

Seroprevalence of Toxoplasma Gondii among Pregnant Women in Kirkuk / Iraq

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Abstract

Toxoplasma gondii is an intracellular parasite of many types of tissues, including muscles and intestinal epithelium. The infection may be acquired or congenital, the congenital form is most severe when maternal infection occurs early in pregnancy. *Toxoplasma* serological tests have been used successfully to determine whether the infection acquired in the recent or more distant past.

The aim of this study was to detect *Toxoplasma gondii* antibodies among pregnant women in Kirkuk city by using different serological tests.

The study was carried out in Kirkuk Hospitals, and Primary Health Care Centers to detect *Toxoplasma* antibodies among 319 pregnant women aged from less than 18 to more than 35 years old. The period of study was from beginning of November 2003 to end of May 2004.

The study showed that (117) cases out of (319) were positive for *Toxoplasma gondii* (36.6%) by using LAT, and 54 case positive for IgM- ELISA (16.9%).

The highest rate of *Toxoplasma* seropositivity was among the age group 19-35 years (38.3%) by LAT and (18.75%) by IgM-ELISA.

The distribution of *Toxoplasma* seropositivity was higher in housewives than officials and was higher in rural area than urban area by both methods.

The pregnant women who were in contact with animals showed higher rate of seropositivity than those not in contact with animals. There was no relationship between number of abortions and *Toxoplasma* seropositivity.

Introduction

Toxoplasmosis is a zoonotic protozoal disease caused by tissue parasite, *Toxoplasma gondii*. *Toxoplasma* is a parasite of cosmopolitan distribution present in hot, humid countries able to develop in a wide variety of vertebrate hosts. Cats and other members of felidae are the definitive hosts, while human and wide range of animals, birds and rodents act as intermediate hosts (John &Petri, 2006).

Toxoplasma gondii is one of the most successful parasites on earth. Although the burden of this parasite varies greatly from one country to another, it remains a global public health problem which affects about one billion individuals (WHO, 1990).

The transmission of the disease to human beings is either by eating raw or uncooked meat or through ingestion of oocysts introduced into the environment by cats and congenitally during primary mothers disease (Collee *et al.*, 1996). The infection is also transmitted by blood transfusion and organ transplantation (Beaver & Jung, 1985).

Acquired toxoplasmosis is often asymptomatic, but may present with fever, general malaise, enlarged lymph nodes, splenomegaly, headache and maculopapular rash. Congenital infection may lead to abortion or still birth and give rise to abnormalities of the central nervous system (Roberts & Janovy, 2005).

Women can transmit the infection transplacentally to their unborn fetus, this often can occur during an acute infection acquired during pregnancy. The risk of congenital disease is lowest (10-25%) when maternal infection occurs during first trimester but is more likely to produce serious damage, and highest (60-90%) when maternal infection occurs during the third trimester. The overall risk of congenital infection from acute Toxoplasma gondii infection during pregnancy ranges approximately (20-50%) (Neilson, 1999).

Habitual abortion is one of the most distressing problem in obstetrics, particularly in those who have no successful pregnancies. Habitual abortion is generally defined as three or more consecutive spontaneous abortions. Spontaneous abortion has been associated with maternal transmission of Toxoplasma gondii to the fetus (Golledge & Beaman, 1990).

Patients with habitual abortion had higher antibody titers than the normal pregnancy. Although there is a positive serological test for Toxoplasma gondii in women with no history of habitual abortion, the connection of serological test for the evidence and isolation of Toxoplasma gondii from the endometrium, placenta, or the products of conception is essential. Serological tests before and during pregnancy for specific IgM would also confirm the diagnosis of Toxoplasma gondii (Jones, 1969).

The present study is aimed to show the prevalence of Toxoplasma gondii seropositivity among pregnant women in Kirkuk city, by using serological tests.

Materials and methods

A cross sectional seroprevalence study was conducted on 319 pregnant women attending Kirkuk Hospitals, and Primary Health Care

Centers, for the period from 1 st of November 2003 to 30 th of May 2004. The pregnant women were healthy, and their ages were ranging from less than 18 to more than 35 years old.

