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SEROPREVALENCE AND RISK FACTORS OF SPECIFIC *TOXOPLASMA GONDII* ANTIBODY AMONG PREGNANT WOMEN

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ABSTRACT

Toxoplasmosis during pregnancy has an adverse effect on pregnant women, fetus and neonatal. The infection is generally asymptomatic but can cause severe effect on the fetus and immunocompromised women. Control study conducted on 77 pregnant women categorized according to the risk factors influencing toxoplasmosis infection. Specific anti *Toxoplasma gondii* IgG and IgM were evaluated using Enzyme-linked immunosorbent assay (ELISA) test kits. Among 77 pregnant women, (57.1%) women had toxoplasmosis, seropositive for latent infection with specific *Toxoplasma gondii* immunoglobulin G (IgG) antibodies were (54.54 %), whereas acute infection immunoglobulin M (IgM) were only in (2.59 %) cases. The seroprevalence of Toxoplasma was higher in older pregnant women (> 60%) than younger ones (<50%). The specific IgG antibody was higher in pregnant women working in farms (65.7 vs 45.23; P=0.053). Also, the seropositive IgG antibody was low in the first trimester and high in rural areas. Pregnant women need to educate more about toxoplasmosis and prevention to exposure in order to reduce the risk of congenital toxoplasmosis.

***KEYWORDS**:* Anti-Toxoplasma Immunoglobulin G (Igg), Immunoglobulin M (Igm), Pregnancy, Risk Factors, Seroprevalence, *Toxoplasma Gondii*.

1. INTRODUCTION

Toxoplasmosis is the most common zoonotic disease affecting mammalian around the world. The causative agent *Toxoplasma gondii*, is an intracellular protozoan parasite (Dubey, 2013; Hill et al., 2018). The serological evidence of *Toxoplasma gondii* encountered about one third of people throughout the world. Host mainly infected with toxoplasmosis by ingesting contaminated food, water or soil with oocysts shed by the cats (Dubey, 2013).

Toxoplasmosis is the main opportunistic pathogen in immune-compromised hosts. The parasite is mostly in a dormant stage and transforms to the active form in a compromised immune system. Several factors attribute to reduce host immunity as in case of disease or response to physiological change such as pregnancy. Infection is typically subclinical, but may be life threatening or neurological damage in patients with low immune-response (Montoya et al., 2008). The risk of infection by *Toxoplasma gondii* increases in pregnant women with the development of pregnancy (Teweldemedhin et al., 2019). In case of toxoplasmosis in fetus which is known as congenital toxoplasmosis, the fetus is infected with parasites through the placenta from infected pregnant women and could cause severe damage to the brain of fetus. The frequency of vertical transmission disease is approximately 0.1% of pregnant women. Diagnosis and treatment during early stage of pregnancy could reduce the risk of toxoplasmosis in fetus (Wilson et al., 2011).

Previous epidemiological studies reported that the prevalence of *Toxoplasma gondii* infection in pregnant women varies from country to country, from 9 to 67 % in European countries (Nash et al., 2005), 34.1% in Sudan (Elnahas et al., 2003) and 33% in New Zealand (Morris et al., 2004), 70.9% in Cuba (González-Morales et al., 1995). The toxoplasmosis prevalence was low (28.6%), however, acute toxoplasmosis diagnosed by seroconversion during pregnancy showed 9 women out of 12 had an acute infection with 5 (41.6%) infant congenital toxoplasmosis (Muñoz Batet et al., 2004).

Recently, the results from analysis study throughout the world confirmed that the seroprevalence of toxoplasmosis is approximately

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33.6 % in pregnant women (Rostami et al., 2020). The frequency of toxoplasmosis infection in pregnant women varies among geographical region ranged from low (0.7% in South Korea) to high (92% in Ghana) incidence, suggesting that the variation of climate, contact with pet animal, contaminated environment and food with oocysts, play important role in the prevalence of *Toxoplasma gondii* infection. However, little information is available to identify the variation of toxoplasma infection within the country. Variation in prevalence among regions within the country could help to assist and identify specific risk factors (Rostami et al., 2020). The various prevalence of toxoplasmosis in Iraq and surrounding countries are shown in Table 1.

