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Sero-prevalence and immunological parameters of toxoplasmosis in women attending maternity teaching hospital in erbil city, northern Iraq.

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Abstract

Toxoplasmosis is a zoonotic disease One third of the world population has been estimated to be infected with Toxoplasma gondii (T. gondii) parasite. Generally, it does not cause serious illness in healthy adults, but causes severe diseases in immune-compromised patients. Dimness of the host immune role may allow reactivation of infection, sometimes and results in widespread organ damage. The present study was aimed to investigate the prevalence of toxoplasmosis among women in Erbil and to assess serum level of Interleukin 12 (IL-12 and Interleukin 6 (IL-6) in women with recurrent abortion. A total of 300 women at their reproductive age who attended Maternity Teaching Hospital were enrolled in this study. Anti- toxoplasma IgG and IgM antibodies were detected by ELISA technique. Serum IL-12 and IL-6 were assessed by enzyme-linked immunosorbent assay. Anti-toxoplasma IgG and IgM were seropositive in 129 (43%) and 14 (4.7%) women, respectively, and only 9(3%) of them for both anti-toxoplasma IgG and IgM. Significant association of toxoplasmosis and history of miscarriage was observed, while no significant association of toxoplasmosis and educational level, socioeconomic level, age, and residency were observed. The results also revealed that IL-6 level (2.51±0.06 pg/ml vs. 2.50±0.03pg/ml) was non-significantly (P > 0.05) changed in infected women in comparison with negative control group. However, serum IL-12 level (232.81± 51.58 pg/ml vs. 152.96±37.90 pg/ml) was significantly (P < 0.05) elevated in infected women in comparison to the control group. Seroprevalence of toxoplasmosis is relatively high in Erbil and IL-12 level was significantly elevated in the sera of women with toxoplasmosis.

Keywords: Toxoplasma gondii, cytokines, interleukin -12 (IL-12), interleukin -6 (IL-6).

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Introduction

Toxoplasma gondii (a member of the phylum Apicomplexa) is a ubiquitous apicomplexan parasites of human and others warm-blooded animals which have been measured as the cause of the most prevalent parasitic zoonosis and is the causative representative of significant morbidity and mortality among human worldwide.

Nearly one third of the world's population is infested by this obligate intracellular protozoan parasite Infections with T. gondii typically occur by ingestion of water or food contaminated by oocysts shed by T. gondii-infected cats or by assimilation of tissue cysts in meat from T. gondii-infected animals while the congenital transmission is also expansively reported and represents a public health problem Congenital infection arises only when a woman becomes infected during pregnancy About one-third of all the women who gain infection with T. gondii during pregnancy spread the parasite to the fetus After primary maternal infection by Toxoplasma gondii during gestation, the parasite may arrive the fetal circulation by infection of the placenta. The risk and severity of infection in the child rely on the time of gestation in which the mother acquires the infection.

Fetal toxoplasmosis, predominantly in early pregnancy can cause miscarriage, stillbirth, and birth defects. Early first-trimester maternal infection is less prospective to result in congenital infection, but the squeal is more severe.The recognition of recently acquired infection in pregnant women is, therefore, dangerous for clinical management of the mother and her fetus. The clinical spectrum of T. gondii infections differs from asymptomatic to life-threatening disease Since the infection is associated with no signs or non-specific symptoms in the great majority of individuals, mainly those with efficient immune systems and pregnant women, the diagnosis of toxoplasmosis primarily relies on serological tests. The detection (and quantification) of T. gondii antibodies in serum is used to found whether the infection acquired recently or in the distant past Enzyme linked immunosorbent assay (ELISA) is the most common test accessible as commercial kits and automated platform. is a biochemical technique used to distinguish the presence of antibody or antigen in a sample. The discovery of specific IgM & IgG antibodies has been used serological marker for diagnosing current toxoplasmosis. As an obligate intracellular parasite, T. gondii must attack host cells to survive and develop the infection.

During cell invasion, two subcellular organelles, micronemes and rhoptries, sequentially discharge their contents at the apical end of the parasite to mediate entry. As the parasite invades the host cell, a parasitophorous vacuole (PV) membrane is formed and surrounds the parasite. After the parasite finishes entry, other organelles, termed as dense granules (DG), secrete proteins into the PV. Dense granule proteins are supposed to function in modification of the PV for nutrient acquisition. The T. gondii infection results in a strong and persistent T-helper-1

(Th1) response considered by creation of proinflammatory cytokines including interleukin 12 It is known that IL-12 is produced by human dendritic cells, neutrophils and monocytes in response to T. gondii. Resistance to the intracellular protozoan parasite Toxoplasma gondii is originated by the induction of interleukin-12 (IL-12), which stimulates interferon (IFN)-γ synthesis by natural killer (NK) cells and T lymphocytes.

