinical complications during pregnancy, including spontaneous abortion, preterm labor, stillbirth, and fetal abnormalities ^[8] .

Material & Methods

1. Collection of samples

Histological specimens: collected from the tissues of the uterine shelf curettage and tissues of the placenta for aborted females reviewed to the Salah al-Din Hospital and the Emergency Hospital between the ages of (20 - 45 years), the total number of tissue samples prepared was (38) samples from Uterine shelf , and (20) samples of placental tissue. Part of each sample was placed in a clean, sterile plastic container containing (0.9%) NaCl solution, and then covered with a tight lid and labeled with the patient's name, number, type of sample and date.

Blood samples: (100) blood samples were collected from the patients who suffer from once or several times abortion, by withdrawing 5 ml of venous blood using the Tourniquet band and a 5 ml medical syringe with 23 mm prick. Types of laboratory tubes:

1. Ready tube containing anticoagulant (EDTA) for blood film tests (hemoglobin Hb, PCV, total WBC count, differential WBC count .
2. A tube containing anticoagulant (Tri-Sodium citrate) for the purpose of testing the speed of deposition of erythrocytes (ESR)
3. Disposable tube free of any substance left for 15-30 minutes and then placed in the centrifuge for 5 minutes for the purpose of separating the blood serum and keep the serum frozen until the time of following serological tests:

- A. Widal test
- B. Rose Bengal test
- C. LAT test
- D. IgM ELISA test

Control Group samples:

This group represents a comparative group for evaluating the results of the study and to know the changes that occurred to some blood and serological tests to be investigated in the abortion abnormalities toxoplasmosis through blood and serological tests as well as the absence of any pregnancy or abortion for any reason whatsoever.

Statistical Analysis

The Chi- square test (χ^2) or the so-called good-match test, was used to analyze the data in the experiments according to the existing tables and some of them used the test (t) test. A significant level of 0.05 was selected to test the hypotheses in the tables as being the most commonly used in experimental studies and research, especially life research ^{[9] [10]}.

Results and Discussion

1- Isolate the parasite:

The total number of samples examined was 100 samples of which the parasite was isolated, of which only 6 samples out of 58 samples were fully prepared, including 38 samples of uterine shelf tissues and 20 placenta samples, the parasite was detected in swabs prepared from the stuck of the uterine shelf tissue (Figure 1). and placenta suspension after being staining with Giemsa stain . Although toxoplasmosis is a widespread disease in the world, its clinical symptoms are not apparent and can only be diagnosed by serological tests to detect antibodies formed or to isolate the parasite from body fluids and tissues ^[11].

This study was concerned with the diagnosis of the parasite curved by isolating it from the tissues of the uterine shelf and the placenta of aborted women. Garcia (1968) isolated and diagnosing the parasite from human placentas ^[12]. The inclusion of tissue samples of the uterine shelf and placenta on the cysts indicates a close relationship between the settlement of the parasite and the phenomenon of abortion, which is certainly the result of mother's infection with toxoplasmosis is naturally expected , especially if the infection occurred in a nurse strain, as the decline that occurs in the body's immunity during Pregnancy activates the conversion of slow-

multiplying vesicles into tissue bags into fast-multiplying vesicles that tear apart the cells in which they are free to infect other cells, including fetal cells that reach the placenta leading to miscarriage^{[13] [14]}.



(Figure : 1) Cyst Tissue in the swab prepared from uterine stuck solution shelf tissue and stained with Giemsa stain (oil lens x100) .

2- Comparison of the latex and ELISA-IgM tests:

Table (1) shows a comparison between the two tests used in this study to determine the percentage of infection with toxoplasmosis in aborted women, where the percentage of positive samples tested latex were (44%) which are higher than the percentage of positive samples tested ELISA (7%). In this study, two serological tests were used: latex and ELISA to compare their sensitivity in detecting infection in abortion. Many researches indicated that ELISA is highly specific and sensitive in detecting the disease^[14]. Latex gives only a preliminary visualization of the diagnosis, magnitude of the infection and the probability of an acute or chronic phase^[16]. The manufacturer of the latex test kit indicated that a positive result means the presence of serum toxoplasmosis antibodies indicating pre-infection and that the level of antibody or antibody standard is not used to identify acute infection or stage of the disease. To ensure acute infection of the disease, IgM should be detected. For this, latex testing for IgG and IgM antibodies is used in surveys because of its distinctiveness, ease of conduct, low cost, time and effort^[17]. The results of the current study confirm the importance of toxoplasmosis in Salah al-Din province, such as those conducted in other governorates of Iraq, where the rate of infection outbreaks in abortifacients in the current study 42% using latex test and 7% using ELISA test, a proportion consistent with the global ratio 42%, which was the result of many studies conducted in more than 53 countries in the world and lasted for several years^[18].

