Full Length Research paper

Seroprevalence of *Toxoplasma gondii* between couples in Ramadi city using enzyme linked immunosorbent assay (ELISA)

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Toxoplasma gondii **is an obligate intracellular protozoan parasite that represents an actual public health problem. This study aims to investigate the prevalence of** *T. gondii* **among 91 couples in Ramadi city who were examined for the presence of antibodies against** *T. gondii* **using enzyme linked immunosorbent assay (ELISA). The overall anti-***T. gondii* **(Immunoglobin M (IgM) and Immunoglobin G (IgG)) in both couples were 38.4%, the seroprevalence in wives was only 30.7%, while that in husbands was 13.1% only. This study showed that abortive women and abnormal pregnancy had the highest percentage rates (35.7 versus 57.14%) of toxoplasmosis among those of 26 to 30 years old and the lowest was among those who have the average of age (36 to 40) years old. One group miscarriage was (50%) higher than the other groups. The group of 26 to 30 years old showed high rate of IgM antibodies of about (66.66%). The number of abortion in the first trimester was high in both patterns of antibodies IgM only and (IgM and IgG) (62.5 and 29.16%), respectively. Analysis of variance revealed that there were no significant interactions between IgM and IgG seropositivity and the gestational age of the fetus.**

Key words: *Toxoplasma gondii*, pregnancy, the couples, seroprevalence.

INTRODUCTION

Toxoplasmosis is an important zoonotic parasitic disease that affects millions of people and is caused by the protozoan *Toxoplasma gondii*. In immune competent individuals, *T. gondii* preferentially infects tissues of central nervous systems, which might be a contributing factor to certain psychiatric disorders (Reischl et al., 2003, Xiao et al., 2010). It is a ubiquitous obligate intracellular protozoan parasite, widely prevalent in humans and other animals on all continents (Dodds, 2006; Weiss et al., 2007).

In Iraq, Niazi et al. (1988) found out that the prevalence of *Toxoplasma* antibodies among women in Baghdad was 39%, whereas Niazi et al. (1992) reported low rate of 8.6% positively from eight governorates in Iraq. Mohammed and Al-Nasiry (1996) reported a prevalence rate of 20.4% toxoplasmosis in Iraqi women. In a study carried out in Basrah (Yacoub et al., 2006), the prevalence of toxoplasmosis had been shown to be 41.1 to 52.1%, whereas a previous study by Al-Hamdani and Mahdi (1997) showed low rate of 18.5% of *Toxoplasma* antibodies in Basrah population. In Duhok, North of Iraq, Razzak et al. (2005) found low *Toxoplasma* infections of about 0.97%. This result indicated that the contribution of toxoplasmosis to fetal loss is greatly overestimated. On his side, Karem (2007) found out that the seropositivity was 32.6% by enzyme linked immunosorbent assay (ELISA) between women in Sulaimania. In Baghdad, Juma and Salman (2011) found the infection of *T. gondii* in women to be 19.17%. In Tikrit, Al-Doori (2010) showed the presence of infection around 49 to 95% and higher

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Table 1. Seropositivity of anti-*Toxoplasma* IgM and IgG detected by ELISA in examined samples.

Month	Number of couples	Both couples infected		Wife infected only		Husband infected only		No anti-*Toxoplasma* abs.	
		No.	%	No.	%	No.	%	No.	%
November	10	4	40	2	20	0	0	4	40
December	11	4	63.6	5	45.4	2	18.1	0	0
January	16	5	31.2	6	37.5	3	18.7	2	12.5
February	18	5	27.7	6	33.3	3	16.6	4	22.2
March	11	8	72.7	3	27.2	0	0	0	0
April	13	8	61.5	1	7.1	3	23.0	1	7.1
May	12	1	8.3	5	41.6	1	8.3	5	41.6
Total	91	35	38.4	28	30.7	12	13.1	16	17.5

rate of infection lies among those of 25 to 31 years of age in women and their husbands.

Seroprevalence of *T. gondii* infection in man rises with age and it does not vary greatly between sexes (Montoya and Remington, 2000). The prevalence of toxoplasmosis significantly increases with age and the highest seropositivity rate 35.4% was found among pregnant women in the age group of 35 to 44 years in Slovakia (Studenicova et al., 2006).

