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RESEARCH PAPER

Seroprevalence of anti-*Toxoplasma gondii* antibodies among women of childbearing age in Zakho City, Kurdistan Region/Iraq

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A B S T R A C T:

Toxoplasma gondii is the causative agent of toxoplasmosis. That makes serious health problems among immunocompromised patients which comprise pregnant women as well. The present study aimed to investigate the seroprevalence of anti-*Toxoplasma gondii* Antibodies among women of childbearing age and their associations to some demographic factors in Zakho City, Kurdistan Region/Iraq. Blood samples were collected randomly from 630 women aged 15-45 which were divided into subgroups (15-20), (21-26), (27-32), (33-38) and (39-45) years after taking their consent for the detection of anti-*Toxoplasma* IgG and IgM antibodies. A special questioner sheet was designed for the study containing full information about each participant. The diagnoses were done using ELISA-IgG and IgM kits and Rapid Test (RT) method. The prevalence of anti-*Toxoplasma* antibodies was 78/630 (12.38 %), including 73 (11.58 %) for ELISA IgG, 4 (0.63 %) for ELISA IgM and 1 (0.15 %) for RT method. The age group 33-38 years and married females showed the highest seroprevalence of 19/93 (20.43 %), 67/535 (12.52 %), respectively using ELISA IgG. Statically there were significant relations regarding ages, educational status, contact with cats and consumption of undercooked meat. This investigation indicated that the seroprevalence of anti-*Toxoplasma* antibodies among women at childbearing age is still a high rate. Furthermore, the association of some risk factors must be taken into consideration and this requires the introduction of health education programs to the community.

KEY WORDS: *Toxoplasma gondii*; Seroprevalence; ELISA IgG/IgM; Zakho
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1. INTRODUCTION:

Toxoplasma gondii, is an obligatory intracellular protozoan parasite with a cosmopolitan distribution, infecting human and other warm blooded animals and causing community health problems (Al-Kadassy *et al*., 2018). It is suggested that one-third of the global population is infected with this parasite, even though it is a latent form of a disease and is non-fatal (Dubey, 2010; Tenter *et al*., 2000).

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A high frequency of this disease has been reported between pregnant women and women of childbearing age from different parts of the world (Pappas *et al*., 2009). Many techniques have been used for detecting toxoplasmosis such as serological, histological, and molecular or their recombination. The serological tests include, indirect fluorescent antibody assay (IFA), the latex agglutination test (LAT), the indirect haemaglutination assay, Sabin–Feldman dye test, immunosorbent agglutination assay test (ISAAT), but the more common and accurate serological test is the enzyme linked immunosorbent assay (ELISA) (Frenkel, 1970; Remington *et al*., 1995; Mawlood, 2017). The seroprevalence rate of toxoplasmosis among women of childbearing age

in Zakho City, Kurdistan Region of Iraq was poorly studied, consequently, the purpose of this study was to estimate the seroprevalence of anti-*Toxoplasm gondii* antibodies among Women of childbearing age and their relation with some demographic factors (age, marital status, educational status, occupation, contact with cats, application of hygienic habit, and consumption of undercooked meat in Zakho city, Kurdistan Region/Iraq.

2. MATERIALS AND METHODS

2.1 Sample collection and processing

Six hundred and thirty blood samples were taken randomly from women at childbearing age, who visited Zakho Maternity Hospital after taking their consent and permission from health authority, during the period from July 2018 to July 2019, the women were divided into subgroups (15-20), (21-26), (27-32), (33-38) and (39-45) years. From each patient, 5 ml of blood was withdrawn, using a sterile disposable syringe; the collected blood was transferred to a clean tube, without anticoagulant, each tube was labeled clearly. From each participant full information was taken, included: age, occupation, residency, marital status, educational status, time of gestation in pregnant women, history of abortion, cat in neighbor, application of hygiene, and method of cooking meat. Each of the collected blood samples was transferred to centrifuge tube and centrifuged at 4000 rpm for 4 minutes, and then the separated serum was dispensed into two Eppendorf tubes using micropipette and stored at -20^{o}c until to be used. The seroprevalence of anti-toxoplasma IgG and IgM antibodies was determined using ELISA and RT techniques. The ELISA kits used were from Bioactiva diagnostica (Germany), The RT test cassette was from Bio Tina GmbH (Germany). The procedures were done according to the instructions supplied with the kit. The serological tests were performed in Zakho General Hospital/ Zakho City. Regarding ELISA test the sample was considered Positive: if the ratio >1.1, and it was considered Negative: if the ratio <0.9 for both IgG and IgM antibodies.

