

SEROPREVALENCE OF ANTI-TOXOPLASMA ANTIBODIES AMONG COUPLE REFERRED TO A PRE-MARRIAGE SCREENING CENTER IN DUHOK PROVINCE, IRAQ

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ABSTRACT

Toxoplasma gondii is a wide spread parasitic infection of human and animals which associated with high morbidity and mortality rate. Due to the lack of previous study estimating the prevalence of *T. gondii* in unmarried couple in Duhok. This study aimed to measure the seroprevalence rate of anti-*Toxoplasma* antibodies and its associated risk factors in couples referred to a pre-marital screening center in Duhok province from July to October 2022. A total of 376 serum samples from couples attending premarital screening center were tested for the presence of anti-*Toxoplasma* IgG and IgM antibodies using Elecsys Toxo IgG and Elycsys Toxo IgM kit with Cobas e 411 analyzer. Out of 376 samples examined, 52 (13.8%) were seropositive for both anti-*Toxoplasma* antibodies; 48 (12.8%) were tested seropositive for IgG, and 9 (4.2%) were found seropositive for IgM. The seroprevalence of *T. gondii* in male (32/188, 17.02%) was higher than that in female (20/188, 10.63%). With regard to occupation, the highest seroprevalence rate was seen in employee (25 %) followed by worker, students and non-worker (13.2%, 11.7%, 11.1%) respectively. The rate of anti-*Toxoplasma* antibodies among rural participants 16.3% was higher than that among participant from urban area 12.3%. Concerning the education level, the highest seroprevalence (31.5%) observed in illiterate group. Regarding risk factors, the seroprevalence of *T. gondii* among participant in contact with cat (52.4%) was higher than those with no contact with cat 11.5%. The highest seroprevalence of anti-*Toxoplasma* antibodies was seen among 36 and more year old participant.

KEY WORDS: *T. gondii*, Unmarried couple, IgM, IgG, Cat contact, Rural and Urban

INTRODUCTION

Toxoplasma gondii (*T. gondii*) is a facultative intracellular protozoan that can infect a variety of warm-blooded mammals and birds, and regarded as an important zoonotic disease. The life cycle of *T. gondii* requires a definitive and intermediate host, domestic and wild cats which belong to the Felidae family act as a definitive host where the sexual parasitic cycle takes place. To complete the life cycle a range of intermediate hosts are required including humans, birds, and other mammals (Tenter, Heckeroth et al. 2000, Polley and Thompson 2009, Lüder and Rahman 2017). *T. gondii* infection in human occurs mainly through utilization of raw or under cooked meat containing tissue cyst or by consumption of contaminated water or vegetables by oocysts. However, it may spread congenitally via placenta, organ transplantation and through blood transfusion (Dubey and Jones 2008,

Kijlstra and Jongert 2008, Lüder and Rahman 2017). In healthy immune competent people, the infection is generally asymptomatic. While, in pregnant women it can lead to abortion, stillbirth, mental or physical retardation, hydrocephalus and blindness of the fetus (Dubey 1996). In immunocompromised patient, infection with *Toxoplasma gondii* associated with severe encephalitis which lead to significant morbidity and mortality (Mamidi, DeSimone et al. 2002).

A variety of diagnostic tests are accessible for detection of *T. gondii* infection, among those, serological tests are the most regular test (Robert-Gangneux and Dardé 2012), followed by molecular technique and then histological technique (Hill and Dubey 2002, Hill, Chirukandoth et al. 2005).

Prevalence of *T. gondii* infections are different among different geographical area and population. It is evaluated that more than 70% of the world's population are infected with *T. gondii* (Torgerson and Mastroiacovo 2013). Most of the studies on the prevalence of *T. gondii* in human have been conducted on pregnant women (Doudou, Renaud et al. 2014, MIKAEEL and AL-SAEED 2019), and only few researches have been published demonstrating the prevalence of *T. gondii* in unmarried couples in Ramadi city and in Erbil (Al-Daoody 2012, Mohanad, Shehab et al. 2013).