The overall seroprevalence of toxoplasmosis in South Africa was 29/160 (18.1%). Seroprevalence in males and females were 7/42 (16.7%) and 22/118 (18.6%), respectively and the difference was not statistically significant (P > 0.05). The age distribution was 0.63% (1/160) for individuals of 20 years old and below, 10.6% (17/160) for those between 21 and 35 years old, and 6.9% (11/160) for individuals who were 36 years old and above (Bessong and Mathomu, 2010).

The serologic evidence of toxoplasmosis in Ethiopia was found in 60% (39/65) of them. A large number of the seropositive were females (64.1%), while in male it was (53.8%) (Negash et al., 2008). The overall anti-*T. gondii* immunoglobin G (IgG) prevalence in china was 12.3%, while the seroprevalence was 10.5% in men versus 14.3% in women (Xiao et al., 2010).

The purpose of this research is to investigate infections with *T. gondii* in couples by ELISA.

MATERIALS AND METHODS

ELISA is one of the few frequently used methods in detection of *T. gondii* infection in humans and animals. In the ELISA test, soluble antigen is coated to micro titer plates and sample serum is added to form an antigen-antibody complex (if specific antibodies are present). A secondary enzyme-linked antibody specific to the host species is added to detect antigen-antibody complex. This test requires an ELISA reader and also enzyme conjugation to the secondary antibodies. Numerous modifications of ELISA have been reported to enhance specificity and simplify the protocol of the conventional ELISA (Dubey and Beattie, 1988).

Serum samples were collected from 91 clinically and laboratory confirmed *Toxoplasma* infected patients. The samples were collected during the period from November 2010 to May 2011 from

the clinical laboratory in Ramadi Hospital. Collected samples were stored at -20°C until we started ELISA test.

This assay was performed by using two kits, one for detection of IgG and another one for detection of immunoglobin M (IgM) specific antibodies against *T. gondii* antigens in the patient's serum (Biokit Diagnostics Company, Spain).

Detection of IgG and IgM titers in all samples were analyzed for *T. gondii* by the titer of IgG and IgM antibodies by ELISA kit as described (Biokit Diagnostics Company, Spain). The optical densities (OD) of the samples were measured at 450 nm, using the OD value of the blank well to correct all the OD reading from test wells (Biokit Diagnostics Company, Spain).

RESULTS

Seroprevalence data obtained are shown in Table 1. The overall percentage of positive reaction to *T. gondii* in both couples was 38.4 (35/91), while for wife infected it was only 30.7% (28/91) and husband infected was only 13.1% (12/91). The most frequent age group for abortive women and abnormal women was among those of 26 to 30 years old and it represents 35.7 and 57.14% of the total number of each group, respectively. Moreover, it was not observed to have a significant difference in the prevalence of toxoplasmosis between the age groups (P < 0.05) (Table 2).

In Table 3, the samples of wives were divided into three groups (abortive women, abnormal pregnancy, and normal pregnant women), and each of them was subdivided into four groups (0, 1, 2, and >3 miscarriages). A rate of one miscarriage in abortive women was 50% (35/70), while 40% (28/70) and 10% (7/70) are for the two and three miscarriages, respectively. The statistical analysis revealed a high significant difference (P < 0.05).

The prevalence of IgM only in the age group of 26 to 30 years shows a high percentage (66.66%) and in the age group of 36 to 40 it shows a high prevalence of IgG (60%), while in the age group of 31 to 35 it shows a high prevalence (50%) for both (IgM and IgG). Its correlation to the different age groups was not statistically significant (Table 4). The prevalence of IgM recorded the highest number with two miscarriages, it was 58.83% (10/17),

Table 2. Age group distribution in examined samples.

Age groups (year)	Abortive women		Abnormal pregnancy		Normal pregnant women	
	No.	%	No.	%	No.	%
≤ 20 - 25	21	30	1	14.28	3	21.42
26 - 30	25	35.71	4	57.14	3	21.42
31 - 35	15	21.42	1	14.28	3	21.42
36 - ≥40	09	12.85	1	14.28	5	35.71
Total	70	100	7	100	14	100
Statistical analysis	Cal. χ^2 = 5.817; Tab. χ^2 = 14.06; P ≤ 0.05					

Table 3. Ratio of previous miscarriages in examined samples.

