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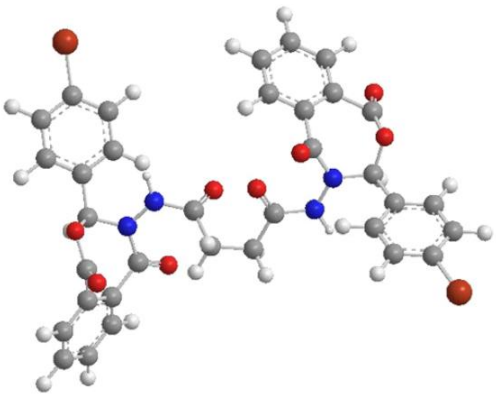

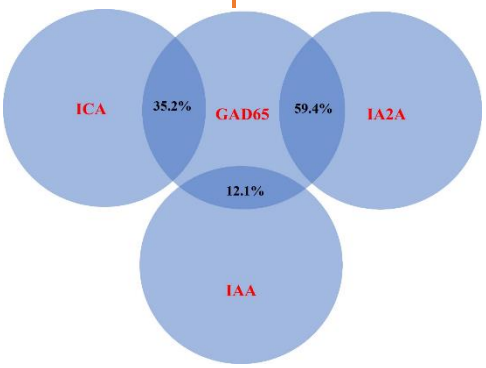

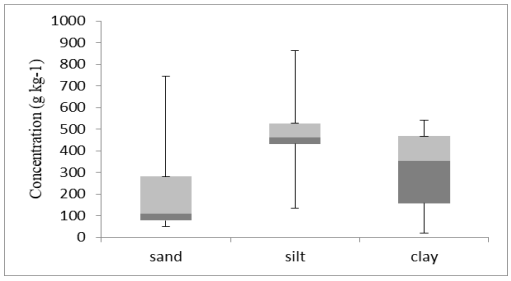

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Seroprevalence of *Toxoplasma gondii* among Aborted Women Admitted to Erbil Maternity Teaching Hospital

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Abstract

Background: *Toxoplasma gondii* is an obligate intracellular parasite responsible for causing congenital toxoplasmosis. Congenital infection during the first and second trimesters of pregnancy may result in spontaneous abortion, mental retardation, and cerebral calcifications, while the severity of fetal damage is decreased during the third trimester. **Objectives:** This study aimed to determine the seroprevalence of toxoplasmosis and its association with clinical-obstetric determinants and sociodemographic factors in aborted women. **Materials and Methods:** The present study was a Hospital-based Cross-sectional Study comprised 100 women who admitted to Erbil Maternity Hospital with a spontaneous abortion. A questionnaire was filled by the researchers after verbal consent, and 5 ml of venous blood was collected from each participant, centrifuged at 3500 rpm, and stored in 2 Eppendorf tubes, and tested by ELISA and Electrochemiluminescence Immunoassay by Cobas e 411 for Toxoplasma IgG and IgM according to Manufacturer's instructions. **Results:** The highest seroprevalence for toxoplasmosis was observed in illiterate females aged 26-35 and living in Erbil City's urban areas. A statistically significant association was recorded regarding seropositivity to Toxoplasma IgG and having a history of abnormal babies (P=0.023), Toxoplasma IgG seropositivity and a history of having toxoplasmosis (P=0.016), and seropositivity to Toxoplasma IgM and parity of the females (P=0.037). **Conclusions:** Screening for toxoplasmosis during pregnancy is crucial for all women for the prevention of disease progression. Although there are various screening methods for the detection of toxoplasmosis, not all the methods are applicable and financially suitable for the patients.