Toxoplasmosis only causes asymptomatic or mild infection in pregnant women, but it can cause severe complications in immunocompromised pregnant women and fetus. Identification of toxoplasmosis in susceptible women is indispensable to minimize the congenital transmission. The demonstration and isolation of *Toxoplasma gondii* regarded to be difficult (Hung et al., 2007) and mostly diagnosed by detection of antibodies either by Latex agglutination test, indirect fluorescent antibody test or by the specific *Toxoplasma gondii* IgM and IgG antibody using ELISA test (Montoya et al., 2004).

Table (1):. Seroprevalence of toxoplasmosis in pregnant women in Iraq and neighbor region.

Region	Total %	IgG %	IgM%	References	Test
Duhok Iraq	29.4		0.97	(Razzak et al., 2005)	Latex ELISA
Erbil Iraq	47.9	34.8	12.93	(Abdullah et al., 2017)	ELISA
Kirkuk Iraq	36.6		16.9	(Kadir. et al., 2011)	Latex
Tikrit Iraq		30	26.7	(B. A. Mohammed, 2018)	Chromatographic immunoassay
Baghdad Iraq	37.7	28.8	23.8	(T. K. Mohammed, 2011)	ELISA
Muthana Iraq	44.5			(Al Se´adawy, 2010)	Latex
Wassit Iraq	53.8	32.4	22.2	(Tawfeeq et al., 2012)	ELISA
Najaf Iraq		36	5	(Fadhil Al Kalaby et al., 2016)	ELISA
Centre Turkey	-	22.3	0.64	(Sert et al., 2019)	ELISA
Turkey	32.6 (28.5-36.9)			(Yilmaz1. et al., 2018)	Review
Syrian refugees		28.3	0.1	(Altunal, 2018	ELISA
Jorden		31.7		(Jumaian, 2005)	ELISA
South Iran	27.8	27	0.8	(Khademi et al., 2019)	ELISA
Iran	41 (33-53)			(Foroutan-Rad et al., 2016)	Review
Saudi Arabia	24.1	20	6.2	(Aqeely et al., 2014)	ELISA
Saudi Arabia	27.8 (20.6 –36.3)			(Alzaheb, 2018)	Review
Kuwait	63.8	53.1	13.8	(Iqbal et al., 2007)	VIDAS
Throughout world	33.6 (0.7-92)			(Rostami et al., 2020)	Meta-analysis Review

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Serological screening for toxoplasmosis antibodies in seronegative women and during early stage of pregnancy each month would be ideal for recently infected women and initiating early treatment. Such serological testing for *Toxoplasma gondii* IgG and IgM antibodies is authorized by law in some countries (Montoya et al., 2008). Specific *Toxoplasma gondii* IgM antibody can be detected earlier than IgG antibody after infection and reduced after recovery (Wilson et al., 2011).

Low income, illiterates, farm workers, and contaminated regions are factors attributing to increase the seroprevalence of toxoplasmosis factors. This study performed in Akra and surrounding districts where factors influencing toxoplasmosis suspected to be high. There are little or no available information recording the prevalence of *Toxoplasma gondii* in the pregnant woman of a particular population in this specific geographical location. The study aimed to explore the demographic factors, and risk factors associated with the incidence of *Toxoplasma gondii* infection in pregnant women. It will be valuable to evaluate the prevalence of disease in specific areas considering; the profile of pregnant women regarding the incidence of Toxoplasmosis with the risk factors such as previous miscarriage, stage of pregnancy, residency, age, contact with pet animals and working on the farms.

2. MATERIAL AND METHODS

Study populations consist of 77 pregnant women aged 15 years old and above. The study was designed to identify the risk factors of seroprevalence of *Toxoplasma gondii* infection in pregnant women by linking the participants with the guide of questionnaire interviews. The participants were grouped according to their age, residential area, pregnancy stage, history of miscarriage, presence of pet animals and working on farms.

Sample collection

Blood samples (5ml) were collected in test tubes from pregnant woman after obtaining information considering risk factors. Samples then transported to the molecular lab in a local hospital. Serum separation was performed by centrifuged blood samples at 3000 rpm for 10 minutes at room temperature. Serum samples then transferred to new labeled tube and kept at −20 °C for further immunoassay processing.