Number of miscarriage	Abortive women		Abnormal pregnancy		Normal pregnant women	
	No.	%	No.	%	No.	%
0	0	0	5	100	2	12.5
1	35	50	0	0	7	43.7
2	28	40	0	0	6	37.5
≥3	7	10	0	0	1	6.2
Total	70	100	5	100	16	100
Statistical analysis	Cal. X^2 = 67.16; Tab. X^2 = 9.488; P ≤ 0.05					

Table 4. Seropositivity of anti-*Toxoplasma* IgM and IgG in relation to participant age.

Age group (year)	IgM$^+$ve		IgG$^+$ve		IgM$^+$ve and IgG$^+$ve		Total	
	No.	%	No.	%	No.	%	No.	%
≤ 20 - 25	7	58.33	2	16.66	3	25	12	100
26 - 30	8	66.66	2	16.66	2	16.66	12	100
31 - 35	3	50	0	0	3	50	6	100
36 - ≥40	1	20	3	60	1	20	5	100
Statistical analysis	Cal. X^2 = 8.59; Tab. X^2 = 14.06; P≤ 0.05							

then 23.52% (4/17) for IgG antibodies, while the percentage of both (IgM and IgG) recorded the highest number of one miscarriage, which was 44.44% (8/18) (Table 5). Statistical analysis revealed a non significant interaction between IgM or IgG seropositivity and the gestational age of the fetus. Comparable results of seropositivity of both IgM and IgG obtained in the first trimester gave a high percentage in IgM 62.5% (15/24) and both (IgM and IgG) 29.16% (7/24), while the number of women in their third trimester indicated no anti-*Toxoplasma* antibodies (Table 6).

DISCUSSION

The main purpose of this study was to evaluate the seroprevalence of *T. gondii* antibodies between couples of Ramadi city. The overall seroprevalence of *Toxoplasma* in this study among both couples was 38.4%, but when the previous results were compared with the published data, a decreasing seroprevalence was observed in pregnant women (Yacoub et al., 2006; Al-Rawi, 2009; Al-Doori, 2010; Juma and Salman, 2011), while the results of other studies were in agreement with the results of this study (Al-Khafajy, 2004; Al-Musauy, 2008). These variable results may be due to the differences in the specimens used by each researcher and their variable condition and data of studies.

This study found out that seroprevalence of toxoplasmosis in wives was higher than in husbands. These results were similar to the results of Negash et al. (2008), Bessong and Mathomu (2010), Xiao et al. (2010), and Sroka et al. (2010). One of the reasons for this high prevalence is related to the fact that women handle raw meat more frequently than men due to the fact that they spend more time cooking at home.

There are several causative factors responsible for both habitual and sporadic abortions. However, the prevalence of toxoplasmosis in women with bad obstetrics history is known to be significantly higher than

Table 5. Ratio of anti-*Toxoplasma* antibodies according to the number of miscarriages in couples infected.

Number of miscarriage	Pattern of antibody							
	IgM+ve		IgG+ve		IgM+ve and IgG+ve		Total	
	No.	%	No.	%	No.	%	No.	%
0	0	0	0	0	0	0	0	0
1	7	38.88	3	16.66	8	44.44	18	100
2	10	58.83	4	23.52	3	17.64	17	100
≥3	0	0	0	0	0	0	0	0
Statistical analysis	Cal. X^2 = 2.9; Tab. X^2 = 3.841 ; $P \le 0.05$							

Table 6. Seropositivity of Anti-*Toxoplasma* IgM and IgG in relation to gestational age.

Gestational age	Pattern of antibody							
	IgM+ve		IgG+ve		IgM+ve and IgG+ve		Total	
	No.	%	No.	%	No.	%	No.	%
1st trimester	15	62.5	2	8.33	7	29.16	24	100
2nd trimester	4	36.36	3	27.27	4	36.36	11	100
3rd trimester	0	0	0	0	0	0	0	100
Statistical analysis	Cal. X^2 = 2.97 ; Tab. X^2 = 3.841 ; $P \le 0.05$							

in normal. The seroprevalence in pregnant women on worldwide scale varies from 7 to 51.3% and in women with abnormal pregnancies and abortions; the seroprevalence varies from 17.5 to 53.3% (Kumar et al., 2004). The seropositivity rate of abortive women in age group (26 to 30) years old was obviously higher (53.71%) than in other groups, which was similar to the results reported in Iraq (Shani, 2004; Kadhim, 2006; Al-Rawi, 2009; Juma and Salman, 2011). This is presumably due to the high presence of cats, climatic, hygienic, and socioeconomic conditions in the regions. However, it is acknowledged that seroprevalence increases with age, as seen in studies conducted in various countries (Dodds, 2006).