Introduction

Toxoplasma gondii (*T. gondii*) is a parasite that belongs to the phylum Apicomplexa, it is an obligate intracellular parasite responsible for causing toxoplasmosis; a zoonotic disease infecting all warm-blooded animals (1). *T. gondii* has one life cycle divided into: Sexual and Asexual stages. The sexual stage occurs in domestic felids which are considered definitive hosts of the parasite. The parasite will form oocysts as a result of sexual reproduction in the epithelial cells of the small intestine of the definite host which then will be shed in their feces into the soil (2, 3). The asexual stage occurs in humans and other non-feline mammals harboring the parasite as intermediate hosts (1, 4). During the asexual stage, oocysts or tissue cysts housing bradyzoites are ingested by consuming contaminated food or undercooked contaminated meat. The bradyzoites grow slowly and remain within a tissue cyst in the host throughout its life. Asexual reproduction of *T. gondii* occurs

when the bradyzoites enter a cell and reproduce by endodyogeny within vacuoles inside infected cells. The cells eventually burst and tachyzoites are released (2).

Toxoplasmosis can be symptomatic during the acute stage of infection giving flulike symptoms, however, most toxoplasmosis infections are asymptomatic (2, 5). Infection occurs upon ingestion of undercooked meat containing tissue cysts or foods contaminated with oocysts or drinking milk contaminated with tachyzoites (6). Toxoplasmosis can be transmitted through sexual contact or having contact with soil contaminated with the feces of infected cats (1, 7). Congenital transmission, blood transfusion, and organ transplantation are also caused by transmission (6).

Congenital toxoplasmosis is one of the most serious forms of toxoplasmosis. In the United States, 500-5000 infants are born every year with congenital toxoplasmosis (8). Acute congenital toxoplasmosis occurs when the tachyzoites of *T. gondii* in the blood of a seropositive mother cross the placental tissue and cause fetal infection (9). Congenital toxoplasmosis predominantly occurs after the primary infection of a pregnant woman. However, transmission from women infected shortly before becoming pregnant, transmission from immunosuppressed women undergoing reactivation, and transmission from women infected with a serotype developing infection during pregnancy have been clinically observed (10).

Women who are infected with *T. gondii* during pregnancy and left untreated have a 25% incidence of fetal infection in the first trimester, 54% in the second trimester, and 65% in the third trimester of pregnancy (11). The gestational age at which infection occurs is considered a key variable affecting the clinical fetal outcome. So, clinical management of infected pregnant women is dependent on identifying the gestational age at primary infection because as the gestational age increases, the transmission rate increases and the severity of toxoplasmosis for the fetus decreases (12). Congenital infection during the first and second trimesters of pregnancy may result in spontaneous abortion, mental retardation, and cerebral calcifications, while the severity of fetal damage is decreased during the third trimester (9). Treating pregnant women infected with *T. gondii*, especially during the acute phase of infection with antibiotics such as spiramycin can reduce the concentration of the parasites in the placental tissue and therefore decrease the risk of transmission from mother to fetus (11).

In 2013, the World Health Organization (WHO) explained that the seroprevalence of toxoplasmosis during pregnancy is estimated to be around 15.4-24.3% worldwide (13). The incidence of acquiring *T. gondii* ranges from 12% to 90% increasing with sanitary habits, age, contact with infected animals, eating undercooked meat, poor education, socioeconomic status, and ethnicity (14). Higher IgG and lowest IgM seroprevalence rates for toxoplasmosis have been recorded in Iraq among all Arab countries (15). Maternal diagnosis of toxoplasmosis is based on immunological tests targeting circulating antibodies or antigens specific for *T. gondii* in titers, Polymerase Chain Reaction (PCR), and Ultrasound Imaging, while the diagnosis of fetal toxoplasmosis relies on the direct identification of the parasite through inoculation of the amniotic fluid in experimental animals or cell cultures taking note that both procedures are time-consuming (16).

The first antenatal visit can happen between 8 - 12 weeks of pregnancy serological tests for the presence of toxoplasmosis can be done if requested by the patient. The presence of *Toxoplasma* Immunoglobulin M (IgM) is an indication of a possible ongoing infection, while the presence of *Toxoplasma* IgG confirms a previous or ongoing infection. However, distinguishing between past and ongoing infection can be challenging because IgM antibodies may even persist in serum for a long time after the acute phase of infection (17). This study aimed to determine the seroprevalence of toxoplasmosis in aborted women who were admitted to Erbil Maternity Hospital and to associate the seroprevalence of *T. gondii* in aborted women with clinical-obstetric determinants and sociodemographic factors.