No previous studies have been conducted to measure the seroprevalence of *T. gondii* in unmarried couples in Duhok province. Therefore, this study will be carried out to estimate the seroprevalence of anti-*Toxoplasma* antibodies and its associated risk factors in couples referred to a pre-marital screening center in Duhok province.

AIMS OF THE STUDY

1. To investigate the seroprevalence of anti-*Toxoplasma* antibodies among couples attending the pre-marital screening center in Duhok.
2. To study the correlation between toxoplasmosis and some risk factor such as age, occupation, education level, residency and cat contact.

OBJECTIVES

To measure the anti -*Toxoplasma* IgG and IgM antibodies in the serum of couples who are going to get married using ELISA.

STUDY RATIONALE

The serologic tests to be performed before pregnancy allow the early diagnosis of infection. Thus, early treatment will reduce the risk of congenital transmission of the disease to the fetus during pregnancy.

It will also decrease the costs of long-term care for the babies in which disability develops.

METHODOLOGY

Study design and population

A cross sectional study was carried out on 376 couples (188 male and 188 female) attending the laboratory of Duhok pre-marital screening center in the period from July to

October 2022. Inclusion criteria for the women participant include being married for the first time, having no serious illness, and willing to participate in the study. The exclusion criteria include being married before, being pregnant or not willing to participate in the study.

Ethical consideration

This research study was approved by the College of Health Sciences committee, and by the Research Ethical Committee in the General Directorate of Health in Duhok governorate on 7 of August 2023, reference number: 1342022-2-11 R2

Data analysis:

The data entered in, managed, and analyzed using the SPSS software Package (IBM SPSS Statistics 22). Descriptive analysis (frequency, percent, and central tendency) of variables were calculated. Chi square or fisher's exact test used to compare the proportions. A *p* value of < 0.05 assigned to be significant for the differences between the variables.

Sample collection

Serum sample (50 µl) from each participant was collected and stored at -20°C until used. Sera were already separated for viruses screening which is one of the requirements of pre marriage screening test and used in this study. Serum separated from whole blood by centrifugation of blood at 3000 rpm for 5 minutes.

Questionnaire administration

All participants of this study were asked to answer some question regarding the risk factor associated with *Toxoplasma* infection. The questionnaire contained simple closed ended question such as age, occupation, contact with cat, education level and residential area.

Serological assay

All serum samples were tested for the presence of anti-*Toxoplasma* antibodies (IgG and IgM) using Elecsys Toxo IgG and Elycsys Toxo IgM kit (Roche, Germany) with Cobas e 411 analyzer (Roche Diagnostic) as per manufacturer's instruction. The concentration of anti-*Toxoplasma* IgG and IgM were expressed as IU/ml and COI respectively. IgG level of <1 IU/ml were considered seronegative and concentration > 3 IU /ml were considered seropositive. IgM Concentration of < 0.8 COI were considered seronegative and values ≥ 1.0 COI were considered seropositive. All of the laboratory works were conducted in the medical laboratory of private Wan Global hospital in Duhok.

RESULTS

Sociodemographic characteristic of the study participants

In the current study, 376 serum samples from couples attending the laboratory of Duhok premarital screening center were tested for the presence of anti-*Toxoplasma* IgG and IgM antibodies. All of the participants were categorized into three age groups (18-25, 26-35 and 36 and over). Among those 185 (49.2%) were 18-25 years, 160 (42.6 %) were 26-35

years and 31 (8.2 %) were 36 and over years old. With regard to occupation, 135 (35.9%) were non worker, 129 (34.3%) were worker, 60 (16%) were students and 52 (13.8%) were employee. In term of residential area, 141 participants (37.5%) were from rural areas and 235 (62.5%) were lived in urban areas. Out of the total participant, 16 (4.3%) were illiterate, 53 (14.1%) had bachelor degree, 128 (34%) from intermediate school, 96 (25.5%) secondary and 83 (22.1%) were from primary school (Table 1).