Serological detection of Toxoplasmosis

The total serum samples from 77 pregnant women were tested serologically for anti-*Toxoplasma gondii* IgG and IgM antibodies using ELISA kits (Biorex Diagnostic Limited, UK. DEIA379 for IgG ; Bioactive diagnostic, GmbH, Germany. MBS494234 for IgM). Samples were tested according to the manufacture's instructions. Briefly, all serum samples and kit components were brought into room temperature before use. Then 100 µl of diluted sera samples (1; 21 IgM: 1; 101 IgG), positive control, negative control, blank (Sample diluent) and standards added into appropriated wells. Samples then mixed thoroughly and incubated (20 minutes IgM and 60 minutes IgG) at room temperature. Liquids from wells were removed and the plate was washed three times with 300 µl of wash buffer and blotted into absorbent paper. Followed by adding 100 µl of conjugate into wells and incubated for 20 to 30 minutes. Then the plate was washed three times and blotted into absorbent paper. Next adding 100 µl of substrate into each well and incubated for 10 to 20 minutes. To stop the reaction, 100 µl of stop solution were added into wells and mixed thoroughly. Finally, optical densities were measured by a microplate reader at 450 nm to determine the IgM and IgG concentrations.

Statistical analysis

The statistical analyses were conducted to investigate the association between risk factors and toxoplasmosis infection. In the first analysis, the pregnant women were categorized according to the risk factors (Age, residential area, pregnancy stage, history of miscarriage contact with cat and farm working). The effect of the risk factors was analyzed by logistic regression model binomial distribution, using GenStat (GenStat 19th edition software, 2017). The outcome of Toxoplasmosis considered as number of successes, whereas the model to be fitted was risk factors and the binomial total or number of subjects were the total number of pregnant women.

In the second analysis, the variations between/among the mean within group were analyzed by generalized linear regression. The results were converted into percentage then the odd ratio and P. value were presented and

used to indicate the significant differences between/among groups.

3. RESULTS

Study conducted on 77 pregnant women in different age ranged from 15 to 45 years old. The participants were classified into 4 groups according to their age; younger than 20, 20 to 29, 30 to 39 and older than 40 years old. The majority of the participants within the group were aged 20 to 29 (44.2%) and 30 to 39 (44.2 %) years, whereas the percentage of the youngest and oldest participants were 2.6% and 9.1% respectively.

The information of pregnant women categorized according to the risk factors, (Age, resident location, stages of pregnancy, miscarriage, contact with pet animals and working in the farm), affecting the prevalence of seroprevalence of toxoplasmosis in pregnant women shown in Table 2.

The frequency of *Toxoplasma gondii* infection in pregnant women was 57.13% for anti-Toxoplasma. Statistically, the prevalence of Toxoplasma antibodies within the risk factor was higher in pregnant women which had miscarriages compared to normal (70.2% *vs* 36.7%; P=0.004) and working in farm than not working in farm (71.4% *vs* 45.2%; P=0.02; Table 2).

The results were further analysed depending on the type of immunoglobulins diagnosed in serum. Specific antibodies against *Toxoplasma gondii* IgG (54.5%) in pregnant women were significantly (P<0.001) higher than IgM (2.6%). The prevalence of IgM antibodies recorded only on two serum samples of pregnant women aged 30 to 39 years, living in rural areas, at the second stage of pregnancy, with previous miscarriage, working in farm and contact with pet animals. The frequency of specific IgM antibodies in all previous risks factor was lower (P<0.001) than IgG.

The highest frequency of IgG antibodies detected in older pregnant women (>40 years; 71.4%; P=0.053), followed by a younger woman. Whereas, pregnant woman aged lower than 20 years old had the lowest percentage (50%) of infection with *Toxoplasma gondii* IgG antibody Table 3.