Women who may get infection during pregnancy may show a variety of clinical signs and symptom depending on many factors, such as the number of parasites, virulence of strain, and the time period the mother acquires infection (Tenter et al., 2000). If the mother is infected in the first trimester, the result is abortion, stillbirth or severe disease of fetus (Lin et al., 2000).

On the other hand, IgM antibodies titer to *T. gondii* was found to be more than IgG antibodies. Clearly, the overall prevalence of IgM antibodies was interpreted as a diagnosis of the acute form of the disease. In the present survey, it was shown that chronic form (shows prevalence of IgG antibodies) increased with age (16.66 to 60%). These results reflected the contact with cats or infected materials and vegetables in these age groups.

This results pointed out that most IgM and both IgM and IgG patterns of antibodies were increased in first trimester (62.5 and 29.16%, respectively), these patterns of antibodies were absent in the third trimester. The severity of disease decreases if the infection occurs in the second or third trimester, but the risk for transmission from mother to fetus increase (Romand et al., 2001). In pregnant women, the primary infection of *T. gondii* may cause abortion, neonatal malformation, neonatal death, or severe congenital deficiency, such as mental retardation, retinochoroiditis, and blindness (Kravetz and Federman, 2005). In addition, toxoplasmosis is one of the main causes of fetal abortion, stillbirth, and neonatal mortality in domestic animals, resulting in significant economic loss in the farming industry (Mcallister, 2005).

Congenital toxoplasmosis is most severe when the mother becomes infected in the first trimester, then approximately 10 to 20% of fetuses are infected. If the infection is acquired in the second trimester, 30 to 40% of fetuses are infected, but the disease is mild or asymptomatic at birth. These differences in transmission may be related to the placental blood flow, size of uterus, virulence of the parasite or to the immunocompetence of the mother (Singh, 2003).

REFERENCES

Al-Doori MA (2010). Epidemiological study of *Toxoplasma gondii* between couples in Tikrit city and experimental trial about possibility of sexual transmission of infection in mice. M.Sc. Thesis, college of Education, University of Tikrit.

Al-Hamdani MM, Mahdi NK (1997). Toxoplasmosis among women with habitual abortion. Med. East J., 3: 310-315.

Al-Khafajy AH (2004). Cytogenic, immunological and biochemical studies on women infected with *Toxoplasma gondii* with a history of abortion. M.Sc. Thesis. College of Medicine, Al-Nahrain University.

Al-Musauy AS (2008). A comparative study for *Toxoplasma gondii* infection in pregnant women diagnosed by enzyme linked immunosorbant assay (ELISA) and polymerase chain reaction (PCR).

M.Sc. Thesis. College of Medicine. Al-Mustansiriyah University.

Al-Rawi KH (2009). Detection of B1 gene from blood of pregnant and abortive women infected with *Toxoplama gondii* . Ph.D. Collage of science, University of Baghdad.

Bessong PO, Mathomu LM (2010). Seroprevalence of HTLV1/2, HSV1/2 and *Toxoplasma gondii* among chronic HIV-1 infected individuals in rural northeastern South African. Afr. J. Microbiol. Res., 4(23): 2587-2591.

Dodds EM (2006). Toxoplasmosis. Curr. Opin. Ophthalmol., 17(6): 557-61.

Dubey JP, Beattie CP (1988). Toxoplasmosis of animals and man. CRC Press, Inc., Boca Raton, Fla.

Juma AS, Salman S (2011). Correlation between apoptosis and *Toxoplasma* in abortion induction: Relevance of caspase 8. Int. J. Med. Sci., 3(6): 181-192.

Kadhim MA (2006). Study of some immunological parameters of women sera infected with toxoplasmosis. M.Sc. Thesis. College of Science, University of Baghdad.

Karem LO (2007). Seroepidemiological study of *Toxoplasma gondii* for aborted women sera in Sulaimania city. M.Sc. Thesis, College of Science, University of Baghdad.

