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# Serodiagnosis Of Toxoplasmosis By Using ELISA And IgG Avidity Test In Relation To Some Risk Factors Among Women at Childbearing Agein Zakho City, Duhok Province/Iraq.

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## Serodiagnosis of Toxoplasmosis by Using ELISA and IgG Avidity Test in Relation to Some Risk Factors among Women at Childbearing Age in Zakho City, Duhok Province/ Iraq.

#### **Authors Names ABSTRACT** Sarwin S. M. Mizuri a This study intended to evaluate the seroprevalence of anti-Toxoplasma IgG and IgM Wijdan M. S. Mero b antibodies in the sera of 630 women at childbearing age, and to link the outcomes with some risk factors. The enrolled women visited Zakho Maternity Hospital from July 2018 to July 2019. Their ages ranged from 15 to 45 years. All samples were examined using Article History ELISA to detect immunoglobulin G and M, in addition to performing IgG Avidity test for Received on: 5/6/2021 seropositive pregnant women. Revised on: 15/6/2021 Accepted on: 17/6/2021 The differences between seropositivity and age was significant (p<0.05), the highest rate (20.43%) for anti-Toxoplasma IgG antibodies in the age group 33-38 years. Women who had more contact with cats showed higher IgG and IgM seropositivity rates (16.45% **Keywords:** and 1.26%, respectively). Married women had higher IgG Abs seropositivity than single childbearing Age; ones (12.52% vs 6.31%, respectively), moreover, only married women were seropositive Immunoglobulin G and M; for IgM Abs. Pregnant women presented higher IgG Abs seropositivity than non-IgG Avidity; pregnant (15.21% versus 10.49%), with almost equal seropositivity for IgM Abs (0.65% Toxoplasmosis; and 0.86%, respectively). Women Anti-Toxoplasma IgG Abs seropositivity was higher in women underwent miscarriages than those with normal pregnancies (18.44 vs. 8.81%), however IgM Abs was only found **DOI:**https://doi.org/10.29350/ among women who had miscarriages (0.97%). Women with triple miscarriages jops. 2021.26. 4.1331 presented the highest IgG Abs seropositivity (37.03%). Chronic infection was found in 68.75% of pregnant women, whereas acute infection was found in 31.25 %. Following up the pregnancy resulted in 15 healthy births, 9 miscarriages, and 10 women did not show up. The finding of this study demonstrates the relationship between toxoplasmosis and risk factors in women at childbearing age, with the aims of decreasing infection rates through the introducing of health education programs and the application of hygienic measures in the community by health authority.

#### 1. Introduction

The causative agent of Toxoplasmosis is *Toxoplasma gondii*, is an obligate intracellular protozoan parasite with worldwide distribution manipulating both animals and humans particularly in moist andwarm environment. <sup>[5, 9]</sup> Warm-blooded animals act as intermediate hosts, whereas, cats and other

felines act as definitive hosts. <sup>[11, 29]</sup> The transmission occurs by ingestion of sporulated oocyst passed with cat feces that contaminate vegetables, water, or ingestion of undercooked meat containing tissue cysts. <sup>[30]</sup> Toxoplasmosis at its acute stage can be transmitted transplacentally from mother to fetus. Immunocompetent individual does not show any sign and symptoms, while, severe consequences can occur in pregnant women and immunocompromised individuals. <sup>[17]</sup> The pathological effects of toxoplasmosis in the developing fetus includes microcephaly, hydrocephaly, brain disorder, blindness, and even intra uterine death. <sup>[20]</sup> After one week from the onset of infection IgM can be estimated in the serum, consequently, is considered an initial diagnostic indicator for acute toxoplasmosis. Conversely, it may also exist serologically for several months or years. <sup>[13]</sup>

The presences of specific antibodies are more important for the diagnosis of *T. gondii* in the laboratory. <sup>[3]</sup> Toxoplasmosis is diagnosed by many tests, including histological, serological, and molecular, or their combinations. The global variation in the seropositivity rate of toxoplasmosis may be due to prevalence of stray cats in the community, methods of consuming meat, residency, level of education, poor sanitation, socioeconomic status etc. <sup>[20, 30]</sup> The purpose of current investigation was to evaluate the rate of Anti-*Toxoplasma* IgG and IgM Abs in women at reproductive age and to correlate the seropositivity rate with some risk factors, in addition to following seropositive pregnant women up to giving birth.