Methods and Materials

Subjects

A cross sectional study was carried out in Erbil city in which 300 women in their reproductive age who attended Maternity Teaching Hospital from October 2018 to march 2019 were enrolled. An informative close end questionnaire including Age, residency, socioeconomic level, history and number of abortions, history and number of abnormal parities, gestational age, was obtained through direct interview. The participants were apparently healthy and women with autoimmune diseases such as rheumatoid arthritis, SLE, and those with UTI (vaginal discharge, burning during urination, dysuria), were excluded from the study.

Ethical considerations

The study was approved by the Research Ethical Committee of the College of Medicine, Hawler Medical University, Erbil. A verbal consent was obtained from each participant before collection of blood samples.

Sample collection

5ml of blood was withdrawn using a sterile syringe and transferred to a clean fully labeled tube, then centrifuged at 3000 rpm for 10 minutes to separate the serum. The separated, serum was dispensed into two Eppendorf tubes fully labeled with the required information according to the questionnaire sheet designed for the study and stored at -20oC until used. The seroprevalence of Toxoplasma antibodies was determined using ELISA technique. The ELISA kits were supplied by Bioactive Company (Germany). The procedure was done according to the manufacturer's instructions supplied with the kit.

CRP (C- reactive protein)

One hundred sera samples were examined by CRP for control group: the samples which showed serological tests negative to anti-T. gondii IgG/IgM antibody were enrolled in the control group. C-reactive protein was estimated by latex fixation method via a kit (Spinreact SA, Spain) following the manufactures instructions.

Quantitative estimation of human il-10 and il6 by enzyme-linked immunosorbent assay (ELISA)

The Human IL-10 and IL6 ELISA Kits ((Komabiotech, Korea) were used for quantitative estimation of IL-10 and IL6 in the sera samples of the case group which showed (serological tests positive to anti-T. gondii IgG/IgM antibody), and control group which showed (serological tests negative to anti-T. gondii IgG/IgM antibody by CRP) in accordance with the leaflet provided by the manufacturer.

Statistical analysis

The Statistical Package for Social Science (SPSS version 21.0) was used for data entry and analysis. The student t-test and Chi – square were used to compare the data. P value of ≤0.05 was considered as statistically significant.

Results and Discussion

A total of 300 women agreed to be involved in the study. The age of the participants ranged between 18 to 48 years with a mean age of 27.36±6.28 years. Half of the participants 152 (50%) had antibodies for toxoplasma gondii. Anti-toxoplasma IgG and IgM were seropositive in 129 (43%) and 14 (4.7%) women, respectively, only 9(3%) of women who were seropositive for toxoplasmosis carrying both anti-toxoplasma IgG and IgM as expressed in Fig 1. As it can be seen in Table 1, significant (P< 0.05) association of toxoplasmosis and history of miscarriage was observed on screening for both anti-toxoplasma IgG and IgM antibodies (2.3% vs 0.7%) by ELISA kits Bioactive Company (Germany) in women with and without history of miscarriage, respectively.

Table1. *Seroprevalence of toxoplasmosis among women according to history of miscarriage.*

History of miscarriage	No. of examined samples	IgG	IgM	IgG and IgM
		Seropositive samples (%)	Seropositive samples (%)	Seropositive Samples (%)
Yes	118	48 (16.0)	10(3.3)	7(2.3)
No	182	81 (27.0)	4(1.3)	2 (0.7)
Total	300	129 (43)	14 (4.7)	9 (3.0)
P- value		P= 0.513	P= 0.012	P= 0.017

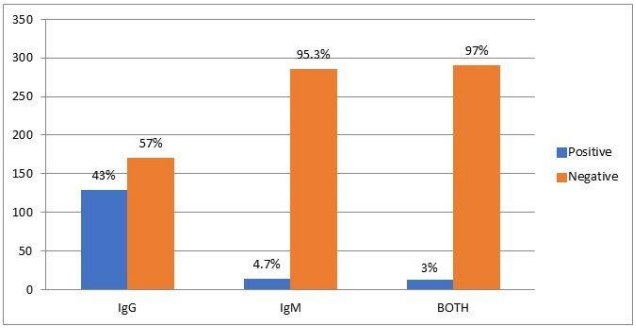

Figure1. *Prevalence of anti- toxoplasma IgM and IgG antibodies among 300 women of Erbil city.*

In respect to the socioeconomic level of the participants, the results revealed highest rate of anti-Toxoplasma gondii IgG (27.7 %), IgM (2.7 %), both IgG and IgM (2.3 %) among women with low socioeconomic level, however, statistically no

significant association was observed between toxoplasmosis and socioeconomic level as revealed in (Table 2). According to educational level of the participants, no significant (P > 0.05) association of toxoplasmosis and educational level was observed (Table 3).