The number of pregnant women in rural area was 49 women, only 4.1% were positive for anti-*Toxoplasma* IgM, whereas no specific IgM antibody in urban zones. In terms of IgG anti-*Toxoplasma gondii*, 42 serum samples had positive specific antibodies against *Toxoplasma gondii* IgG. The seroprevalence of specific *Toxoplasma gondii* IgG antibodies were 57.14% in rural area Table 3. Neither prevalence of risk factors, stage of pregnancy and contact with pet animals had a statistically differences for anti-*Toxoplasma* IgG antibody. However, the frequency of infection was higher in the third trimester of pregnancy (61.1%; P=0.061) compared to first trimester (42.0%) and patient contact with cats (61.1% *vs* 48.8%). Another related risk factor working in farm (65.7%) tends to be statistically significant (P=0.053; Table 3).

Table(2):-Characterization of the pregnant woman participated in study with the seroprevalence of *Toxoplasma gondii* infection related to the risk factors.

Risk Factors	Total number of 77 (%)	Positive No. (%)	Negative No. (%)	chi pr. P. Value
Age				
<20 years	2 (2.6)	1 (50.0)	1 (50.0)	0.648
20-29 years	34 (44.2)	17 (50.0)	17 (50.0)	
30-39 years	34 (44.2)	21 (61.8)	13 (38.2)	
>40 years	7 (9.1)	5 (71.4)	2 (28.6)	
Residency				
Rural	49 (63.6)	30 (61.2)	19 (38.8)	0.339
Urban	28 (36.4)	14 (50.0)	14 (50.0)	
Pregnancy Stages				

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Trimester I	26 (33.8)	11 (42.3)	15 (57.7)	0.159
Trimester II	33 (42.9)	22 (66.7)	11 (33.3)	
Trimester III	18 (23.4)	11 (61.1)	7 (38.9)	
Miscarriage				
Yes	47 (61.0)	33 (70.2)	14 (29.8)	**0.004**
No	30 (39.0)	11 (36.7)	19 (63.3)	
Contact with pet animals				
Yes	36 (46.8)	24 (66.7)	12 (33.3)	0.112
No	41 (53.3)	20 (48.8)	21 (51.2)	
Farm work				
Yes	35 (45.4)	25 (71.4)	10 (28.6)	**0.020**
No	42 (54.6)	19 (45.2)	23 (54.8)	
Total	77 9 (100)	44 (57.1)	33 (42.9)	

Table(3):- The prevalence and odd ratio of specific *Toxoplasma gondii* IgG antibody in pregnant woman distributed according the risk factors

Risk Factors		**Total**	**IgG**	**IgG %**	**p. value**	**Odd ration**
Age	<20	2	1	50	Reference	
	20-29	34	17	50	1.00	1.00
	30-39	34	19	55.9	0.56	1.118
	>40	7	5	71.4	0.053	1.428
Residency	Rural	49	28	57.1	Reference	
	Urban	28	14	50	0.493	0.8757
Pregnancy stages	Trimester I	26	11	42	Reference	
	Trimester II	33	20	60.6	0.068	1.443
	Trimester III	18	11	61.1	0.061	1.455
Miscarriage	Miscarriage	47	31	66	Reference	
	No Miscarriage	30	11	36.7	0.004	0.5561
Pet animals	Pet	36	22	61.11	Reference	
	No Pet	41	20	48.78	0.24	1.253
Farm worker	Farm work	35	23	65.71	Reference	
	No farm work	42	19	45.23	0.053	0.6883
Total		77	42	54.5		

4. DISCUSSION

Toxoplasma risk factors in some regions of northern Iraq are poorly described in pregnant woman, particularly in low income countryside areas. This study conducted to determine the risk factors of Toxoplasmosis in pregnant women in Akra region where most of the population considered to have a high correlation with the risk factors of toxoplasmosis. The current study showed 57.1 % seroprevalence of specific anti *Toxoplasma gondii* IgG and IgM antibodies among 77 pregnant women. Perhaps, the high seropositive infection of Toxoplasma in pregnant women in current study attributed to the low income and/or environmental factors such as moisture and temperature.

The study, supported by meta-analysis results showed that low or middle income countries were at higher risk of Toxoplasma infection (Rostami et al., 2020). Additionally, moisture and temperature played an essential role in sporulation and infectivity of Toxoplasma oocysts and effect significantly on the prevalence of infections (Gao et al., 2012; Mustafa et al., 2019).