Materials and Methods

Study Population

The present study was a hospital-based cross-sectional Study that enrolled 100 women who admitted to Erbil Maternity Teaching Hospital/ Erbil City/ Kurdistan Region/ Iraq with spontaneous abortion, from July 2022 to October 2022. The study population included women undergoing spontaneous abortion at different ages, ethnicities, and gestational ages. Exclusion criteria involved women with planned abortions due to medical conditions unrelated to toxoplasmosis. Individuals who participated in the study were interviewed by the researchers after verbal consent and a questionnaire was used to collect clinical obstetric information (Gestational Age, Gravidity, Parity, and Number of Abortion), epidemiological variables (Age, Residency, and Educational Level) and History of toxoplasmosis or history of births with congenital anomalies.

Ethical Considerations

This study was approved by the Scientific and Ethics Committee of the College of Health Sciences/ Hawler Medical University (No. ScES 5A on 13th March 2023).

Sample Collection and Laboratory Analysis

Five milliliters of venous blood were collected from each participant and transferred to sterile jell tubes (tubes without anticoagulant), left to coagulate at room temperature then centrifuged at 3500 rpm for 5 min. The sera were collected from the tubes with sterile disposable pipettes, placed in two Eppendorf tubes for each sample, and stored at -45º C for further laboratory analysis (35).

Direct Enzyme-linked Immunosorbent Assay (ELISA) test for IgM and IgG antibodies

All serum samples were tested using a commercially available ELISA kit (Dia. Pro Diagnostic Bioprobes, Milano, Italy). Sample analysis was performed according to the manufacturer's instructions. For the Anti-*Toxoplasma* IgM ELISA test, the cut-off was calculated through a Mathematical calculation by adding the Mean absorbance value (OD 450 nm) of the negative control (NC) to 0.250 (Cut-off = NC + 0.250). NC was 1.471 the calculated cut-off was 1.721. The results were interpreted as a ratio of the sample's absorbance value and the Cut-off value (or S/CO). Results < 1.721 S/CO were considered negative and > 1.721 S/CO were considered positive. For the Anti-*Toxoplasma* IgG ELISA test, the cut-off of the device has been set at 50 IU/ml. samples with concentrations higher than 50 IU/ml were considered positive and those with concentrations below 50 IU/ml were considered negative.

Roche Elecsys for Anti-Toxoplasma IgG and IgM Testing System

All samples were also analyzed by Roche Elecsys Anti-*Toxoplasma* IgG and IgM testing system. This system is an automated system based on Electrochemiluminescence Immunoassay designed to be used on Roche "Cobas e 411" and Roche Elecsys systems for a sandwich-based quantitative determination of IgG and IgM antibodies specific to *Toxoplasma gondii* in patient's serum. An aliquot (10 µl of serum at 2-8C^0) was analyzed by Toxo IgM and Toxo IgG Assay (Roche Diagnostics, Mannheim, Germany) using Cobas e 411 Analyzer according to the Manufacturer's instructions.

Statistical Analysis

Descriptive statistical analysis was carried out using IBM SPSS version 26 (SPSS Inc., Chicago, IL, USA). Results were reported in terms of frequency and percentage. The Chi-square test was applied to assess the association between the independent variables and the outcome. *P*-value ≤ 0.05 was considered to be statistically significant.

Results

Socio-demographic Characteristics of the Participants

Most participants who were included in the study were aged between 26-35 years old (46%), living in the urban areas of Erbil City (65%), and many were Illiterate (42%) (Figure 1).