Table (1) Comparison of Latex and ELISA Tests in Assay of Abortifacient .

Total number of samples Screened	Latex test		ELISA test	
	%	Positive samples	%	Positive samples
100	42	24	7	*7

* This number (7) is among the positive samples tested latex.

3- The location of the housing and its effect on the infection rates of toxoplasmosis:

Table (2) shows the difference in the distribution of arthritis infections between the city and the rural areas in Salah al-Din Governorate, where the percentage of positive cases in rural areas according to latex test (56.25%), which is higher than the positive percentage in urban areas (35.29%) , as well as according to ELISA test The percentage of positive cases in the rural areas was (9.67%), which is higher than the rate of infection in urban areas, which amounted to (5.79%). Comparing these results with those of Mohammed and Al-Janabi (2019) The percentage was in rural 25% and in urban 12%. Statistical analysis shows that there are significant differences between infections of toxoplasmosis with patients residences ^[8]. and also results AL-Safe (2018) ^[19]. Consistent with the of this study .

The increase in infection in rural areas is attributed to several reasons, including that the rural areas are wet areas with abundant cultivated lands and orchards, thus increasing the contact of women with the soil through work on farms as well as the presence of cats and animal husbandry at home, as the moderation of the area and increase humidity help On the spawning of egg bags raised with feces of infected cats gives a greater chance of contamination of food and water sources that are the main factor in the transmission of infection ^[20] ^[21] .

Table (2): Distribution of Toxoplasmosis by City and Rural Areas.

Housing	Total number	Latex test		ELISA test	
		Positive samples	%	Positive samples	%
City	68	24	35.29	4	5.79
Countryside	32	18	56.25	3	9.67
Total	100	42	-	7	-

L.S. = 0.05

4 - Educational level and its effect on infection rates:

Table (3) shows the different educational levels of abortion women with toxoplasmosis and the uneducated women were the most affected (54.76% and 9.52%) according to the latex and ELISA tests respectively, while the lowest abortions rates were among the preparatory education women (0%). In the two tests, statistical analysis did not show significant statistical differences between the different educational levels of abortifacients according to significant level (0.05). This finding is consistent with his findings Cook and others ^[22]. Although the rate is not very high, it is noted that the rate of infection decreases with increasing level of education because the knowledge of the disease and how it is transmitted, and the damage caused by the parasite can reduce the rate of infection.

Table (3): Distribution of toxoplasmosis by educational levels.

Educational level of abortifacients	Total number	Latex test		ELISA test	
		Positive samples	%	Positive samples	%
Uneducated	42	23	54.76	4	9.52
Primary education	37	17	45.94	2	5.40
Intermediate Education	5	1	20.00	-	-
Preparatory Education	2	0	0	-	-
University Education	14	1	7.14	1	7.14

L.S. = 0.05

5 - Different age groups and their effect on the rates of infection with toxoplasmosis:

Table (4) shows the relationship between age and infection with toxoplasmosis. The

results showed that the highest percentages were in the age group 20-30 years using the two tests under study. The infection was 54.05% and 13.51% according to the latex and ELISA tests respectively. 38-46 years, which was 14.28% according to the latex test and 0% according to ELISA test? The statistical analysis showed significant differences under the chi-square test (χ^2) and a significant level (0.05). This is identical to what many researchers in different parts of the world have indicated, as the incidence was high in the group aged (34-25) years^{[19] [23] [24]}. The reason that the age (30-25) is the appropriate period of birth and this leads to the risk of the disease due to the transmission of infection from the placenta to the fetus and the birth which led to birth defects if the injury occurred in the last period of pregnancy^[25]. Infants born become infected with *T. gondii* just prior to (less than three months) or during pregnancy are at risk of severe toxoplasmosis^[26].

Table (4) Distribution of toxoplasmosis by age groups of abortifacients.