The diagnoses of the recent and latent infection of toxoplasmosis are based on the detection of specific anti *Toxoplasma gondii* IgG and IgM antibodies. Since *Toxoplasma gondii* IgG antibody usually persist for a long period of life (Calderaro et al., 2009). The seroprevalence of anti-*Toxoplasma gondii* IgG antibody (54.5%) were higher than the seroprevalence of anti-*Toxoplasma gondii* IgM antibody (2.6%) in pregnant women. In Iraq study, seroprevalence of anti-*Toxoplasma gondii* IgG and IgM antibodies in Iraqian pregnant women were 36% and 5% respectively in Najaf city (Fadhil Al Kalaby et al., 2016).

The seroprevalence of toxoplasmosis was higher in the current study compared to that reported from other researchers shown in table 1.

Perhaps, the various prevalence of toxoplasmosis could be due to the variation of environment in different regions. Montoya and Liesenfeld, (2004) suggested that dry climate and high temperature reduce the infectivity of the *Toxoplasma gondii* oocysts. Low prevalence of infection from a hot region such as Southern Brazil (>1.0 %) were reported by (Mozzatto et al., 2003; Segundo et al., 2004).

The prevalence of seropositivity for toxoplasmosis was higher in older pregnant women than younger. Several studies reported higher prevalence of toxoplasmosis infection in older women, suggesting that older women are exposed more to the risk factors than younger women during their life (Al Se´adawy, 2010; Morris et al., 2004; Petersson et al., 2000).

There was no statistical difference in the prevalence of toxoplasmosis in people living in rural and urban (Baril et al., 1999). Possibly, non-significant variations of toxoplasmosis in current studies are that most population in this region had strong correlation with rural woman either by ingestion of contaminated food or contact with pet animals. It has been reported that Toxoplasma infection associated significantly with the consumption of raw vegetable outdoor and undercooked meat as well as having pet cat (Baril et al., 1999). However, Ades *et al*., (1993) reported a higher seroprevalence in urban areas.

The rate of seropositivity of *Toxoplasma gondii* among pregnant women contact with pet animals was higher than women that did not deal with pet animals. People had pet animals are at greater risk to toxoplasmosis, because infected pet animals (cats) can excrete oocysts during infection for two weeks and they become infected within 5 days. Additionally, it can survive for more than a year (Al-Hamdani et al., 1997; AL Omar et al., 1993). Pet animals (cats) defecation outdoors may pose the greatest risk for *Toxoplasma gondii* infection in pregnant women regardless whether they own cat or not. Furthermore, women working outdoors or in farms had higher ($P>0.05$) infection than indoor working. The prevalence of toxoplasmosis were higher in unemployed pregnant women (75%) compared with employed 25% women (Al Se´adawy, 2010). Nash *et al*., (2005) recommended pregnant women to avoid undercooked meat and carefully handling of raw meat. Poor hygiene and environmental contamination could responsible for increasing seroprevalence of toxoplasmosis.

The high seroprevalence of toxoplasmosis could be responsible for miscarriages in pregnant women. The prevalence of anti-*Toxoplasma gondii* IgG was found to be 66%. Similar results (67%) were reported by (El Deeb et al., 2012) and lower prevalence reported by (Tammam et al., 2013).

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The parasite *Toxoplasma gondii* leads to serious health complications such as abortions and still birth. Furthermore, Toxoplasma infections in pregnant women lead to congenital toxoplasmosis in children. Congenital toxoplasmosis affecting fetus could cause mild chorioretinitis to mental retardation, microcephaly, hydrocephalus and seizures (Gavinet et al., 1997; Peyron et al., 2019).

CONCLUSION

In conclusion, the seroprevalence of toxoplasmosis is high among pregnant women in this area. Perhaps pregnant woman did not know about toxoplasmosis. Women should be aware of toxoplasmosis to control or reduce risk factors attaining diseases through educational programs including handling contaminated food, contact with animals and working on farms. Government and/or population should educate about the source of infection, transmission and preventive of toxoplasmosis. The literacy level of pregnant women in society needs to be enhanced, particularly in rural areas. In addition, a large number of samples are recommended from different areas and culture to identify the specific risk factors and cyst viability.

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