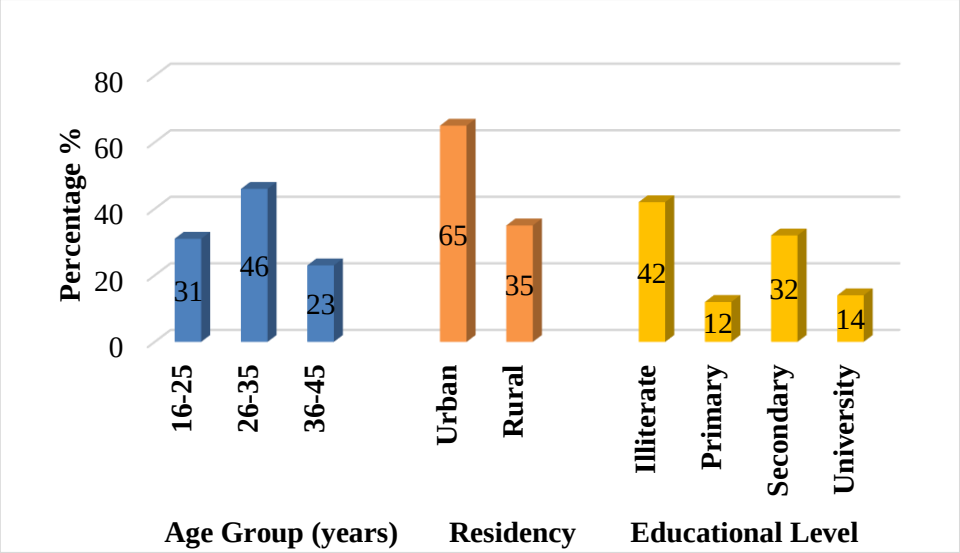

Figure 1: Socio-demographic Characteristics of the Participants

Obstetrical History of the Participants

Among the 100 women tested, only 12% were pregnant for the first time (Primigravida), and the highest percentage (63%) had >1 healthy child at home (Multiparous). Most of the women selected for this study experienced their first or second abortion (78%). Only 4% had a history of abnormal babies and only 2% were previously diagnosed with toxoplasmosis. The highest percentage (59%) of gestational-age individuals participating in the study were in the first trimester of pregnancy (the first 12 weeks of pregnancy) (Figure 2).

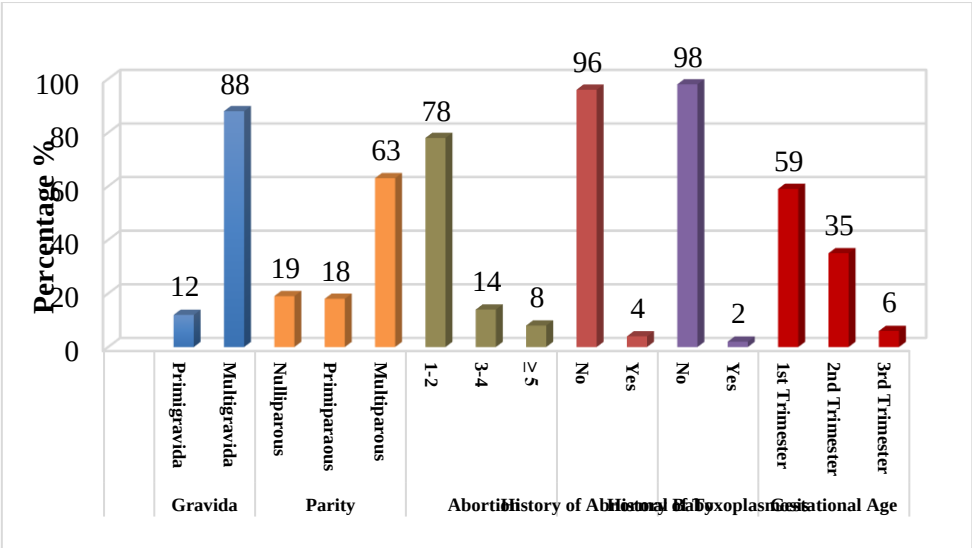

Figure 2: Clinical Obstetrical Information of the Participants

Serological Tests (ELISA and Elecsys by Cobas e 411) Results

Among participants, 26% and 5% were seropositive for Anti-*Toxoplasma* IgG and IgM respectively by ELISA. Collectively, (31%) of the women having spontaneous abortions were seropositive for toxoplasmosis by ELISA. When the 100 samples were re-tested by Cobas e 411, 25% of the patients were seropositive for Anti-

Toxoplasma IgG and only 1 patient was positive for Anti-*Toxoplasma* IgM by Roche Elecsys testing system for *T. gondii*. Collectively, (26%) were seropositive for toxoplasmosis by Cobas e 411 (Figure 3).