#### 2. Materials and Methods

#### 2.1. Samples and the study area

Six hundred thirty blood samples were randomly collected from women at childbearing age (15-45 years) from July 2018 to July 2019. These women attended Zakho Maternity Hospital. From each woman 5 ml of Venus blood was withdrawn after taking her verbal consent and permission from the health authority. Each blood sample was transferred to a fully labeled centrifuge tube with each participant full information according to questionnaire form designed for this study and included, age, and contact with a cat, marital status, gestational state, miscarriage, and number of miscarriages. This study was performed in the laboratory of Zakho General Hospital, in the laboratory, each collected blood sample was centrifuged at 4000 rpm for 4 minutes, after that the serum was separated and transferred into two Eppendorf tubes fully labeled with the participant full information. All samples were kept at -20°c until to be tested.

#### 2.2. Serodiagnosis methods

The rate of Anti-Toxoplasma immunoglobulin G and M were estimated by using ELISA kit (Bioactiva diagnostica/Germany) and IgG avidity test (Bioactiva diagnostica/Germany) were done for pregnant women only with the aim of detect the stage of infection either is acute (recent) or chronic (old). All these tests were carried out according to instruction supplied with the used kits.

#### 2.3. Statistical analysis

The data were statistically assessed by using computer program IBM-SPSS (version 19), along with Open-Epi version (3.01), based on the considered risk factors.

#### 3. Results

#### 3.1. Seroprevalence related to risk factors

The outcomes of this study revealed that the maximum seropositivity percentage presented among age group (33-38) years (20.43%) for anti-*Toxoplasma* IgG Abs, the seropositivity percentage reduced among younger and older ages as indicated in Table (1). Regarding anti-*Toxoplasma* IgM Abs Four cases were recorded, each age group reported one case, excluding the age group 39-40, which was negative for IgM Abs. Seropositivity rate for anti-*Toxoplasma* IgG and IgM were higher between women with direct contacts with cats (16.45% and 1.26%) respectively. In contrast to unmarried women, married women had higher seropositivity rate for IgG Abs, which is was about two folds higher than nonpregnant ones (12.52% and 6.3%), respectively. Furthermore, despite its low prevalence, IgM Ab was only observed among married women (0.75%). Non-pregnant women had a

higher seropositivity rate of IgG Abs than pregnant ones (15.21% vs 10.49%), but both groups had two IgM seropositive cases, at rates of 0.86% and 0.65%, respectively. Women who had experienced miscarriage had a higher rate of seropositivity for anti-*Toxoplasma* IgG Abs than women who had not experienced a miscarriage (18.44% vs 8.81%, respectively), and this disparity was significantly higher than those who had not experienced a miscarriage (p>0.003952). With regard to IgM Abs, only one case was found in each category. Finally, women who underwent three miscarriages had the highest seropositivity rate regarding IgG Abs (37.03%), after that those who had more than triple miscarriages (21.42%). IgM Abs was detected in only one case between triple miscarriages in addition to another case among women with single miscarriage.