Table (1): Sociodemographic characteristic of study participants

Socio-demographic Characteristics		Frequency	Percentage %
Gender	Female	188	50.0
	Male	188	50.0
Age, Median (IQR)	18 - 25 Years	185	49.2
	26 - 35 Years	160	42.6
	36 years and more	31	8.2
Occupation	Employee	52	13.8
	Non worker	135	35.9
	Student	60	16.0
	Worker	129	34.3
Residency	Rural	141	37.5
	Urban	235	62.5
Education Level	Bachelor	53	14.1
	Illiterate	16	4.3
	Intermediate	128	34.0
	Primary	83	22.1
	Secondary	96	25.5
Total		376	100.0

Prevalence of anti-*T. gondii* antibodies among study participants

The results of our study found that around 52 of participants (13.8%) were tested seropositive for *T. gondii* antibodies IgG and IgM (Table 2). The seropositivity for anti-*Toxoplasma* IgG

antibody 12.8% (48/376) was higher than the seropositivity for anti-*Toxoplasma* IgM antibody 2.4% (9/376) as shown in Table 3. Interestingly, 5 of the participants were seropositive for both anti-*Toxoplasma* IgG and IgM antibody.

Table (2): Seroprevalence of anti-*Toxoplasma* antibodies in examined participants

Infection with <i>Toxoplasma</i>		Frequency	Percentage %
<i>Toxoplasma</i> seropositivity	Seronegative	324	86.2
	Seropositive	52	13.8
Total		376	100.0

Table (3): Seropositivity of anti-*Toxoplasma gondii* IgG and IgM in examined couples

Serological Analysis		Frequency	Percentage %
<i>Toxoplasma</i> . IgG	Seronegative	328	87.2
	Seropositive	48	12.8
<i>Toxoplasma</i> . IgM	Seronegative	367	97.6
	Seropositive	9	2.4
Total		376	100.0

Seroprevalence of anti-*Toxoplasma* antibodies and sociodemographic characteristic of participants

It is clear from Table 4 that the seroprevalence of *T. gondii* in male (32/188, 17.02%) was higher than that in female (20/188, 10.63%) with no significant differences ($p=0.073$).

In term of occupation, the seroprevalence of *T. gondii* was 25% (13/52) in employee, 13.2% (17/129) in worker, 11.1% (15/135) in non-worker and 11.7% (7/60) in student group. The highest percentage of anti-*Toxoplasma* antibodies was seen among employee groups. However, no significant differences were observed among the different occupation groups ($p=0.087$).

As expected, the seroprevalence of *T. gondii* among rural participants 16.3% (23/141) was higher than that among participant from urban area 12.3% (29/235), although no significant differences were found between the two residential area ($p=0.280$).

Concerning the education level, a significant difference was seen among the different education level ($p=0.001$) with the highest prevalence 31.5% (5/16) observed in illiterate group, followed by Bachelor, intermediate, primary and secondary level at rate 28.3 %, 12.5%, 10.8% and 7.3% respectively.

With regard to the risk factors, the seroprevalence of *T. gondii* among participant in contact with cat 52.4% (11/21) was higher than those with no contact with cat 11.5% (41/355) and the differences were statistically highly significant ($p < 0.001^*$).

The seroprevalence of *Toxoplasma* was 11.9% in 18-25 year old, 14.4% for 26-35 year old and 22.6% for 36 and over year old. The highest seroprevalence was seen among 36 and over year old. However, no significant differences were seen among the three age groups ($p=0.271$).

Table (4): Seroprevalence of *T. gondii* according to the sociodemographic characteristic of participants

Variables		Infection with <i>Toxoplasma</i>		Total No (%)	Chi-Square	Sig (2-sided) < 0.05
		Seronegative n (%)	Seropositive n (%)			
Gender	Female	168 (89.4)	20 (10.6)	188 (50.0)	3.214	0.073
	Male	156 (83.0)	32 (17.0)	188 (50.0)		
Occupation	Employee	39 (75.0)	13 (25.0)	52 (13.8)	6.563	0.087
	Non worker	120 (88.9)	15 (11.1)	135 (35.9)		
	Student	53 (88.3)	7 (11.7)	60 (16.0)		
	Worker	112 (86.8)	17 (13.2)	129 (34.3)		
Residency	Rural	118 (83.7)	23 (16.3)	141 (37.5)	1.166	0.280
	Urban	206 (87.7)	29 (12.3)	235 (62.5)		
Education Level	Bachelor	38 (71.7)	15 (28.3)	53 (14.1)	17.644	0.001
	Illiterate	11 (68.8)	5 (31.5)	16 (4.3)		

