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Research Article

Serological Study of IgG and IgM Antibodies to Cytomegalovirus and *Toxoplasma* Infections in Pregnant Women in Zakho City, Kurdistan Region, Iraq

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Abstract

Background: *Toxoplasma* gondii and Cytomegalovirus (CMV) can cause a range of diseases in pregnant women and may lead to adverse fetal outcomes. Therefore, the detection of these infections is necessary during pregnancy.
Objectives: The aim of this study was to estimate the seroprevalence of *Toxoplasma* and Cytomegalovirus infections among the pregnant women with history of abortion in Zakho city, Iraq.
Methods: Over a period of five years (2014-2018), blood samples were collected from 500 subjects aged 16-45 years old and tested to identify the presence of specific IgG and IgM to *Toxoplasma* and CMV infections by Enzyme Linked Fluorescent Assay (ELFA) method.
Results: Of 500 pregnant women, 145 (29%) and 7 (1.8%) were seropositive for anti-*Toxoplasma* IgG and IgM, respectively. IgG seropositivityto *T.* gondii infection varied significantly between age groups (P=0.05). Additionally, the seroprevalence of IgG and IgM antibodies for CMV was 475 (95%) and 9 (1.8%), respectively. Estimation of age specific subgroups showed high CMV IgG seropositivity rates for all age groups with no significant difference between them. Altogether, 145 cases were verified seropositive for specific IgG antibody against both pathogens and only 2 cases were positive for specific IgM against both agents.
Conclusion: Anti-*Toxoplasma* and CMV IgG and IgM antibodies positivity rates among pregnant women determined in the present study are quite similar as compared to other studies reported in Kurdistan Region, Iraq. Though, infection with those pathogens is not a major cause of abortion; it is useful to screen women with recurrent abortion for such infections in order to avoid undesirable fetal outcomes and other serious complications.

Keywords: Toxoplasma, Cytomegalovirus, Seroprevalence, Pregnant women, Abortion

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1. Introduction

Maternal infections acquired in utero or through the process of birth are recognized as the most common causes of fetal and neonatal death. Congenitally, acquired infections are caused by different pathogens such as *Toxoplasma* gondii (T. gondii) and Cytomegalovirus (CMV) infections, Rubella, and Herpes Simplex virus (1). Several studies have reported that these pathogens were commonly associated with multiple abortions, still birth, sterility, intrauterine fetal death, congenital malformations and other reproductive failures especially in developing countries (2-4). Hence, the screening of these infections is essential during pregnancy. Primary or recent infection is associated with higher morbidity rate than previous infection. Primary infection can be revealed by IgM antibodies as IgM antibodies indicate recent or recurrent infection.

Toxoplasmosis is a parasitic infection caused by an intracellular tissue protozoan *T.* gondii, which is transmitted by contaminated water, food, and undercooked meat (1). According to the geographical area, the seroprevalence rates of *T.* gondii in pregnant women ranged from 10% to 75% (1). Congenital toxoplasmosis is globally estimated to be 1.4-1.6 cases per 1000 live births (5). *T.* gondii is acquired during pregnancy and generally an asymptomatic and benign infection especially in immunocompetent people, but may be associated with deleterious consequences if affects the fetus in utero leading to mental retardation, stillbirth, congenital malformation, and blindness (6).

Globally, CMV infection is considered one of the common viral infections that predispose to congenital infection with an incidence rate of 0.3–2.4% of all live births. A study reported that in adult population, the serological prevalence rate of CMV ranged from 50%-90% in several developed and developing countries, respectively (7). During pregnancy, primary CMV infection carries the highest risk of intrauterine

transmission, which can seriously cause fetal damage, including microcephaly with intracerebral calcifications, hearing loss, anaemia, hepatosplenomegaly, jaundice, and growth retardation (8, 9).

Diagnosis of chronic or acute stages of infections in pregnant women is usually established by demonstration of specific IgG and IgM antibodies (10). Enzyme Linked Fluorescent Assay (ELFA) has been assessed by a recent study that shows *Toxopplasma* and *CMV* IgM and IgG with a sensitivity ranging from 90.91%-96.00% and specificity ranging from 89.47%-95.59% for the detection of specific IgM and IgG antibodies (11). Due to the lack of screening national program, limited data has been provided from the pregnant women in Zakho City, Kurdistan Region, Iraq. Therefore, this study aimed to study the seroprevalence rate of anti-Toxoplasma and anti- CMV IgG and IgM antibody in pregnant women in this region.

2. Methods

2.1 Study Design

This cross-sectional study was conducted on pregnant women with a history of spontaneous abortion, intrauterine fetal death, still birth, and growth retardation who referred to a private clinical health centre in Zakho city, Kurdistan Region, Iraq. Over a four-year period (2014 -2018), a total of 500 serum samples were collected from women aged 16-45 and tested to detect specific IgG and IgM antibodies against Cytomegalovirus and Toxoplasma infections in the studied area.