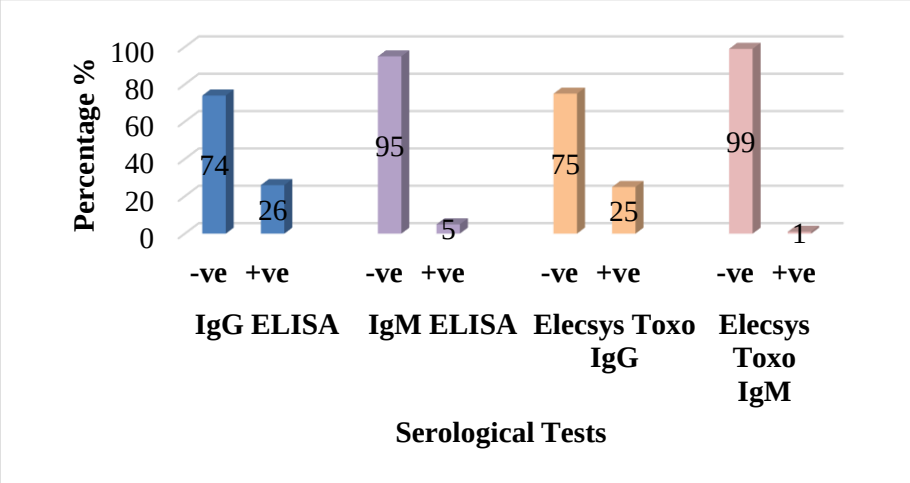

Figure 3: Enzyme-Linked Immunosorbent Assay (ELISA) and Roche Elycsys *Toxoplasma* Results

Serological Results for Anti-Toxoplasma IgG Concerning Socio-demographic Factors

Most of the women (30.4%) belonged to the age group 26-35 years old, followed by 26.1% in the age group 36-45 years, and the lowest seropositive (19.4%) were in age 16-25 years old. 65 women out of the 100 who participated in this study were living in urban areas and 18 (27.7%) were seropositive for Anti-*Toxoplasma* IgG meanwhile, 35 of them lived in rural areas, and out of the 35, only eight (22. 9%) were positive when tested for Anti-*Toxoplasma* IgG. 26.2% individuals who tested positive for Anti-*Toxoplasma* IgG were illiterate, whereas (33.3%) finished their primary school, and 21.4% have completed their university education. No significance was observed concerning seropositivity for Anti-*Toxoplasma* IgG and socio-demographic factors of the individual who participated in this study (Table 1).

Table 1: Association between IgG Serology Results and Socio-demographic Factors of the Participants

Demographic Characteristics		IgG Seropositivity by ELISA				p-value
		Negative		Positive		
		No.	%	No.	%	
Age Group (Years)	16-25	25	80.6	6	19.4	0.554
	26-35	32	69.6	14	30.4	
	36-45	17	73.9	6	26.1	
Residency	Urban	47	72.3	18	27.7	0.599
	Rural	27	77.1	8	22.9	
Educational Level	Illiterate	31	73.8	11	26.2	0.918
	Primary	8	66.7	4	33.3	
	Secondary	24	75	8	25	
	University	11	78.6	3	21.4	

Serological Results for Anti-Toxoplasma IgG Concerning Clinical Obstetrical History

Twenty-four (27.3%) participants who were seropositive for IgG have been pregnant more than once regardless of the viability of the fetus (Multigravida) and 19 (30.2%) have given birth to>1 viable fetus (Multiparous), 5 have given birth to only one viable fetus (Primiparous), and only 2 were pregnant for the first time (Nulliparous). Most of the women who were seropositive for Anti-*Toxoplasma* IgG (25.6%) were experiencing their first or second abortion and only 3 women had 3 - 4 abortions and three had >5 abortions. Meanwhile, out of 4 individuals with a history of abnormal babies (75%) were seropositive for Anti-*Toxoplasma* IgG. Two

women had a history of toxoplasmosis and both were seropositive for Anti-*Toxoplasma* IgG. The highest number of seropositive women (28.6%) were having an abortion in the second trimester, but only 16.7% were detected in the third trimesters. Significant values were observed concerning seropositivity to Anti-*Toxoplasma* IgG and having a history of an abnormal baby (*P*=0.023 and *P*=0.016, respectively) (Table 2).