	Intermediate	112 (87.5)	16 (12.5)	128 (34.0)		
	Primary	74 (89.2)	9 (10.8)	83 (22.1)		
	Secondary	89 (92.7)	7 (7.3)	96 (25.5)		
Contact with cat	No	314 (88.5)	41 (11.5)	355 (94.4)	< 0.001*	
	Yes	10 (47.6)	11 (52.4)	21 (6.6)		
Age Group	18 - 25 Years	163 (88.1)	22 (11.9)	185 (49.2)	2.615	0.271
	26 - 35 Years	137 (85.6)	23 (14.4)	160 (42.6)		
	36 years and more	24 (77.4)	7 (22.6)	31 (8.2)		
Total		324 (86.2)	52 (13.8)	376 (100)		

* Exact's test Sig. (2-sided)

DISCUSSION

Toxoplasma gondii is a high prevalent parasite among world's population that is associated with fatal consequences specially in congenitally infected child and immunocompromised people (Mamidi, DeSimone et al. 2002). Girls willing to get married are considered at high risk for congenital toxoplasmosis when they become pregnant. Therefore, we sought to determine the infection rate of *Toxoplasma* by measuring the anti-*Toxoplasma* antibodies in the serum of couple attending pre-marital screening center in Duhok province. Early diagnosis of *Toxoplasma* infection is useful to decrease and prevent congenital toxoplasmosis in women before marriage. Our results revealed that the seroprevalence of anti-*Toxoplasma* antibodies among the couples was 13.8%. The seroprevalence rate in the current study was lower than that found in couples from Erbil city (29.19%), and in Ramadi city (38.4%) (Al-Daoody 2012, Salih, Mero et al. 2020). The differences in the seroprevalence rate may be due to the different diagnostic test used, and different geographical areas and climatic changes. In the present study Elecsys Toxo IgG and Elycsys Toxo IgM kit with Cobas e 411 analyzer was used to detect anti-*Toxoplasma* antibodies, and the study population was from rural and urban area.

The seroprevalence of *T. gondii* IgM (2.4%) was lower than the IgG antibodies (12.8%), this finding is consistent with other studies conducted on unmarried women in Iran (Mohammadi, Shojaee et al. 2015) and in male in Erbil (Salih, Mero et al. 2020). Interestingly we found that the seroprevalence of *T. gondii* in male (17.02%) was higher than that in female

(10.63%). Which is in agreement with the previous study conducted in China (Xin, Su et al. 2020), Iran (Mostafavi, Ataei et al. 2011), but in contrast to a study done in Iraq (Mohanad, Shehab et al. 2013). These conflicting data may be due to the low number of *Toxoplasma* researches on males as compared to females. However, this finding points out the need for more research to be performed on males. In addition, diagnosis of *T. gondii* in male is important to roll out the possibility of sexual transmission of the disease (Hlaváčová, Flegr et al. 2021). Moreover, the reason for the high prevalence of *Toxoplasma* infection among male may be related to the quality of life as men are more exposed to risk factors like soil contact, animal contact and eating outside home.

Our results indicated no association between *Toxoplasma* infection and the occupation of the participants. Our result was similar to other findings (Al-Daoody 2012, Salih, Mero et al. 2020). These differences may be due to the age of the participants.

Toxoplasma seroprevalence in illiterate couple was significantly higher than that in educated couple. Our result is in agreement with other study that found an inverse relation between *T. gondii* infection and education (Sharif, Ajami et al. 2006, Saraei 2010, Mostafavi, Ataei et al. 2011). Education may have consequence on the quality of life and behaviors of people leading to increase the awareness of the educated people toward the source of infection and transmission and subsequently lower the incidence of the infection.