Table 1. The prevalence of *Toxoplasma gondii* and its relationship with some variables

Variables	No:	ELISA IgG+		ELISA IgM+	
	Tested	No:	%	No:	%
Age groups					
15-20	92	2	2.17	1	1.08
21-26	188	20	10.63	1	0.53
27-32	174	21	12.06	1	0.57
33-38	93	19	20.43	1	1.07
39-45	83	11	13.25	0	-
Total	630	73	11.59	4	0.63
		p-value	=0.03418*		
Cat contact					
Yes	237	39	16.45	3	1.26
No	393	34	8.65	1	0.25
	'	p-value=	= 0.003244*	-	<u> </u>
Marital status					
Married	535	67	12.52	4	0.74
single	95	6	6.31	0	0
	'	p-valu	ie=0.1468	-	<u> </u>
Gestational status					
Pregnant	305	32	10.49	2	0.65
Non pregnant	230	35	15.21	2	0.86
		p-valu	ie=0.2482		
Miscarriage					
Having	206	38	18.44	2	0.97
Without	329	29	8.81	2	0.60
		p-value=	=0.003952*		
Miscarriage no.					
Single	119	18	15.12	1	0.84
Twice	46	7	15.21	0	0
Triple	27	10	37.03	1	3.70
≤ Triple	14	3	21.42	0	-
		p-valu	ie=0.1021		

#### 3.2. Anti-Toxoplasma Seroprevalence and IgG Avidity

The results of the follow-up of 32 seropositive pregnant women for ELISA IgG, showed that 31.25% (10/32) of them had acute or recent infection (low avidity result), and 68.75% (22/32) revealed chronic/ old infection (high avidity result) as indicated in Table (2).

Table 2. The rate of IgG avidity test among seropositive pregnant women

Total	Recent infection		Past infection	
	No.	%	No.	%
32	10	31.25	22	68.75

#### 3.3. Following up of seropositive case

Table (3) illustrate the results of follow up of certain cases among seropositive pregnant women throughout their pregnancy. Among 34 seropositive pregnant women, 15 of them delivered healthy babies (three of them were seropositive for IgG during first trimester, seven of them were seropositive during second trimester, and six of them were seropositive through third trimester), regarding miscarriages, 9 cases had miscarriages (7 in the first and 2 in the second trimesters), also 10 of them did not show up to be followed.

Table 3. Following up of a seropositive case of Toxoplasmosis among pregnant women

Trimesters	No.	Miscarriages	Delivered normal	Unknown
	followed	No.	babies No.	No.
1 <sup>st</sup>	13	7 IgG	3 IgG	3 IgG
2 <sup>nd</sup>	13	2 IgG, 1 IgM	6 IgG	4 IgG
3 <sup>rd</sup>	8		6 IgG	2 (1 IgG, 1 IgM)
Total	34	10 (9 IgG, 1IgM)	15 IgG	9 (8IgG, 1 IgM)

#### 4. Discussion

Cats have an important role in contaminating the environment with *Toxoplasma* oocyst. Moreover, there are several factors which have a great impact on transmission of *T. gondii* such as, ecological, cultural, social, and economic situations. Socio-demographic status, lifestyle, and environment are risk factors for toxoplasmosis; for the reason of interdisciplinary cooperative studies are necessary to decrease the transmission risks, which improve and support the public health's role in controlling and to reducing the rate of infectious diseases. [21, 22, 27] Furthermore, toxoplasmosis has global distribution, the frequency rates start from 0% to 100% even in regions of the same State and in different countries. High rates of seropositivity of anti-*Toxoplasma* Abs let women to be at higher risk of acquiring toxoplasmosis especially during early pregnancy with having risk of intrauterine transmission of the disease. Whereas, chronic toxoplasmosis, is considered as a reason of frequent miscarriages because during chronic infection reactivation will occur in women who didn't receive any medication after their first miscarriage. Such situation can cause the rupture of the tissue cyst that liberate tachyzoites which cross the placenta and damage the fetus and finally causing miscarriage.