2.2 Statistical analysis of the data

The data were statistically treated using computer program (IBM-SPSS Static) version 19, besides, (Open-Epi) version (3.01) program achieved to detect chi-square and any significant differences of *T. gondii* seroprevalence in the tested groups. *P*-value 0.05 (5 %) demonstrated statistically significant (Sokel and Rohlf, 2009).

3. RESULTS

3.1 Seroprevalence of anti-*Toxoplasma gondii* antibodies using ELISA and RT

The overall seroprevalence of anti-*T. gondii* IgG and IgM antibodies were 78/630 (12.38 %) of the tested blood samples, using Enzyme-Linked Immunosorbent Assay (ELISA) and Rapid Test (RT). The maximum rate 73/78 (11.58 %) was recorded by ELISA for Anti- IgG Abs. Regarding IgM only 5/78 (0.79 %) sera samples were positive, 4 by ELISA and only one by RT that is why this result unnoticed in the tables. Statistically the difference between ELISA IgG/IgM and Rapid test was highly significant (*P-value*=<0.0000001) as presented in table (1).

Table 1. The overall seroprevalence of anti-*Toxoplasma gondii* antibodies by using ELISA and RT (No. =630).

Type of Test	No: of positive sample	% of positive
ELISA IgG	73	11.58
ELISA IgM	4	0.63
RT IgG	0	-
RT IgM	1	0.15

Total	78	12.38

X^2 =180.6 df=3 *P*-value= <0.0000001 Significant*

3.2 Seroprevalence of anti-*T. gondii* antibodies according to age:

Table (2) shows the seroprevalence of anti-*Toxoplasma* antibodies among women of different age groups. The maximum seroprevalence (20.43 %) was recorded for anti-IgG among the age group 33-38 years. The seroprevalence rate increased with age until the age of 38 years and then declined after age 39 yrs. Regarding IgM only 5 positive cases were recorded, four by ELISA and one by RT as indicated in Table (2). Furthermore, the maximum rate of ELISA IgM was found among age groups 15-20 and 33-34 years, which were (1.08 %) and (1.07 %), respectively. Whereas, the minimum rate (0.53 %) was recorded between the age group 21-26 years. statistically there were significant relationships among different age groups related to ELISA tests (P>0.05).

Table 2. The relation between seropositivity of anti-*T. gondii* antibodies and age by using ELISA

Age (years)	No: Tested	ELISA IgG+ No:	%	ELISA IgM+ No:	%
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	0
Total	630	73	-	4	-

X^2=16.63 df=8 *P*-value=0.03418 Significant*

3.3 Association between anti-*Toxoplasma gondii* Abs and marital status:

The maximum seroprevalence rate (12.52 %) for anti-*Toxoplasma* IgG a ntibodies was recorded among married women, followed by single women (6.31 %). Regarding ELISA IgM, the recorded cases (0.74 %) were among married women only as shown in Table (3).

Table 3. Seroprevalence of anti-*T. gondii* antibody marital status by using ELISA.

Marital status	No: tested	ELISA IgG+ No:	%	ELISA IgM+ No:	%
Married	535	67	12.52	4	0.74
Single	95	6	6.31	0	0

Total	630	73	11.58	4	-

X^2=3.838 df=2 *P*-value=0.1468 Non-Significant

3.4 The relation between anti-*Toxoplasma gondii* antibodies and educational status:

Concerning the educational status, the highest seroprevalence (17.75 %) was observed among illiterate, then decreased among women who studied until different school levels (11.98 %). On the other hand, the rate decreased among women who completed their university education (7.18 %). Regarding IgM antibodies, they were recorded at a rate of (1.16 %) among women with school education. Statistically, significant differences were observed among different educational level groups (*P*-value=0.02779) as revealed in table (4).

Table 4. Seropositivity of anti-*T. gondii* antibody and educational status by using ELISA.