Table 2: Association between IgG Serology Results and Obstetrical History of Participants

Obstetrical History		IgG Seropositivity by ELISA				p-value
		Negative		Positive		
		No.	%	No.	%	
Gravidity	Primigravida	10	83.3	2	16.7	0.432
	Multigravida	64	72.7	24	27.3	
Parity	Nulliparous	17	89.5	2	10.5	0.228
	Primiparous	13	72.2	5	27.8	
	Multiparous	44	69.8	19	30.2	
Abortion	1-2	58	74.4	20	25.6	0.702
	3-4	11	78.6	3	21.4	
	≥ 5	5	62.5	3	37.5	
History of Abnormal Baby	No	73	76	23	24	0.023*
	Yes	1	25	3	75	
History of Toxoplasmosis	No	74	75.5	24	24.5	0.016*
	Yes	0	0	2	100	
Gestational Age	1st Trimester	44	74.6	15	25.4	0.818
	2nd Trimester	25	71.4	10	28.6	
	3rd Trimester	5	83.3	1	16.7	

Serological Results for Anti-Toxoplasma IgM Concerning Socio-demographic Factors

The percentage of women who tested positive for Anti-*Toxoplasma* IgM and aged between 36-45 years old was 13%, while 2.2% were aged 26-35 and 3.2% were aged between 16-25 years old. 6.2% of the seropositive females lived in urban areas and 2.9% lived in rural areas. 3.1% were illiterate, (3.1%) had finished secondary school education, and 7.1% had a university degree among the IgM seropositive women. No statistical significance was observed for the association between the seropositivity of Anti-*Toxoplasma* IgM and the socio-demographic factors noted in this study (Table 3).

Table 3: Association between IgM Serology Results and Socio-demographic Factors of the Participants

Demographic Characteristics		IgM Seropositivity by ELISA				p-value
		Negative		Positive		
		No.	%	No.	%	
Age Group (Years)	16-25	30	96.8	1	3.2	0.128
	26-35	45	97.8	1	2.2	
	36-45	20	87	3	13	
Residency	Urban	61	93.8	4	6.2	0.471
	Rural	34	97.1	1	2.9	
Educational Level	Illiterate	39	92.9	3	7.1	0.703
	Primary	12	100	0	0	
	Secondary	31	96.9	1	3.1	
	University	13	92.9	1	7.1	

Serological Results for Anti-Toxoplasma IgM Concerning Clinical Obstetrical History

Concerning gravidity and abortion, all the women (5.7%) who tested positive for Anti-*Toxoplasma* IgM have been pregnant >1 regardless of the viability of the fetus (Multigravida), three of them (16.7%) had either their first or second abortion, and only one (7.1%) had 3-4 abortions, and only one (12.5%) had ≥5 abortions. About parity, three of the seropositive women (16.7%) have given birth to only one viable fetus (Primiparous), and two (3.2%) have given birth to more than two viable fetuses (Multiparous). Concerning having a history of abnormal babies and toxoplasmosis, all the seropositive aborted women tested for Anti-*Toxoplasma* IgM did not have a history of abnormal babies or have been diagnosed previously with toxoplasmosis. Concerning gestational age, two aborted women were in their first trimester (3.4%), two aborted women were in their second trimester (5.7%), and only one woman (16.7%) was in her third trimester of pregnancy when they tested positive for Anti-*Toxoplasma* IgM by serological testing. Significant value was observed concerning seropositivity to Anti-*Toxoplasma* IgM and Parity of the aborted women (P=0.037) (Table 4).