Kravetz JD, Federman DG (2005). Toxoplasmosis in pregnancy. Am. J. Med., 118: 212-216.

Kumar A, Arora V, Mathur M (2004). *Toxoplasma* antibody levels in females with habitual or sporadic abortions and normal pregnancies. Indian J. Med. Microbiol., 22(4): 276- 277.

Lin M, Chen T, Kuo T, Tseng C, Tseng C (2000). Real time PCR for quantitative detection of *Toxoplasma gondii.* J. Clin. Microbiol., 38: 4121-5.

Mcallister MM (2005). A decade of discoveries in veterinary protozoology changes our concept of "subclinical" toxoplasmosis. Vet. Parasitol., 132: 241–247.

Mohammed NR, Al-Nasiry SA (1996). Toxoplasmosis among Iraqi women with a history of abortion: Serological study. J. Comm. Med., 9: 207-214.

Montoya J, Remington JS (2000). Toxoplasma gondii. In: Mandell G, Beenett J, Dolin R. Principle and practice of infectious diseases. 5ed. Philadelphia, Churchill Livingstone Press, pp. 2858-2881.

Negash T, Tilahum G, Medhin G (2008). Seroprevalence of *Toxoplama gondii* in Nazaret town, Ethiopia. East Afr. J. Pub. Health Vol. 5, No. 3.

Niazi AD, Nasif WH, Abbass SA, Gzar SF (1992). Prevalence of *Toxoplasma* antibodies in Iraqi population. J. Fac. Med. Baghdad, 34(3): 355-361.

Niazi AD, Omer AR, Al-Hadithi TS, Aswad A (1988). Prevalence of *Toxoplasma* antibodies in Iraqi pregnant women in Baghdad. J. Fac. Med. Baghdad, 30(3): 323-329.

Razzak AH, Wais SA, Saeid AY (2005). Toxoplasmosis: the innocent suspect of pregnancy wastage in Duhok, Iraq. Eastern Mediterr. Health J., 11(4): 625- 632.

Reischl U, Bretagne S, Krüger D, Ernault P, Costa JM (2003). Comparison of two DNA targets for the diagnosis of toxoplasmosis by real-time PCR using fluorescence resonance energy transfer hybridization probes. BMC Infect. Dis., 3: 7.

Romand S, Franck J, Thulliez P, Peyron F, Dumon H.(2001). Prenatal diagnosis using polymerase chain reaction on amniotic fluid for congenital toxoplasmosis. Obstet. Gynecol., 97: 296-300.

Shani WS (2004). Humoral and cellular immune response in women afflicted with toxoplasmosis. Ph.D. Thesis, University of Basrah.

Singh S (2003). Mother to child transmission and diagnosis of *Toxoplasma gondii* infection during pregnancy. Indian J. Med. Microbiol., 21(2): 69-74.

Sroka J, Wojcik-fatla A, Szymanska J, Dutkiewicz J, Zajac V, Zwolinski J (2010). The occurrence of *Toxoplama gondii* infection in people and animal from rural environment of Lublin region- estimate of potential role of water as a source of infection. Ann. Agric. Environ. Med., 17: 125-132.

Studenicova C, Bencaiova G, Holkova R (2006). Seroprevalence of *Toxoplama gondii* antibodies in a health population from Slovakia. Eur. J. Int. Med., 17: 470-473.

Tenter AM, Heckeroth AR, Weiss LM (2000). Toxoplasma gondii: from animal to human. Int. J. Parasitol., 30: 1217-58.

Weiss LM, Kim K (2007). *Toxoplasma gondii.* The model apicomplexan: perspectives and methods. London: Academic Press.

Xiao Y, Yin J, Jiang N, Xiang M, Hao L, Lu H, Sang H, Liu X, Xu H, Ankarklev J, Lindh J, Chen Q (2010) Seroepidemiology of human *Toxoplasm gondii* infection in China. BMC Infect. Dis., 10: 4.

Yacoub AA, Bakr S, Hameed AM, Al-Thamery AA, Fartoci MJ (2006). Seroepidemiology of selected zoonotic infections in Basra region of Iraq. La Revue de Sante de la Mediterranee orientale, 12(1/2): 112-118.