Educational status	No:	ELISA IgG+		ELISA IgM+	
		No:	%	No:	%
Illiterate	107	19	17.75	0	0
High school	342	41	11.98	4	1.16
University level	181	13	7.18	0	0
Total	630	73	-	4	-

X^2=10.89 df=4 *P*-value=0.02779 Significant*

3.5 The Relation between seroprevalence of anti-*T .gondii* antibodies and occupation:

The seroprevalence of anti-*Toxoplasma* antibodies according to occupation, showed the maximum seroprevalence (16.66 %) of IgG among employed women, followed by students (12.90 %). On the other hand, the lowest seroprevalence (11.02 %) was observed among housewives. While IgM antibodies were recorded only among housewives (0.78 %). There was non-significant relation between the prevalence of the parasite and the occupation (P>0.05) as revealed in table (5).

Table 5. Seropositivity of anti-*T. gondii* Abs according to occupation by using ELISA

Occupation	No:	ELISA IgG+		ELISA IgM+	
		No:	%	No:	%
Housewife	526	58	11.02	4	0.76
Student	62	8	12.90	0	0
Employed	42	7	16.66	0	0
Total	630	73	11.58	4	-

X^2=2.071 df=4 *P*-value=0.7228 Non-Significant

3.6 The relation between seroprevalence rates of anti-*T. gondii* Abs and contact with cats

Table (6) shows the relationship between seroprevalence of anti-*T. gondii* antibodies and contact with cats. The results indicate that the highest rate of IgG was 39/237 (16.45 %) in women who had contacts with cats. While regarding ELISA IgM 3/237 (1.26 %) had a history of contact with cats. A significant difference noticed among both groups (*P-value*=0.003244).

Table 6. Seropositivity of anti-*T. gondii* Abs and contacts with cats by using ELISA

Cat Contact	No:	ELISA IgG+		ELISA IgM+	
		No:	%	No:	%
Yes	237	39	16.45	3	1.26
No	393	34	8.65	1	0.25
Total	630	73	-	4	-
X^2=11.46	**df=2**	***P*-value=0.003244**		**Significant***	

3.7 Association between seropositivity of anti-*T. gondii* Abs and consumption of undercooked meat

The seroprevalence rate of anti-*Toxoplasma* IgG antibodies was the highest (100 %) among women who consumed undercooked meat than others as shown in Table (7). While the IgM cases (0.64 %) were recorded among those who do not consume undercooked meat.

Statistically this difference was highly significant (*P*-value<0.0000001).

Table 7. Association between seropositivity of anti-*T. gondii* Abs and consumption of undercooked meat by using ELISA

Consumption Of undercooked Meat	No:	ELISA IgG+		ELISA IgM+	
		No:	%	No:	%
Yes	5	5	100	0	0
No	625	68	10.88	4	0.64
Total	630	74	-	4	-
X^2=38.46	**df=2**	***P*-value=0.0000001**		**Highly Significant***	

3.8 Association between prevalence of anti-*T. gondii* Abs and application of hygienic habit.

Table (8) shows the relationship between prevalence of anti-*Toxoplasma* and application of hygienic habit. The rate of ELISA IgG Abs was (3.84 %) among women who applied the hygienic habit, while the rate increased (11.92 %) among women who did not apply hygienic habit. In contrast, the ELISA IgM Abs only present among

women who do not apply hygienic habits (0.66 %). Statistically these differences were statistically non-significant between both groups (*P*-value=0.4083).

Table 8. The relation between seropositivity of anti-*T. gondii* Abs and application of hygienic habit

Application of hygienic habit	No:	ELISA IgG+		ELISA IgM+	
		No:	%	No:	%
Yes	26	1	3.84	0	0
No	604	72	11.92	4	0.66
Total	630	73	-	4	-

X^2=1.719 df=2 *P*-value=0.4083 Non-Significant

4. DISCUSSION

Nowadays, the importance of toxoplasmosis extended as opportunistic pathogens particularly in immunocompromised persons, which include; pregnant women, AIDS patients, immunosuppression organ transplant patients and malignant patients (James, 1989; Koltas *et al*., 1992; Breecher, 2004). Toxoplasmosis detection is very important particularly throughout pregnancy, for the reason that if a woman infected with toxoplasmosis for the first time during her pregnancy, the infection can pass to her fetus, and this leads to numerous severe consequences and damage of the fetus (Kadhim and Mohammed, 2011).