Full information was obtained from each pregnant woman in special questionnaire form, including age, occupation, address, duration of pregnancy, gravidity, parity, abortion,etc.

Five ml of venous blood was taken from each woman in a 5ml size disposable syringe then transferred to 10 ml disposable sterile test tube. The blood samples were then centrifuged at 3000 rpm for 5 minutes and serum samples then transferred to 3 ml sized micro test tube with screw cap and stored at 4-8 °C for 24-48 hrs. If longer period of storage was required sera kept in deep freeze at -20 °C.

Two serological tests used for detection of *Toxoplasma* antibodies were direct latex agglutination test (LAT) and Pathozyme *Toxoplasma* IgM (ELISA IgM test).

The latex kit received from (Biokit) Spain. Pathozyme *Toxoplasma* IgM (antibody capture enzyme immunoassay for the detection of IgM antibodies against *Toxoplasma gondii* antigen in human serum), were received from Omega Diagnostics, United Kingdom.

Results

Table 1, shows the rate of seropositivity of toxoplasmosis in pregnant women, using both LAT and ELISA. It shows that the rate of seropositivity by LAT (36.6%) was significantly higher than ELISA (16.9%).

Table (1): Comparison between the seropositivity of toxoplasmosis using Latex (LAT) and enzyme linked immunosorbant assay (ELISA) tests.

No. examined	LAT		ELISA	
	No. positive	Positive%	No. positive	Positive%
319	117	36.6	54	16.92

t =5.76

P < 0.01

Regarding the distribution of *Toxoplasma* antibodies in pregnant women according to age, Table (2) revealed that the rate of seropositivity among different age groups, less than 18, 19-35 and more than 35 years old by using LAT were 30.6%, 38.3% and 33.3% respectively, while by using ELISA were 10.2%, 18.75% and 13.3% respectively. Statistically there was no significant difference in the rate of seropositivity between difference age groups by using both LAT and ELISA.

Table (2): Distribution of *Toxoplasma* seropositivity according to age, using LAT and ELISA tests

Age (years)	No. examined	LAT		ELISA	
		No. +ve	%	No. +ve	%
Less than 18	49	15	30.6	5	10.2
19-35	240	92	38.3	45	18.75
More than 35	30	10	33.3	4	13.3
Total	319	117	36.6	54	16.9

LAT $\chi^2 = 6.01$ d.f = 2 $p > 0.05$
 ELISA $\chi^2 = 5.61$ d.f = 2 $p > 0.05$

The distribution of *Toxoplasma* seropositivity according to occupation of pregnant women is indicated in table (3). It was found that the rate of seropositivity in housewives was higher than officials by using both LAT (38.1%, 15.0%) and ELISA (17.39% and 10.9%) respectively. Statistically there was significant difference between housewives and officials by using LAT.

Table (3): Distribution of *Toxoplasma* seropositivity according to occupations

Occupation	No. Examined	LAT		ELISA	
		No. positive	Positive%	No. positive	Positive %
House wives	299	114	38.1	52	17.39
Officials	20	3	15.0	2	10.0
Total	319	117	36.67	54	16.9

LAT $\chi^2 = 4.317$ d.f = 1 $p < 0.05$
 ELISA $\chi^2 = 0.728$ d.f = 1 $p > 0.05$

Comparison between the distribution of *Toxoplasma* seropositivity among pregnant women in rural and urban areas using LAT and ELISA tests is indicated in table (4). In LAT the rate of seropositivity in rural area (50%) was higher than urban area (33.5%) and also in ELISA test, the seropositivity rate in rural area (23.3%) was higher than urban area (15.4%). The difference in the rate of seropositivity between rural and urban areas was significant by using LAT ($P < 0.05$), while the difference between them was not significant by using ELISA.