***Table 2**. Sero-prevalence of toxoplasmosis among women according to socioeconomic level.*

Socioeconomic level	No. of examined samples	IgG	IgM	IgM and IgG
		Seropositive samples (%)	Seropositive samples (%)	Seropositive samples (%)
Low	183	83(27.7)	8(2.7)	7 (2.3)
Medium	105	42 (14)	4(1.3)	1 (0.3)
High	12	4 (1.3)	2 (0.7)	1 (0.3)
Total	300	129 (43)	14(4.7)	9 (3)
P- value		P= 0.533	P= 0.152	P= 0.136

***Table 3.** Sero-prevalen ce of toxoplasmosis among women according to educatiional level.*

Education	No. of examined samples	IgG	IgM	IgG and IgM	Total
		Seropositive samples (%)	Seropositive samples (%)	Seropositive samples (%)	Seropositive (%)
Primary	64	25(8.3)	1 (0.3)	1(0.3)	27 (17.7)
Secondary	75	28(9.3)	2(0.7)	0(0.0)	30 (19.7)
University	52	53 (17.7)	8 (2.7)	6(2.0)	67 (44)
Illiterate	109	53 (17.7)	8 (2.7)	6(2.0)	67 (44)
Total	300	129 (43)	14 (4.7)	9 (3.0)	152 (50.6)
P- value		P= 0.419	P= 0302	P= 0.123	P= 0.113

In the current study, the age of the participants ranged between 18 to 48 years with a mean age of 27.36±6.28 years. Comparably, the results revealed that, highest rate of anti-toxoplasma (IgG, 22.7 %; IgM, 3%; IgG and IgM, 2%) antibodies were among women with ages ranged between 21-30 years old.

However, statistical analysis revealed no significant, differences in the levels of IgG (P= 0.355), IgM (P= 0.575), both IgG and IgM (P= 0.571) antibodies among the studied age groups. As it can be seen in Table 5, the sero-prevalence of toxoplasmosis among studied women in relation to their residency, the highest rates, 65 (21.7 %), 9 (3 %), 5(1.7) were observed among women who were rural inhabitants by IgG, IgM, both IgG and IgM respectively.

However, statistical analysis revealed no significant differences among urban and rural inhabitants for anti-toxoplasma IgG, IgM, both IgG and IgM (P= 0.604, P= 0.231, P= 0.467) antibodies.

***Table 4.** Age distribution of anti-toxoplasma antibodies among women with toxoplasmosis.*

Age	No. of examined samples	IgG	IgM	IgG and IgM
		Seropositive samples(%)	Seropositive samples (%)	Seropositive samples (%)
≤20	52	18 (6.0)	1 (0.3)	0 (0.0)
21-30	156	68 (22.7)	9 (3)	6 (2)
31-40	87	41 (13.7)	4 (1.3)	3 (1)
≥41	5	2 (0.7)	0(0)	0 (0)
Total	230	129 (43)	14 (4.7)	9 (3.0)
P- value		P= 0.355	P= 0.575	P= 0.571

***Table 5.** Sero-prevalence of toxoplasmosis among women according to history of residency.*

Residency	No. of examined samples	IgG	IgM	IgG and IgM
		Seropositive samples (%)	Seropositive samples (%)	Seropositive Samples (%)
Urban	155	64 (21.3)	5 (1.7%)	4(1.3%)
Rural	145	65 (21.7)	9 (3.0%)	5 (1.7%)
	300	129 (43)	14 (4.7%)	9 (3.0%)
P- value		P= 0.604	P= 0.231	P= 0.467

Table 6 illustrates the mean concentration of IL-12, IL-6 in women with toxoplasmosis and women of healthy control group. IL-12 level (232.81± 51.58) was significantly elevated in the sera of women with toxoplasmosis when compared with those of healthy control group (152.96± 37.90).

***Table 6.** Immunological parameter in women with toxoplasmosis and control geoups.*

Immunological parameter	Infected women (n=56) (Mean ±SE)	Control group (n=33) (Mean ±SE)	P value
IL-12 (pg/ml)	232.81±51.58	152.96±37.90	0.042
			<0.05
IL-6 (pg/ml)	2.51±0.06	2.50±0.03	0.215
			>0.05