Table 4: Association between IgM Serology Results and Obstetrical History of Participants

Obstetrical History		**IgM seropositivity by ELISA**				**p-value**
		Negative		**Positive**		
		No.	**%**	**No.**	**%**	
Gravidity	Primigravida	12	100	0	0	0.397
	Multigravida	83	94.3	5	5.7	
Parity	Nulliparous	19	100	0	0	0.037
	Primiparous	15	83.3	3	16.7	
	Multiparous	61	96.8	2	3.2	
Abortion	1-2	75	96.2	3	3.8	0.522
	3-4	13	92.9	1	7.1	
	≥ 5	7	87.5	1	12.5	
History of Abnormal Baby	No	91	94.9	5	5.1	0.640
	Yes	4	100	0	0	
History of Toxoplasmosis	No	93	94.9	5	5.1	0.743
	Yes	2	100	0	0	
Gestational Age	1st Trimester	57	96.6	2	3.4	0.357
	2nd Trimester	33	94.3	2	5.7	
	3rd Trimester	5	83.3	1	16.7	

Discussion

Untreated congenital toxoplasmosis can lead to serious fetal damage or other consequences (20). Therefore, to avoid intrauterine infection and complications, it is important to test pregnant women for Anti-*Toxoplasma* IgG and IgM. In France and some other European countries, a policy has been established for the prevention of congenital toxoplasmosis by systematic serological screening of the pregnant women where the first serological testing for *T. gondii* is performed before the end of the first trimester of pregnancy. If the pregnant woman is IgM positive, further tests are performed for a definite diagnosis of acute toxoplasmosis resulting in treatment administration. In negative cases, serological testing is repeated every month until delivery (21). Toxoplasmosis prevalence changes among countries depending on lifestyle, traditions, and customs (19).

To measure *T. gondii*'s seroprevalence, Anti-*Toxoplasma gondii* IgM and IgG quantification through ELISA and Electrochemiluminescence Immunoassay were performed. The difference in seropositivity tested by both techniques was noted when testing for *Toxoplasma*-specific IgM, this could be explained by the ability of antibiotic treatment to change the maturation of Immunoglobulins and the antibody response of Immunoglobulins as explained by (17, 22). Prusa et al. reported that using Elecsys for screening *Toxoplasma gondii* infections is considered useful and has 97.1% sensitivity and 100.0% specificity, a positive predictive value of 100.0%, and a negative predictive value of 81.3% compared to the Sabin-Feldman dye test (20).

Other serosurveys conducted in Erbil city with roughly close results are 22 which showed 29.19% seropositivity by ELISA in females who tested for toxoplasmosis before marriage and 23 which stated that the seropositivity in apparently healthy females was 25.6% tested for toxoplasmosis by Latex Agglutination Test (LAT). Also, other studies that recorded higher results in Erbil city were 24 with seropositivity of 45.1% for *T. gondii* in pregnant women and 25 with 45.2% positive pregnant women for toxoplasmosis by LAT. The difference in seroprevalence of *T. gondii* in these researches might be related to the sample size, screening technique, and the different socio-demographic factors selected for determining the study population.

Research done in other regions in Iraq with different results is 28.5% in Duhok by ELISA (26), 35.56% for Anti-*Toxoplasma* IgG using ELISA technique in Kirkuk (27), 17.8% in Al-Kut in aborted females by ELISA (19), and 2.8% for Anti-*Toxoplasma* IgM in aborted females by Cobas e 411 in a cross-sectional study in Kurdistan region between 2015 and 2018 (28). Results in other countries include 25.94% in Turkey (29), 48.8% in female blood donors in Iran (30), 24.8% in Pakistan (31), 21.2% in Yemen (32), and 21.8% in Saudi Arabia (33). The variation in the results mentioned can be attributed to the study population, cultural differences, socioeconomic status, sanitary and eating habits, and different screening techniques with the origins of the kits used (18, 19, 23).

