

Full Length Research Paper

## Seroprevalence of *Toxoplasma gondii* in 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* (IgM and IgG) in both couples were 38.4%, the seroprevalence in wives was only 30.7%, while 13.1% in husbands only. The study showed that abortive women and women with abnormal pregnancies had the highest percentage rates, 35.7 vs. 57.14%, of toxoplasmosis within the age range of 26 and 30 years old, while the lowest rate was found among those within the average age range of 36 and 40 years old. One 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 an adding factor of 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 across continents (Dodds, 2006; Weiss and Kim, 2007).

In Iraq, Niazi et al. (1988) found that the prevalence of *Toxoplasma* antibodies among women in Baghdad was 39%, whereas Niazi et al. (1992) reported low rate (8.6%) of positivity 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 by 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. In Sulaimania, Karem (2007) found out that by using ELISA, the seropositivity was 32.6% in women. In Baghdad, Juma and Salman (2011) found that the infection of *T. gondii* in women was 19.17%. In Tikrit, Al-Doori (2010) showed the presence of infection of about 49 to 95% and higher rate of infection lies among those of 25 to 31 years old in the 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

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

Month	Number of couples	Both		Wife infected only		Husband infected only		No anti- <i>Toxoplasma</i> Abs.	
		Couples infected		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	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

seropositivity rate, 35.4% was found among pregnant women in the age group of 35 to 44 years old in Slovakia (Studenicova et al., 2006).

The overall seroprevalence of Toxoplasmosis in South African 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 seropositives were females (64.1%), while in male was 53.8% (Negash et al., 2008).

The overall anti-*T.gondii* IgG prevalence in China was 12.3%, 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 as a result of *T. gondii* in couples using Enzyme Linked Immunosorbent Assay (ELISA).

## MATERIALS AND METHODS

One of the few methods frequently used in the detection of *T. gondii* infection in humans and animals is Enzyme-linked Immunosorbent assay (ELISA). In the ELISA test, soluble antigen is coated to microtiter 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 clinical laboratory in Ramadi Hospital. Collected samples were stored at -20°C until we started the ELISA test.

This assay was performed by using two kits. One for detection of IgG and another one for detection of 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 using ELISA kit as described by 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), wife infected was only 30.7% (28/91), while infected husband was only 13.1% (12/91). The most frequent age group for abortive women and abnormal pregnancy 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, each of them was subdivided into four groups (0, 1, 2 and 3 miscarriages). The 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 age group 26 to 30 years shows a high percentage (66.66%) and in age group 36 to 40 years shows a high prevalence of IgG (60%), while in age group 31 to 35 years shows a high prevalence of 50% for both IgM and IgG. Its correlation to the different age groups was not statically significant (Table 4). The prevalence of IgM recorded the highest number with two miscarriages [58.83% (10/17)], then 23.52% (4/17) for IgG antibodies, while the percentage of both IgM and IgG recorded the highest number of one miscarriage [44.44% (8/18)] (Table 5). Statical analysis revealed a non

**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	9	12.85	1	14.28	5	35.71
Total	70	100	7	100	14	100

Statistical analysis cal.  $\chi^2 = 5.817$ ; tab.  $\chi^2 = 14.06$ ;  $P \leq 0.05$ .

**Table 3.** Ratio of previous miscarriages in examined samples.

Number of miscarriages	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.  $\chi^2 = 67.16$ ; tab.  $\chi^2 = 9.488$ ;  $P \leq 0.05$ .

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

Age groups (year)	IgM +ve		IgG +ve		IgM +ve and IgG +ve		Total	
	No.	%	No.	%	No.	%	No.	%
≤ 20 to25	7	58.33	2	16.66	3	25	12	100
26 to 30	8	66.66	2	16.66	2	16.66	12	100
31 to 35	3	50	0	0	3	50	6	100
36 to 40	1	20	3	60	1	20	5	100

Statistical analysis cal.  $\chi^2 = 8.59$ ; tab.  $\chi^2 = 14.06$ ;  $P \leq 0.05$ .

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

Number of miscarriages	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.  $\chi^2 = 2.9$ ; tab.  $\chi^2 = 3.841$ ;  $P \leq 0.05$ .

significant interaction between IgM and 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

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

Gestational age	Pattern of antibody							
	IgM +ve		IgG +ve		IgM +ve and IgG +ve		Total	
	No.	%	No.	%	No.	%	No.	%
First trimester	15	62.5	2	8.33	7	29.16	24	100
Second trimester	4	36.36	3	27.27	4	36.36	11	100
Third trimester	0	0	0	0	0	0	0	100

Statistical analysis cal.  $\chi^2 = 2.97$ ; tab.  $\chi^2 = 3.841$ ;  $P \leq 0.05$ .

seroprevalence of *T. gondii* antibodies between couples of Ramadi City. The overall seroprevalence of *Toxoplasma* in this study among both couples was 38.4% and when compared to the previous results of pregnant women, demonstrated a decreasing seroprevalence compared with the published data (Yacoub et al., 2006; Al-Rawi, 2009; Al-Doori, 2010; Juma and Salman, 2011), while other studies were in agreement with this results (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.

The study found that seroprevalence of Toxoplasmosis in wives was higher than in husbands. These results were similar to 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 women handling 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 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 symptoms 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 (which shows prevalence of IgG antibodies) was increased with age 16.66 to 60%. These results reflected the contact with cats or infected materials and vegetables in these age groups.

These 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 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).

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