During this study, the maximum seropositivity rate was observed among age group (33-38) years (20.43%) for anti-*Toxoplasma* IgG Abs. The outcome of the current investigation to some extend are comparable to the study achieved in Duhok city <sup>[3]</sup> in which a higher seropositive percentage of 45.35% for IgG Abs, was seen between the age group (30-35) years and the lowest rate (14.65%) was between the age group (16-20), while, in the present study the lowest percentage was (2.17%) between the age group (15-20) years. The consequences of the present study are inconsistent with those of a study conducted in Erbil <sup>[23]</sup>, in which the age group (18-27) years revealed the highest seropositive percentage (13.09%) for *T. gondii* Abs. This might be attributed to increased exposure opportunities with the increase of age. <sup>[12]</sup>

Seropositivity rate for anti-*Toxoplasma* IgG and IgM were higher between women with direct contacts with cats (16.45% and 1.26%, respectively). Also, a study in Duhok found women who had direct contact with cats had a higher seropositivity rates for anti-*Toxoplasma* IgG and IgM Abs, which were 30.8% and 40% for IgG and 0.7% and 0.8% for IgM, respectively. <sup>[22]</sup> The prevalence rate of toxoplasmosis of the previous study was higher than the current study, this variation may be attributed to the reproductive period and their contact with cats, at this age they are more mature furthermore, they can realize the danger of getting contact with cats, so they prevent cats from entering their kitchens and houses. <sup>[22]</sup> The disagreements among studies may also be due to the samples type and the stray cat's number in the study area. <sup>[7]</sup>

Married women had higher seropositivity rate for anti-*Toxoplasma* Abs than unmarried women for IgG Abs, which was about two folds higher (12.52% and 6.3%, respectively). These outcomes are in line with those of <sup>[22]</sup> in Duhok who similarly reported higher seropositivity rate for IgG Ab among married women than single ones, accounting for 31.7% and 20%, respectively, while IgM Ab was only recorded among married women. <sup>[22]</sup> With regard to IgG Abs, another study also, in Duhok found that married women had a higher rate of IgG Abs than unmarried women (39.93% vs 17.46%), but IgM was found among both of them. <sup>[21]</sup> The high seropositivity of IgG Abs among married women may be due to their home duties, such as cooking, planting, and handling unwashed vegetables, which might put them at a greater risk of infection. <sup>[26]</sup>

Women who were non-pregnant had a higher seropositivity for IgG Abs than pregnant ones (15.21% vs 10.49%). Even though this rate differs slightly between both groups, the difference may be due to sample size. The current findings contradict with the studies conducted in Colombia <sup>[1]</sup>, Duhok <sup>[3]</sup>, and Erbil <sup>[15, 28]</sup>, subsequently all of these studies reported higher seropositivity rates among pregnant women rather than non-pregnant ones, with rates of 49.7%, 48.3%, 34.8%, and 41.9%, respectively. Regarding ELISA IgG, the rates were 2.8%, 0.9%, 11.2%, and 12.9%, respectively. Regarding to ELISA IgM, only the study performed in Duhok city <sup>[21]</sup> reported nearly equal seropositivity rates between both groups of women. The higher seropositivity rates between pregnant women may be due to an increase in the secretion of sex hormones during pregnancy, especially estrogen and progesterone, as these hormones affect the immune system. <sup>[18]</sup>

Women who experienced a miscarriage had a higher rate of seropositivity for anti-*Toxoplasma* IgG Abs than women who had not experienced a miscarriage (18.44% vs 8.81%, respectively). In other studies, women with a history of miscarriages showed highest seropositive rates for anti-*Toxoplasma* IgG and IgM Abs (18.44%, 40%, and 10.29%), respectively for IgG Abs, while those for IgM Abs were variable (0.73%, 4.18%, and 0.6%, respectively). Using a variety of techniques, some studies found a relation between miscarriage and toxoplasmosis.

With regard to miscarriage, women who underwent three miscarriages in the present study had the highest seropositivity rate for anti-*Toxoplasma* Abs, regarding IgG Abs, the rate was 37.03%, after that, the rate increased in women who had more than triple miscarriages (21.42%). IgM Abs was detected in only one case between triple miscarriages in addition to other case was detected within single miscarriages.