2.2 Ethics

This study was approved by local research ethics committee at College of Medicine, University of Zakho. Written informed consent was obtained from all the participants before sample collection

2.3 Enzyme Linked Fluorescent Assay (ELFA)

In total, 5 mL of blood was taken from the patients aseptically in vacutainer tube without anticoagulant and the serum was then separated by centrifuging blood samples for 5 minutes at 3000 rpm. The serum samples were then stored at -20°C until analysis. Specific IgG and IgM antibodies against the infections were tested using the technique ELFA (VIDAS System, BioMerieux SA, Marcy l'Etoile, France) following the manufacturer's instructions. All ELFA kits were obtained from BioMerieux SA (France). The test used for detection of specific IgG and IgM antibodies was based on two steps of enzyme immunoassay sandwich method with a final fluorescent detection ELFA. The fluorescent was measured at wavelength of 450 nm and the intensity of the fluorescent was proportional to the concentration of antibodies present in the sample. The results were calculated by the VIDAS instrument in relation to the calibration curve stored in memory. Anti-CMV IgG antibody titres ≥14 IU/mL and anti-CMV IgM antibody titres ≥22 IU/mL were considered positive. Anti-Toxoplasma IgG antibody titres ≥8 IU/mL and anti-Toxoplasma IgM antibody titres ≥8.8 IU/mL were considered positive.

2.4 Data analysis

Statistical analysis of the results was performed using GrapPad Prism software package, version 8. The significant difference in the seropositvity was determined by Chi-Square test and Fisher exact test. $P<0.05$ was considered statistically significant.

3. Results

Serological results of specific IgG and IgM antibodies against Cytomegalovirus and *Toxoplasma* infections among examined pregnant women are shown in Table 1. Out of 500 samples, 145 cases (29%) were positive for *anti-Toxoplasma* IgG, while only 7(1.4%) cases were positive for *anti-Toxoplasma* IgM, In terms of the CMV infections, 475 (95%) were seropositive for anti-CMV IgG and only 9(1.8%) were seropositive for anti-CMV IgM. Additionally, 145(29%) out of 500 cases were confirmed positive for specific IgG antibody against both infections and only 2 (0.4%) cases were positive for specific IgM antibody against both agents.

The seropositive results of *Toxoplasma* and CMV infections among various age groups in pregnant women are presented in Table 2. It was found that the highest IgG seropositivity against *Toxoplasm* was observed in women over age 30 (41.3%). While the prevalence in women under age 25 was 20.9%, it was 19.4% in women aged 26-29 years. Statistical analysis indicted that the result of *Toxoplasma* IgG was statistically significant ($P<0.001$) between the age groups. In terms of CMV, the highest IgG seropositivity was reported in age less than 25 (96.9%) and over age 30 (94.2%) with no significant difference between the age groups (Table 2). In addition, the highest anti-CMV IgM antibody was found in the ages between 26-29 years (3.9%) and the lowest was recorded in age more than 30 (1.0%).

Table 1: Serological Evidence of Specific IgG and IgM against *Toxoplasma* and Cytomegalovirus Infections in 500 Pregnant Women

Infection	Type of Antibody	No. of Positive cases (%)	No. of Negative cases (%)
Toxoplasma	IgG	145 (29)	358 (71.0)
	IgM	7 (1.4)	493 (98.6)
Cytomegalovirus	IgG	475 (95)	25 (5)
	IgM	9 (1.8)	491 (98.2)

Table 2: Seropositive Results of *Toxoplasma* and Cytomegalovirus Infections in Terms of Age among 500 Pregnant Women

Infections	Type of Antibody	Age Groups (Year)			*P value
		≤25	26-29	≥30	
		% (n=191)	% (n=103)	% (n=206)	
Toxoplasma	IgG	40 (20.9)	20 (19.4)	85 (41.3)	**0.001
	IgM	2 (1.05)	3 (2.9)	2 (0.97)	0.341
Cytomegalovirus	IgG	185 (96.9)	96 (93.2)	194 (94.2)	0.219
	IgM	3 (1.6)	4 (3.9)	2 (0.97)	0.184

*The data was analyzed using Chi-Square test; ***Toxoplasma* IgG was statistically significant (P<0.001)

4. Discussion

Toxoplasma and CMV are well-known to cause infection in utero predisposing to abortions, premature delivery, still birth and congenital malformation in women (2-4). In various geographical areas, there is a significant variation in the seroprevalence rate of these infections among the women of reproductive age; this may be due to diverse rates of immunization from the age group study to another and from one geographical area to another. The aim of this project was to evaluate the seroprevalence rate of acute and chronic against toxoplasmosis and CMV in pregnant women, in Zakho city, Kurdistan Region, Iraq.

It is estimated that 20-90 % of the world adult populations show the evidence of infection with *T.* gondii. In a study conducted in Northern part of Iraq, the overall seropositivity of anti-IgG and IgM for *T.* gondii was 41.3% and 1.1%, respectively (12). In another study conducted in Northern-Iraq, *T.* gondi IgM antibodies were detected in 2.82% of women with abortion (13). The same study demonstrated that *T.* gondii infection is not the main cause of abortion in women in the studied area. In addition, Rasti and co-workers found that 27.2% and 1.2 % of women at childbearing age exhibit IgG and IgM antibodies to *T.* gondii infection in Iran (14).