In our study, the highest IgG seropositivity was noted in the 26-35-years age group (30.4%) as demonstrated in Table (1). This result was similar to (18, 24, 34) and different from (19) which recorded the highest seropositivity in 20-29 years of age groups, (26) with the highest seropositivity in 18-28 years. The highest IgM seropositivity was noted in the 36-45-years age group (13%) that might be attributed to an increase in exposure to infection sources i.e. animals, soil, rate and frequency of meat consumption (18, 23).

There was a difference in the ratio of IgG and IgM seropositivity for toxoplasmosis noted in urban more than in rural areas. The results of this study agreed with another studies (22, 34) whereas disagreed with (26, 30, 31). This could be attributed to eating raw or undercooked meat (34) and Erbil city borders growing larger and sub-rural areas eventually becoming urban. Regarding lack of education, the highest percentage of toxoplasmosis was noted in illiterate females (7.1%) for IgM and (26.2%) for IgG (33.3%). These findings were consistent with another studies (22, 24, 26) where housewives had the highest rates of toxoplasmosis due to their little knowledge regarding the disease and its prevention.

Concerning the gravidity and parity of the females, most of the seropositive aborted females (33%) had multiple pregnancies where 27.3% were positive for IgG, and 5.7% were positive for IgM. The highest IgG seropositivity was noted in multiparous females (30.2%), while the highest IgM seropositivity was observed in primiparous aborted females (16.7%). An association was noted (P=0.037) between Anti-*Toxoplasma* IgM seropositivity and parity as documented and this result could be accidental due to random sampling. Many researchers focused on the gestational age rather than the gravidity and the parity of the patients only (34) mentioned parity and disagreed with IgG seropositivity stating that women with no previous pregnancy had the highest seroprevalence of toxoplasmosis. Regarding the history and number of spontaneous abortions, the highest *Toxoplasma* seroprevalence (29.4%) for IgM and 27.7% for IgG was recorded in females having their first or second abortion. Other studies (24, 26, 18, 34) explained that a strong association was present between spontaneous abortion and seropositivity of both Anti-*Toxoplasma* IgM and IgG. An association was found between Anti-*Toxoplasma* IgG seropositivity and a history of having an abnormal baby (P=0.023) and a history of having toxoplasmosis (P=0.016). This result can be clarified by the ability of *T. gondii* to cause fetal damage during pregnancy in different severities (9, 20).

Regarding the gestational age, the highest seroprevalence for toxoplasmosis was observed in the first trimester (25.4%) for IgG and 3.4% for IgM, and second trimester (28.6%) for IgG and 5.7% for IgM (28.8% and 34.3% respectively). These findings were compatible with other studies (31, 24) but disagreed with others (34, 25) which observed higher seropositivity for *T. gondii* in the third trimester of pregnancy. Unfortunately, routine

screening for toxoplasmosis is not performed for pregnant women in Erbil city as the screening test is not mandatory mentioning that most families find the screening test costs high. Additionally, the lack of promotion for the disease public health and poor education explains the high percentage of females being positive for *T. gondii* without previously having information about getting screened for toxoplasmosis upon knowing that they are pregnant i.e. only 2/100 females tested stated that they have been previously tested for toxoplasmosis.

Conclusions

In conclusion, the present study recorded 26% and 5% seropositivity for *T. gondii* of IgG and IgM respectively by ELISA in females having a spontaneous abortion. A total of 31%. Whereas by Elecsys, 25% were positive for IgG and 1% for IgM, a total of 26% in the population selected for this study. The highest seroprevalence was noted in group 26-35-years, in urban areas, and also in the illiterate individuals. Also, statistically significant values were observed concerning seropositivity to Anti-*Toxoplasma* IgG and having a history of an abnormal baby and toxoplasmosis. Having high IgG titers could also indicate that the patient is continuing in the primary infection stage, therefore it is necessary to involve screening for *T. gondii* during the first antenatal visits and throughout pregnancy to prevent later complications for both the mother and her fetus.

Acknowledgments

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Conflict of Interest

There is no conflict of interest as this research was a personal non-profit work.

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