Table (4): Distribution of *Toxoplasma* seropositivity according to residency

Residency	No. Examined	LAT		ELISA	
		No. positive	Positive%	No. positive	Positive%
Urban	259	87	33.5	40	15.4
Rural	60	30	50	14	23.3
Total	319	117	36.67	54	16.9

LAT $\chi^2 = 5.648$ d.f = 1 $p < 0.05$
 ELISA $\chi^2 = 2.156$ d.f = 1 $p > 0.05$

Table (5), shows the distribution of *Toxoplasma* seropositivity among patients according to animal contacts. The seropositivity among those keeping animals at home was higher than those not keeping animals at home by both LAT (48.57% and 22.85%) and ELISA (33.33% and 15.26%) respectively.

Table (5): Distribution of *Toxoplasma* seropositivity according to presence of animals at home.

Animal at home	No. examined	LAT		ELISA	
		No. positive	Positive%	No. positive	Positive%
Present	70	34	48.57	16	22.85
Absent	249	83	۳۳,۳۳	38	15.26

LAT $\chi^2 = 5.46$ d.f = 1 p < 0.05
 ELISA $\chi^2 = 2.241$ d.f = 1 p > 0.05

It is indicated in table (6) that the rate of *Toxoplasma* seropositivity in pregnant women by LAT was highest in first trimester of pregnancy (41.66%) followed by second (35.29%) and third trimester (32.45%) respectively, although statistically there was no significant difference between seropositivity and stage of pregnancy.

The highest titer was 1/64 in one case while the lowest titer was 1/2. The frequency was highest in 1/16 (37) cases followed by 1/2(35), 1/4(22), 1/8(16), 1/32(6) and the lowest was in 1/64(1) respectively.

Table (6): Distribution of *Toxoplasma* seropositivity titers according to trimester of pregnancy by using LAT.

Trimester of pregnancy	No. examined	No.+ve	+ve%	1/2	1/4	1/8	1/16	1/32	1/64
First	120	50	41.66	15	10	5	15	5	-
Second	85	30	35.29	10	5	4	10	1	-
Third	114	37	32.45	10	7	7	12	-	۱
Total	319	117	36.67	35	22	16	37	6	۱

* 1/2 dilution is positive titer $\chi^2 = 2.23$ d.f = 2 p > 0.05

Table (7) shows the distribution of *Toxoplasma* seropositivity among pregnant women by ELISA .The rate of seropositivity among pregnant women in first, second and third trimester was 19.1%,18.8% and 13.1% respectively. Statistically there was no significant difference between them. The highest titer (3.1-3.5) and the lowest titer was (1.1-1.5). the frequency was highest in 1.1-1.5 (30), 1.6-2.0 (13), 2.1-2.3 (5) and the lowest was in 2.6-3.0(3) and 3.1-3.5(3) respectively.

Table (7): Distribution of *Toxoplasma* seropositivity titers according to trimester of pregnancy by using IgM ELISA.

Trimester of pregnancy	No. Examined	No +ve	+ve %	1.1-1.5	1.6-2.0	2.1-2.5	2.6-3.0	3.1-3.5
First	120	23	19.1	15	5	2	2	3
Second	85	16	18.8	10	5	2	1	–
Third	114	15	13.1	5	3	1	–	–
Total	319	54	16.9	30	13	5	3	3

$\chi^2 = 0.035$ d.f = 2 p > 0.05

Table (8) shows the rate of *Toxoplasma* seropositivity according to number of abortions. It is indicated that rate of seropositivity in single, 2-4, and 5-8 by LAT was 55.17%, 50.0%, 33.33% respectively. While by ELISA was 24.13%, 26.66%, 33.33% respectively. Although statistically there was no significant difference in the rate of seropositivity and the number of abortions, the highest rate of seropositivity by LAT was among those with single abortion (55.17%) and by ELISA was among those with 5-8 abortions (33.33%).

Table (8): Distribution of *Toxoplasma* seropositivity according to number of abortions.

Number of abortion	No. examined	LAT		ELISA	
		No. +ve	+ve%	No. +ve	+ve%
Single	58	32	55.17	14	24.13
2-4	60	30	50.0	16	26.66
5-8	3	1	33.33	1	33.33
Total	121	63	52.0	31	25.61

LAT $\chi^2 = 0.75$ d.f = 2 p > 0.05
 ELISA $\chi^2 = 0.195$ d.f = 2 p > 0.05

Discussion

In the present study, two serological tests were used to achieve the best diagnostic criteria for toxoplasmosis among pregnant women in different stages of pregnancy (first, second and third trimesters).