However, IL-6 level was non-significantly (P > 0.05) altered in response to toxoplasma gondii infection. The causative agent of toxoplasmosis is Toxoplasma gondii which is an intracellular protozoan. It has an important role in abortion and congenital diseases in pregnant women, which lead to infant's defectiveness birth when pregnant, are exposed during pregnancy. Toxoplasmosis is one of the most prevalent and most successful parasitic infectious disease worldwide, due to its efficient transmission. In the present study serological screening of toxoplasmosis among 300 pregnant and nonpregnant women with and without history of abortion, revealed that 129 (43%) of the samples were positive for IgG, 14 (4.7%) for IgM and 9 of the cases (3%) were positive for

both IgG and IgM. Comparably, our finding was similar to that observed by previous studies, carried out in Erbil. Abdulla reported that sero-prevalence rate of toxoplasmosis was 92 (34.98%) and 34 (12.93%) for IgG and IgM, respectively among women who referred to Erbil Teaching Maternity Hospital in Erbil over the period from December 2015 to April 2016. While Bakre observed 15.3% and 5.3% positive cases for IgG and IgM, respectively. In Duhok province, Iraq the seroprevalence rate was found to be 282(35.61%) for IgG and 6 (0.76%) for IgM among females and their relation to some demographic factors by ELISA. In Basra a study involving pregnant women reported a seroprevalence rate for IgG and IgM at 11.3% and 1.13%, respectively among female university students who were close to childbearing age. Another study carried out in Baghdad- Iraq on 120 women, who had spontaneous abortion reported that 43.33% of abortive women were positive for anti-Toxoplasma antibodies, 4.16% of them had IgM, 25.83% had IgG, and 13.33% revealed positive reactions for both IgM and IgG Subsequently accordingly, the authors have clarified the possible association of toxoplasmosis and occurrence of abortion. Furthermore, the infection rate also reported in some surrounding countries as indicated in Iran, (23.2%) and (7.2%) of women were obtained to have chronic and acute infections, respectively as detected by Sharifi, et al. Another study done by Aqeely et al. (2014) in Saudi Arabia reported that the prevalence of anti- T. gondii IgG and IgM antibodies was 20% and 6.2%, respectively with the use of ELISA Toxoplasmosis is one of the major causes of miscarriage in pregnant women. Most cases of miscarriage occur in the acute phase of infection and early pregnancy. In this study, a significant association of toxoplasmosis and history of miscarriage was detected on screening for both anti-toxoplasma IgG and IgM (2.3 % vs 0.7 %) antibodies in women with and without history of abortion (Table 1) a study that was made in Southwest of Iran shows different results to our study, after testing the sera of 130 abortive and 130 non abortive women by ELISA no statistical difference (P >0.05%) was detected between toxoplasma infection and abortion.

Women of low socioeconomic state could be at risk of repeated infections attributable to the unhygienic environment in which they reside However, in the present study no significant association of anti-toxoplasma antibodies and socioeconomic level was observed. Regarding educational level, our findings revealed no statistical differences (P>0.05) among participant pregnant women. Such result was in accordance with that obtained by Kamal, et al 2015 in Egypt. A study done by Malarvizhi, etal 2012 in India was observed that the infection is more prevalent among illiterate women. The absence of statistical differences of toxoplasmosis among pregnant women and educational levels does not indicate that this factor has no an effect on the reducing of infection in educated women.

Regarding the age of the participants, our study observed highest rate of anti-toxoplasma (IgG, 22.7%; IgM, 3%; IgG and IgM 2%) antibodies were among women with ages ranged between 21-30 years old. However, statistical analysis revealed no significant association. A study that was made in Sudan estimated that the seroprevalence of toxoplasmosis increased in ages ranged between 21-30 years old this due to women in this age are high risky and the most fertile period of childbearing age. Another study that was carried out in Saudi Arabia concluded that the highest rate of toxoplasmosis was observed in women with increasing age (63%) this due to increases the risk of exposure to T. gondii parasite.

In present study the highest rates of toxoplasmosis, 65 (21.7%), 9 (3 %), and 5 (1.7%) of toxoplasmosis were observed among women who were rural inhabitants by IgG, IgM, and both IgG and IgM respectively. The frequency of toxoplasmosis in the human population is related with contact to risk factors. An increased prevalence of toxoplasmosis in the rural area could be attributed to the consumption of vegetables treated with untreated water was also in identified among other risk factors forgetting toxoplasmosis or at least to expose to the parasite infective stages, in addition to some other risk factors, such as personal hygienic practices, feeding habits and socio-economic status.

Regarding immunological parameters IL-12 level was significantly increased in response to toxoplasmosis, but no significant change was observed in IL-6 level. Some previous studies in vitro revealed that exposure to toxoplasmosis stimulated IL-12 secretion in human monocytes, IL-12 induces the production of IFN-γ, a key mediator of immunity in humans IFN-γ cytokine is the most important cytokines that produced in response to T.gondii infection. In addition to activating T cell-mediated immunity, IFN-γ functions in a cell-autonomous manner to control intracellular parasites. IFN-γ increases tryptophan degradation in human fibroblasts, inhibiting parasite replication. Otherwise (IL-6) secretion is measured critical for retaining of usual pregnancy and increased risk of recurrent spontaneous abortions.In conclusion, Seroprevalence of toxoplasmosis is relatively high in Erbil and IL-12 level was significantly elevated in the sera of women with toxoplasmosis.

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