Age group of years	Total number	Latex test		ELISA test	
		Positive samples	%	Positive samples	%
20-30	37	20	54.05	5	13.51
30-38	49	19	38.77	2	4.08
38-45	14	2	14.28	0	0

L.S. = 0.05

6 - Effect of toxoplasmosis on pregnancy duration of abortion:

Table (5) shows the relationship between the effect of toxoplasmosis and the duration of pregnancy in which abortion occurred, where the most abortions were within the first trimester of pregnancy (32 cases, 62.26%) and the lowest rate of abortion in the last three months of pregnancy (11.11%). The presence of statistically significant differences under the chi-square test (χ^2) and a significant level (0.05). These results are consistent with those recorded^[26]. when the prevalence of toxoplasmosis during the first period of pregnancy was higher than those aborted during the second and third period of pregnancy, and the results of the current study confirms the findings of Shahida and others^[28]. Toxoplasmosis infected cases in first, second and third trimesters, respectively, 54.34 % were seropositive (acute infection) in first trimester, 21.56 % seropositive in second trimester, and 14.81% seropositive in third trimester. Although the

toxoplasmosis seropositive test is positive because the initial gestation period is critical and requires caution and caution from the mother.

Table (5): Duration of pregnancy in which abortion occurred.

The duration of pregnancy in which the abortion occurred	Total number	Positive samples	%
First trimester of pregnancy	53	33	62.26
The median trimester of pregnancy	29	7	24.13
The last three months of pregnancy	18	2	11.11

L.S. = 0.05

7- Effect of toxoplasmosis on the frequency of abortion:

Table (6) shows the distribution of toxoplasmosis for cases that have suffered repeated abortions, with the highest positive percentage in two cases of abortion, 20 cases, 54.05% and the lowest in abortion four times and more, where the rate of infection was 25.00% according to the latex test. In the case of ELISA test, the highest positive percentage in abortion was also twice (10.81%), and the lowest percentage within abortion (three times, four times or more) and 0%, and the statistical analysis did not show any significant differences (0.05).

The current study also showed that the number of abortions had an effect on serological positivity among aborted women. The percentage of women who had suffered from twice abortion was higher than the rest of women who suffered once, three or four times and more, and this finding, agree with the findings of AL-Maqdisi ^[29]. Shani. noted that the highest rate of infection was among women who had abortions ^[30]. Research suggests that the toxoplasmosis parasite causes a one-time abortion in premature women if the mother is infected during pregnancy, in the acute phase of the disease ^[31].

Table (6): Distribution of toxoplasmosis in relation to frequency of abortion

The number of time		Latex test	ELISA test
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Abortion	Total number	Positive samples	%	Positive samples	%
Once	43	17	39.53	3	6.97
Twice	37	20	54.05	4	10.18
Three	12	3	25.00	-	-
Four and more	8	2	25.00	-	-

L.S. = 0.05

8 - The relationship between anti-toxoplasmic norms and concentrations and the frequency of miscarriage:

Table (7) shows the criteria and concentrations of toxoplasmic antibodies for positive cases for serological examination using the latex test and the highest infection rate was within the criterion 1/160 at the rate of 47.61% and the lowest in the criterion 1/640 at the rate of 7.14%, and the highest concentration of toxoplasmosis antibodies appeared in Miscarriage twice, with a rate of 20.00% and by 4 cases. Statistical analysis did not show the presence of statistically significant differences with a significant level (0.05). As for the criteria and concentrations of antibodies to toxoplasmosis and its relationship to the frequency of miscarriage, the current study showed that the prevailing standard is 1/160 between the opposites criteria and that this may be due to the fact that chronic infection is the prevalent infection that was relatively low, and this result was consistent with the study conducted by ^[16] [33]. Who found that the highest incidence of infection was at the 160/1 standard, which indicates that miscarriages occurred due to a previous chronic infection.

]Table (7) Criteria and concentrations of toxoplasma antibodies and their relationship to miscarriage.

The number of times Abortion	Antibody standards per the latex test	Concentrations of opposites by Elisa test IU / ml
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	Positive samples	1/80 %	1/160 %	1/320 %	1/640 %	Positive samples with a concentration of (1) and more%
Once	17	2	7	4	3	3
		11.76	47.05	23.52	17.64	17.64
Twice	20	4	9	7	-	4
		20.00	45.00	35.00	-	20.00
Three	3	1	2	1	-	-
		33.33	66.66	33.33	-	-
Four and more	2	-	2	-	-	-
		-	100.00	-	-	-
Total	42	7	20	12	3	7
		16.66	47.61	28.57	7.14	16.66

L.S. = 0.05

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