Results of the current study revealed that the rate of seropositivity increases with the age of the participants. This was similar to the results of other studies (Sharif, Ajami et al. 2006,

Mostafavi, Ataei et al. 2011). This is possible as older peoples have more chance to come in contact with infectious agents. In study done in Venezuela the highest infection rate was observed in children aged less than 15 years (Chacín-Bonilla, Sánchez-Chávez et al. 2003). A study conducted in Duhok found that the seropositive rate of *Toxoplasma* decreases with increasing the age of the male (Salih, Mero et al. 2020). The differences in the susceptible age for acquisition of *Toxoplasma* in different region may be due to various climate condition and different behavioral, cultural and nutritional life pattern.

Several studies from various countries indicated no significant differences in the prevalence of *Toxoplasma* between urban and rural population (Sharif, Ajami et al. 2006, Saeedi, Veghari et al. 2007, Mostafavi, Ataei et al. 2011, Al-Daoudy 2012). However, a study carried out on women in Duhok found that the prevalence of *Toxoplasma* was significantly higher in rural dwellers (AWAD and AL-MUFFTI 2020). Other study in Iran demonstrated a higher prevalence in urban population as compared to rural residents (Sharifi, Hosseini Farash et al. 2019). Results of our study found no significant differences in the prevalence of anti-*Toxoplasma* antibodies between rural and urban residents, in spite of the highest prevalence of antibodies in rural areas. These diversity in the results with regard to residential area may be associated with the life style, majority of the rural resident are farmer and animal breeder so they are more exposed to the infectious agents than urban resident such as direct contact with soil contaminated by oocysts or consumption of contaminated food or fruit.

Seroprevalence of anti-*Toxoplasma* antibody was significantly higher in couples who have contact with cat (52.4%) than those with no contact with cats (11.5%). Our finding is similar to other studies (Nash, Chissel et al. 2005). This is predicted as cat are considered the main source of transmission of *Toxoplasma* by shedding the environmentally resistant oocyst with in the feces which contaminate the soil and vegetables (Dubey, Su et al. 2006).

However, several previous studies found no association between *Toxoplasma* seroprevalence and contact with cat (Jumaian 2005, Nash, Chissel et al. 2005, Ramsewak, Gooding et al. 2008, Al-Daoudy 2012).

CONCLUSION

Results of the current study indicated a low seroprevalence of anti-*Toxoplasma* antibodies among couple before marriage. However, these groups are at high risk of transmitting toxoplasmosis to off spring. The seroprevalence was higher in male, age group more than 36 years old, non-educated and in couple with contact to cat. This necessitates a special preventive program for these groups of people to reduce and control the transmission of the infection in Duhok city.

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پوخته

تۆكسوپلاسما گۆندیا نهخۆشییهکی مشهخۆری بهربلاوی مروّف و ئاژهله كه پهيوهندی ههیه به رپژهیهکی بهرزی نهخۆشی و مردن. به هۆی نه بوونی لیکۆلینه وه ی پيشوو كه مه خمه نده ی بلاوبونه وه ی تۆكسوپلاسما گۆندی له دوو هاوسه ری بئ هاوسه ر له دهۆك ده كات ئامانجی ئهم تويزینهوهیه پيوانهکردنی رپژهی دژه ته نه کانی دژه تۆكسوپلاسما و هۆکارهکانی مهترسی پهيوهست به هاوسهران كه ئاماژهیان پیکراوه بۆ سهنتهريکی پشکنینی پيش هاوسهريگری له پاريزگای دهۆك له مانگی ته موز تا تشرینی یه كه می 2022. 376 نموونهی سیرم لهو جووتانهی كه ئامادهی ناوهندی پشکنینی پيش هاوسهريگری بوون تاقیکراوه بۆبونی