The rate of positive cases by using LAT and IgM-ELISA were (36.67% and 16.92%) from a total of 319 pregnant women. This result reflects that it is possible to depend on LAT in seroepidemiological study for *Toxoplasma gondii*, which is less costly and easy to perform. This finding is in agreement with Khafaf (2001) who compare the positive rate of *Toxoplasma gondii* in pregnant and non pregnant women, using LAT, ELISA, and IFAT, who found the seropositivity in LAT was (86.6%). The result of this study goes with that observed by Mazumder *et al.* (1988) and

Rye *et al.* (1997), who referred that LAT is the best for seroepidemiological study to detect *Toxoplasma gondii* antibodies, this is also agreed with Al-Simani (2000), who found the rate of Toxoplasma seropositivity (39.53%) in pregnant women using LAT.

Comparing the result of this study with that carried on pregnant women by other workers, it was found the rate of seropositivity in Baghdad was 39% by Niazi [1988] using ELISA. While by using LAT it was found 40% in Germany, 60% in Chile, 63% in Panama, 71% in France, 75% in El-Salvador and 78% in Nigeria [2001]. The highest rate of *Toxoplasma* seropositivity was among 19-35 years old pregnant women, by using both LAT and ELISA IgM might be related to child bearing age. This result is in agreement with Al-Doski (2000), and Ageel (2003) who found the highest seropositivity in age 35-60 years (55.9%) followed by 25-35 years (41.2%) respectively. The disease was present in young ages too, but there is higher chance of old ages to be exposed to the infection. The result is also in agreement with Al-Hamdani and Al-Mahdi (1996) who found that the seropositivity to *Toxoplasma gondii* significantly increased with age reaching 23.7% in age group 35-45 years. Regarding the distribution of *Toxoplasma* seropositivity according to occupation, it was found that the housewives exposed to infection higher than officials which was (38.1%, 17.39%) by LAT and ELISA respectively. The percentage of seropositivity in officials was (15.0%, 10.0%) in both methods. This result is in agreement with Al-Waely (1998) who found the *Toxoplasma* seropositivity among housewives in Baghdad province was (49.5%), but not in agreement with Al-Hamdani and Al-Mahdi (1996), who did not find relationship between *Toxoplasma gondii* and occupations. The higher rate of infection among housewives than officials might be related to housewives being in direct contact with infection through handling and preparing of food (contaminated meat and vegetables) in addition to cleaning of house garden contaminated with cat feces. The relationship of seropositive toxoplasmosis with residency was also studied. The seropositive *Toxoplasma* was high among pregnant women from rural area (50.0%, 23.3%) by LAT and ELISA than those in urban area (33.5%, 15.4%). This result is in agreement with Ageel (2003) who found that the seropositivity was higher in rural area (36.36%) than urban area (32.05%). This might be due to lack of health education in rural area, inadequate treatment and direct contact with animals as they breed animals in houses. The result is not in agreement with Al-Maqdisy (2000) who found that there is no relation between *Toxoplasma* seropositivity and residency. This study revealed that there was a relationship between animal contact and *Toxoplasma* seropositivity. The prevalence among pregnant women who were in contact with animals

was high (48.57%, 22.85%) by LAT and ELISA respectively. The distribution of disease might be due to stray cats which get infection from eating birds, rodents, and aborted fetus in slaughter house and shedding their oocysts which lead to contamination of gardens, animal food stuff, granaries and thus lead to infection [1990a].

In the present study there was no significant difference in the rate of seropositivity among pregnant women in different trimesters of pregnancy. This reflects that all stages of pregnancy have the same chance of acquiring infection. This result was supported by Al-Dugaily (1998) and Al-Siman [2000] who found higher prevalence among those with first trimester than other trimesters. According to the antibody titers, the highest number of seropositive pregnant women was recorded in titer 1/16, 37 pregnant women from 117 positive cases (31.6%). This result is in agreement with Ageel [2003] who found the highest number of seropositivity was recorded in titer 1/16, and not in agreement with Al-Simani (2000) who found that the highest titer of seropositive pregnant women was in 1/4 titer (low titer). The parasite is an opportunistic organism which remains in the body in a latent form; it reactivates during depression of immunity. During pregnancy there will be certain physiological changes in the body and weakness of body which lead to activation of latent parasite during this period [1996].