In the present study, the total prevalence of anti-*Toxoplasma* antibodies was 12.38 % which was lower than that reported by Al-Atroshi (2011) in Duhok City, she reported a rate of 37.8 % by using LAT, (27.7%) by using ELISA IgG and 0nly (0.4 %) by ELISA IgM. On the other hand, using the same test much higher rates of anti-*T. gondii* Abs were recorded by Akreyi (2008) and Hamad (2009) in Erbil City and Al-Ubaydi (2004) in Mosul City which was 8 (54.46 %) and 79 %, respectively. The result of anti IgG in the current study was 11.58 % this result is lower than that reported by Kadhim and Mohammed (2013) in Babylon province, as they reported a rate of 18.9 %. In the present study only 4 (0.63 %) samples were seropositive for anti-IgM among 630 samples; this rate was close to the study performed in the United Arab Emirates in which 3 IgM were detected among patients with fetal loss (Singh, 1998).

On the other hand, Al-Khafajy (2004) in Baghdad reported a very high rate (43.7 %) for IgM, in spontaneously aborted women. This high result could be due to the sources of the samples because in the present instruction the samples were collected randomly not only from spontaneously aborted women. The higher seroprevalence rates of anti-*Toxoplasma* Abs in women might be due to warmer and more humid weather in these parts of the country (Al-Doski, 2000). Regarding age, the highest percentage was seen among age group (33-38) which was 20.43 % for ELISA IgG. The maximum rate among this age group may be due to more exposure of these ages to the risk of infection sources that leads to chronic infection with this agent (Srirup *et al.*, 2011). Much higher rate (45.3 %) among nearly same ages (30-35) years and the minimum rate (14.6 %) among 16-20 years were reported by Al-Atroshi (2011) in Duhok city. The difference in both studies might be due to change in population as both were performed in the same province but during different periods.

On the other hand in a study carried out in Sanandaj City/ Iran, Fatollahpour (2016) reported the highest seroprevalence rate (68.5 %) of anti-*Toxoplasma* IgG Abs among women under the age of 25 years. Illiterate women showed the highest (17.75 %) seroprevalence rate of anti-*Toxoplasma* IgG Abs as compared to women with high school and university levels which were 11.98 % and 7.18 %, respectively. Similarly,

Jones *et al*. (2001) in United State of America and Hashemi and Saraei (2010) in Iran, both of them reported higher seroprevalence of toxoplasmosis in illiterate women and stated that lower levels of education were associated with an increased risk for toxoplasmosis. On the other hand, women with high level of education may have more awareness to adopt appropriate hygienic measures (Jones *et al*., 2001).