Elecsys Toxo IgM و Elecsys Toxo IgG و ب بکارئینانا IgM, IgG دژه توکسو
له کۆی 376 نموونهی پشکنین، 52 (13.8%) پۆزهتیف بوون بۆ ههردوو دژهتهنی دژه تۆكسوپلاسما، IgM او 9 (4.2%) پۆزهتیف بوون بۆ IgG 48 (12.8%) نمونه پۆزهتیف بوون بۆ رپژهی بلاوبوونهوه توکسوبلاسما له نیردا (17.02 - 188/32) بهرزتره له وهی له مینهدا (10.63 - 188/20)%. سهبارت به پيشه، بهرزترین رپژهی بلاوبوونهوه له فهرمانبهراندا بیینی (25%) بهدوایدا کریکار و خویندکار و نا کریکار (13.2%, 11.7%, 11.1%). رپژهی تووشبوون له نیوان بهشداربووانی لادیدا 16.3% بهرزتره له وهی له نیوان بهشداربووان له ناوچهی شارستانی 12.3% سهبارت به ئاستی پهروهده، بهرزترین رپژهی بلاوبوونهوه (31.5%) له گروپی نهخویندهواردایبینی کراوه. سهبارت به هۆکاره کانی مه ترسی له نیوان بهشداربووان له پهيوهنديکردن به پشيلهوه (52.4%) بهرزتره لهو کهسانهی كه پهيوهندی پشيلهیان نییه 11.5% بهرزترین رپژهی تووشبوون به تووشبوون به تۆكسوپلاسما له نیوان بهشداربووانی ته مه ن 36 سال و زیاتردا تۆمارکراوه .
بلاوبوونهوهی دژه ته نه کانی دژه توکسوپلاسما له نیوان ئه و ئن و میردانهی كه ئاماژهیان پیکراوه بۆ سهنتهري سکرینکردنی پيش هاوسهريگری له پاريزگای دهۆك، ئیراق

الانتشار المصلي للأجسام المضادة لمرض التوكسوبلازما بين الأزواج المحالين لمركز فحوص ما قبل الزواج في محافظة دهوك , العراق

الخلاصة

المقوسة الغوندية هي عدوى طفيلية واسعة الانتشار تصيب الإنسان والحيوان وترتبط بارتفاع معدل الأمراض والوفيات. نظرا لعدم وجود دراسة سابقة لتقدير مدى انتشار المقوسة الغوندية لدى الافراد المقبلين على الزواج في دهوك. هدفت هذه الدراسة إلى قياس معدل الانتشار المصلي للأجسام المضادة لمرض التوكسوبلازما وعوامل الخطر المرتبطة بها لدى الأزواج المحالين إلى مركز فحص ما قبل الزواج في محافظة دهوك في الفترة مابين يوليو الى اكتوبر 2022. تم اختبار 376 عينة مصل من الأزواج الذين يحضرون مركز فحص ما قبل الزواج للتأكد من وجود الأجسام المضادة لمرض التوكسوبلازما IgM و IgG باستخدام مجموعة Elycsys Toxo IgM و Elecsys Toxo IgG مع محلل Cobas e 411. من بين 376 عينة تم فحصها، كانت 52 (13.8%) إيجابية مصليا لكلا الأجسام المضادة لمرض التوكسوبلازما؛ 48 (12.8%) عينة كانت إيجابية مصليا لـ IgG ، و 9 (4.2%) كانت إيجابية مصليا لـ IgM . كان معدل انتشار المقوسة الغوندية في الذكور (188/32، 17.02%) أعلى منه في الإناث (188/20، 10.63%). وفيما يتعلق بالمهنة، كانت أعلى نسبة انتشار مصلي لدى الموظفين (25%) يليهم العاملون والطلاب وغير العاملين (13.2%، 11.7%، 11.1%) على التوالي. كان معدل الأجسام المضادة لمرض التوكسوبلازما بين المشاركين في المناطق الريفية 16.3% أعلى منها بين المشاركين من المناطق الحضرية 12.3%. وفيما يتعلق بالمستوى التعليمي، فقد لوحظت أعلى نسبة انتشار مصلي (31.5%) في المجموعة الأمية. فيما يتعلق بعوامل الخطر، كان الانتشار المصلي للمقوسة الغوندية بين المشاركين الذين كانوا على اتصال بالقطط (52.4%) أعلى من أولئك الذين لم يكن لديهم اتصال بالقطط (11.5%). شوهدت أعلى نسبة انتشار مصلي للأجسام المضادة لمرض التوكسوبلازما بين المشاركين الذين تبلغ أعمارهم 36 عامًا أو أكثر.