This association among anti-*Toxoplasma* Abs and the miscarriages number in studied women is in agreement with former studies performed in Duhok, which reported the highest seropositivity rates for IgG Abs also by using ELISA test between women underwent three miscarriages which were 62.96% and 48.4%, respectively. [3, 21] This might be due to reactivation of latent infection, which triggers the tissue cysts rupture in the infected uterus also the tachyzoites release, causing loss of

pregnancy in women who have not been treated for toxoplasmosis after the first miscarriage. <sup>[15]</sup> Within the same country, variation in the outcomes can be affected by numerous factors, such as, age, occupation, climatic conditions, source of serological test kits, laboratory facilities, type of consumed food, hygienic application, and contact with cats while working or cleaning the house and gardens. <sup>[6]</sup>

Concerning ELISA IgG Avidity test, the outcome in the current study, revealed that 31.25% (10/32) of the test women sera had acute infection (low avidity result), and 68.75% (22/32) shown chronic infection (high avidity result). A similar pattern was documented in a study in Duhok, which recorded 82.22% for old toxoplasmosis infections (high avidity), whereas, regarding recent infection (low avidity) was 17.77% (11). Furthermore, a study performed in Turkey also stated that during the reproductive cycle, women had a higher avidity rate (84.6%) than low avidity (12.3%) (100). Several studies indicated the significant role of IgM Abs as a key for identifying acute *Toxoplasma gondii* infection. (100) While, some other studies indicated that ELISA IgG avidity test is a more precise test for estimating the infection time. (100) As a result, for the differentiation between recent and old 1000. (1000) 1000

This study showed that the results of follow up of certain cases of pregnant women which were seropositive for anti-*Toxoplasma* Abs during their pregnancy. Which were 34 seropositive pregnant women, 15 of them delivered healthy babies, concerning miscarriages nine cases had miscarriages. In general, there are limited studies in this direction, in Kurdistan, only two studies were achieved in Duhok city. At the beginning, 10 cases were followed up; 1 case was seropositive for both (ELISA IgG and IgM) she delivered healthy baby after taking medication, whereas the second case delivered a dead baby and their result shows seropositive for ELISA IgG and seronegative for IgM during her pregnancy, and another four IgG positive cases as well, delivered dead babies. [22]

In the second study, 20 cases were followed up, 2 of them were seropositive for IgG Abs and seronegative for IgM Abs during first trimester, both of them delivered dead babies. On the other hand, 6 seropositive positive cases for anti-*Toxoplasma* IgG Abs and seronegative for IgM underwent miscarriages, 3 of them during first trimester, and another 3 cases during second trimester. In addition, twelve positive cases of IgG Abs and negative IgM Abs accomplished their gestation with normal babies. <sup>[21]</sup>

The differences in the pregnancy outcome in general of both studies and the present study might be due to other factors, such as viral or bacterial infections, that may affect the pregnancy outcome as both previous studies have suggested. [3, 21] The current study indicate that toxoplasmosis has a great effect on pregnancy outcome if it is acquired at an early pregnancy stage.

#### Conclusion:

The current investigation showed the presence of high seropositivity rate for anti-Toxoplasma IgG Abs among women at their 30 th years of age, married one, and those who were in contact with cats. Moreover, IgG Abs seropositivity rates were highest in women with triple miscarriages. IgG Abs seropositivity was twice as high in married women as it was in single women. On the other hand, IgM Abs were only found among married women. Avidity test indicated that two-third of the followed up pregnant women had a past infection, and one-third of them had recent infection. Following up of 34 infected pregnant women resulted in 15 full-term healthy deliveries and 9 miscarriages. Therefore, prenatal examination and IgG avidity analysis are important to minimize the rate of miscarriages and postnatal complications of the foetus.

**Conflict of Interest:** The authors declare that they have no conflicts of interest.

The author has signed on animal welfare statement

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