While, the present results contradict with many studies, such as Al-Atroshi and Mero (2013) in Duhok; Hamad and Kadir (2013) and Mawlood (2018) in Erbil; Fatollahpour (2016) in Iran, they did not find any significant association among different educational levels. In this study, the prevalence of anti-toxoplasmosis IgG Abs increased among employed women (16.66 %) followed by students and housewives which was 12.90 % and 11.02 %, respectively. This may be due to the type of jobs and poor application of hygienic habits. Additionally, not all housewives are illiterate and live in low socioeconomic levels besides not all employed women are educated. This outcome was highly contradicted with previous studies in Duhok, United State of America and in Erbil (Al-Atroshi, 2011; Jones *et al*., 2013; Mawlood, 2018), respectively they reported that housewives had greater proportion of anti-*Toxoplasma* Abs than students and employed women as they were exposed more to risk factors (handling contaminated raw meat, drinking raw milk, direct contact with oocysts through farming or gardening as well as the ingestion of the oocysts with inadequate washing of vegetables) than other women (Alvarado-Esquivel *et al*.,2009). Saida and Nooraldeen (2014) reported that vegetables had major epidemiological role for transmission of protozoan cyst and oocyst, toxoplasma oocyst revealed (18.3 %) in Erbil city. A significant relation was observed among seroprevalence rate and cat contacts since presences of felines increases the risk of this infection (Avelino *et al*., 2004). This consequence is similar to studies carried out by Al-Khaffaf (2001); Al-Delamy (2002); and Al-Ubaydi (2004) in Mosul City, (Hatam *et al.,* 2005) in Fasa/Iran, (Ayi *et al*., 2009) in Ghana, all of them observed significant relations between exposure to cat and prevalence rate of this disease. On the other hand, the present finding disagrees with the study of Cook *et al*. (2000) in Europe, Al-Atroshi (2011) and Al-Doski (2000) in Duhok; Al-Najjar (2005) and Al-Harbi (2009) in Mosul, they did not report any significant association between contacts with cats and infections. This could be attributed to the fact that now cats are not used for hunting mice, and they spend most of their time outside houses and are not allowed to enter kitchens (Al-Doski, 2000). In the present study the seroprevalence rate of anti-*Toxoplasma* IgG Abs was significantly higher (*p*-value=0.0000001) among women who eat undercooked meat than those who eat well cooked meat (100 %) and (10.88 %), respectively. While IgM Abs were reported at a low rate (0.64 %) only among women who did not consume undercooked meat, these women may be acquired the infection through another route. This result contradict with studies performed in Duhok City in Iraq, Brazil, Iran, Makkahcity in Saudia Arabia, Venezuela and in Ghana, in all of these studies they did not find any significant differences related to consuming uncooked meat (Al-Doski, 2000; Avelino *et al*., 2004; Hatam *et al*., 2005; Al-Harthi *et al*., 2006; Diaz-Suarez and Estevez, 2009; Ayi *et al*., 2009), respectively.

Correspondingly, this outcome agrees with the studies of some researchers in which they found significant association between consumption of raw meat and infection with toxoplasmosis by Al-Delamy, (2002) in Mosul; Studenicova *et al*. (2006) in Slovakia; Spalding *et al.* (2005) in Brazil; Baril *et al*. (1999) and Wilson and McAuley (1999) in France and Mead *et al.* (1999) in United State of America. They stated that in most of these countries the habit of eating undercooked meat is common especially in developed countries.

However, Al-Doski, (2000) in Duhok City in Iraq; Diaz-Suarez and Estevez, (2009) in Venezuela; Ayi *et al*. (2009) in Chana; Al-Harthi *et al*. (2006) in Makkah City in Saudia Arabia; Hatam, *et al*. (2005) in Iran, they stated that the habit of eating undercooked meat is rare in their community as compared with other countries like Europe and may be restricted to those who eat outdoors in restaurants. Regarding to the application of hygienic habits in this study, the seroprevalence of anti-toxoplasmosis IgG Abs among women who use appropriate hygienic measures such as using different cutting board for meats and vegetables and practicing recurrent washing of kitchen utensils and hands during cooking, washing fruits

and vegetables by using antiseptics and salt was 3.84 % lower than those who did not apply most of these measures (11.92 %) for IgG even though this difference was statistically non-significant (P>0.05), while IgM Abs were only present among women who did not apply hygienic methods, but it was at low rate (0.66 %). There is a strong relation between toxoplasmosis and the application of hygienic habit because sometimes the parasite infects women during cleaning the vegetables (Norouzi *et al*., 2017). This is in agreement with the study of Al-Atroshi (2011) in Duhok city in Iraq, she also reported higher seroprevalence rate of anti-toxoplasma Abs among women with poor application of hygienic methods. Also Fouladvand *et al*. (2010) in Iran reported that there was strong and significant relationship between seropositivity of anti-*Toxoplasma* Abs and washing the vegetables.

5.CONCLUSIONS

The present study showed that the total seroprevalence of anti-*Toxoplasma* Abs among women at childbearing age was 12.38) in Zakho City, the majority were seropositive for anti-*Toxoplasma* Abs by ELISA while only one IgM case was recorded by RT, indicating the high specificity of ELISA for diagnosis. Married women showed higher prevalence. The high risk factors contributed to infection with age (33-38 years), education (illiterate's status), occupation (employed group), and most contact with cats eating more undercooked meat and poor application of hygienic measures. Therefore, the community requires an introduction to health education program by health authority, especially for pregnant women.

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