The results of the present study did not support the relation between the increase in the number of abortions and the increasing *Toxoplasma* seropositivity as indicated in table [8]. It was (55.17%) and (24.13%) by LAT and ELISA among pregnant women with single abortion as compared with 2-4 abortion (50%, 26.66%) and 5-8 abortion (33.33%, 33.33%). The difference in the percentage of seropositivity was not significant by using two tests. The high percentage of seropositivity by LAT was among pregnant women with single abortion, while by ELISA the high rate of seropositivity was among the women with 5-8 abortions. This finding was not in agreement with Al-Maqdisy (2000), who found that women with two abortions have higher percent of seropositivity by using LAT (34.14%) and also not in agreement with Al-Dugaily (1998) and Al-Doski (2000). The lowest percentage of *Toxoplasma* seropositivity by LAT was found among those with 5-8 abortions (33.33%). This finding was supported by Fatohi (1985) who found the percent of seropositivity among women with 6 or more abortions was 7.14% by using IHAT and CFT. Our results were not in agreement with Al-Hamdani *et al.* (1996), who found that the women with five or more abortions had the highest prevalence rate of *Toxoplasma* antibodies. It is recommended to screen married girls before and during pregnancy for detection of *Toxoplasma* antibodies (IgM, IgG) by using more advanced techniques such as PCR and others.

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الانتشار المصلي للمقوسات الكوندية بين النساء الحوامل في مدينة كركوك

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الخلاصة

المقوسة الكوندية هي طفيلي داخل الخلايا لعدة أنواع من الأنسجة من ضمنها العضلات والخلايا
الطلائية المعوية و الإصابة ممكن إن تكون وراثية أو مكتسبة، الشكل الولادي هو حاد جدا عندما تكون إصابة
الأم في مراحل الحمل الأولى تستخدم التحاليل السيرولوجية للمقوسات الكوندية بنجاح لتعيين فيما إذا كانت
الإصابة حديثة أو قديمة. الهدف الرئيسي لهذه الدراسة هو إيجاد أضداد المقوسة الكوندية بين النساء الحوامل في
مدينة كركوك باستعمال طرق سيرولوجية مختلفة.

أجريت الدراسة في مستشفى كركوك العام، مستشفى أزادي، المراكز الصحية الأولية لإيجاد أضداد
المقوسات ببيتين ٣١٩ امرأة حامل تتراوح أعمارهن من (اقل من ١٨-٣٥) سنة، فترة الدراسة كانت من بداية
تشرين الثاني ٢٠٠٣-إلى نهاية مايس ٢٠٠٤ وللحصول على المزيد من المعلومات استخدمت استمارة معلومات
خاصة لكل امرأة حامل.

أظهرت نتائج الدراسة إن ١١٧ حالة من ٣١٩ مصابات بالمقوسة الكوندية (٣٦,٦%) باستخدام تالزن
اللاتكس ، ٥٤ حالة موجبة لـ IgM- ELISA (١٦,٩%).

أعلى نسبة للإصابة كانت للأعمار التي تتراوح بين (١٩-٣٥) سنة (٣٨,٣%) باستخدام تالزن اللاتكس و
IgM- ELISA (١٨,٧٥%).

نسبة انتشار الإصابة عالية في ربوات البيوت أكثر من الموظفات وكذلك في المناطق الريفية أكثر من
الحضرية باستخدام الطريقتين أعلاه. النساء الحوامل هن بتماس مباشر مع الحيوانات أظهرت نسبة إصابة عالية
مقارنة مع عدم الملامسات للحيوانات لاتوجد علاقة بين عدد حالات الإسقاط ونسبة الإصابة لجميع المقوسات
الكوندية.