Overall, the current data demonstrate that 29% and 1.4 % of pregnant women with history of spontaneous abortions and stillbirth were seropositive for *T.* gondii IgG and IgM antibodies, respectively. These results were very similar to the prevalence rate of pregnant women reported in the previous studies conducted in Kurdistan Region, Iraq (12, 13). The current results were also consistent to the study performed in Turkey, which reported that the women at the childbearing age were tested positive for anti-toxoplasma IgM (1.34%) and IgG (24.61%). Furthermore, Vilibic-Cavlek and other colleagues observed that *T.* gondii IgG and IgM were detected in 29.1% and 2.4 % in women at the childbearing age in Croatia, respectively (15). Therefore, the prevalence rate of acute toxoplasmosis (IgM) was very low compared with chronic infection in pregnant women in the region of study. Further studies are required to define the role of *T.* gondi infection in pregnant women during acute stage of infections and this will be an important component of epidemiological study in the region.

Regarding the age group, the seroprevalence of *T.* gondii varied significantly between the age groups. High seropositivity was observed in women over age 30. It seems that women over age 30 are at high risk of acquiring *T.* gondii infection. This result is similar to finding of study performed in Iran (14). They found that the highest seropositive rate of *T.* gondii infection was observed in the age group more than 30 years (92.6%) (14). Another study has also emphasized that several factors including age, geographic climatic change, nutritional status, and sociocultural habits can significantly influence the seropositivity of toxoplasmosis (16).

Human CMV infection is a very common with an overall seroprevalence of 50% among adults (17); however, in some population, the prevalence rates

are even higher especially in low and middle-income countries (7). Congenital infection is one of the most imperative consequences of primary CMV infection during pregnancy. Several studies conducted in different regions of Iran, the seroprevalence rate of CMV IgG and IgM was in a range of 23-100% and 2.5-8% among pregnant women (14, 18). In the present study, the seroprevalence rate of CMV IgG and IgM antibodies among pregnant women was 475 (95%) and 9 (1.8%), respectively. It is clear; therefore, that the seropositivity of chronic infection (IgG) was high compared with recent infection (IgM) among the pregnant women. Consistent with the current results, Hussein and Balatay found that 29 out of 1275 (2.27%) of the tested samples were seropositive for anti-CMV IgM antibodies (13). It is well known that primary CMV infection are the most commonly transmitted to the fetus and are more possibly to cause serious fetal damage than recurrent infections (19). This is in agreement with the results of Josheghani and other colleagues, they also observed higher seropositive rate of the CMV specific IgG (98.8%) and lower seropositive rate of the CMV specific IgM (5%) (20). A study carried out in Iran on the prevalence rate of CMV infection in pregnant women suggested a seroprevalence rate of 91.94% and 33.8% for CMV-Specific IgG and IgM, respectively (21). Another study found that out of 92 pregnant women in Northern-Iraq, the seropositivity for anti IgG and IgM CMV were 85.9% and 5.4%, respectively (12). The factors responsible for the transmission of congenital CMV infection to the fetus and the severity of the condition require further studies. Taken all together, the prevalence rate of IgM and IgG CMV infection observed in this study was quite similar to that reported in other developing societies but higher than in the developed societies. This could be attributed to the inclusion of CMV screening program among the pregnancy screening profile and better hygienic standards (3).

In this study, we also found higher seropositivity for CMV IgG in ages less than 25 years (96.9%) and for CMV IgM in ages more than 30 years (94.2%), but no significant difference between the age groups. This result is consistent with the finding of Acharya and co-workers, they also confirmed that there was no significant difference in seropositivity rates of IgG and IgM in relation to age and type of abortion among the study groups (21). No significant difference was found between the age groups and that might be explained by the ubiquity of the infection in our samples. Many studies have reported that co-infections have significantly affect pregnancy outcomes than single infections (22, 23). Consistent with the mentioned hypothesis, the result of this study found that 145 out of 500 cases were positive for specific IgG antibody against both *T.* gondii and CMV infections, but only 2 cases were positive for specific IgM antibody against both infections.

5. Conclusions

In conclusion, both *Toxoplasma* and CMV IgM seropositivity was low in the region. Though, infection with those pathogens is not a major cause of abortion; it is useful to screen women with recurrent abortion for such infections in order to avoid undesirable fetal outcomes and other serious complications.

Authors' Contribution

Conception of study idea: Ibrahim A. Naqid and Shivan H.Yousif; Data collection and ELFA analysis: Shivan H.Yousif; Interpretation of results: Ibrahim A. Naqid and Nawfal R. Hussein; Writing of the main manuscript text: Ibrahim A. Naqid; Review of the manuscript: all the authors.

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Conflict of Interest: None declared.

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