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Seroprevalence of *Toxoplasma gondii,* rubella virus, and cytomegalovirus among pregnant women with previous pregnancy loss

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ABSTRACT

Background. Infectious agents, especially TORCH infections, may contribute to unfavorable pregnancy outcomes, including pregnancy loss, a complex reproductive health issue. There is currently limited information available on the seroprevalence of these infections among pregnant women in southern Iraq.

Objective. To determine the seroprevalence of *Toxoplasma gondii,* rubella virus, and cytomegalovirus (CMV) among pregnant women in Basrah, Iraq, who had experienced a previous miscarriage, and to assess their correlations with clinical factors.

Methods. Between October 2024 and October 2025, 156 pregnant women with a history of spontaneous miscarriage and 100 pregnant women without such a history participated in a cross-sectional analytical study. The enzyme-linked fluorescent assay (ELFA) on the VIDAS automated platform was used for serological testing for IgG and IgM antibodies against *T. gondii,* rubella virus, and CMV (bioMérieux, France). The chi-square test, Fisher's exact test, Spearman correlation, and Cramér's V statistics were used to assess associations.

Results. Among women who had previously experienced pregnancy loss, IgG seropositivity for *T. gondii,* rubella, and CMV was 79.5%, 84.6%, and 74.4%, respectively. No *T. gondii* IgM positivity was detected; 5.1% of women had CMV IgM positivity, and 1.3% had rubella IgM positivity. Moderate positive correlations were observed between age and IgG seropositivity for CMV ($\rho = 0.38$, $p = 0.003$) and T. gondii ($\rho = 0.42$, $p = 0.001$).

Conclusion. In Basrah, pregnant women who had previously experienced pregnancy loss had high IgG seroprevalence rates for *T. gondii,* rubella, and CMV. Further prospective studies using confirmatory diagnostic techniques are needed in light of the observed association between CMV IgM seropositivity and previous pregnancy loss.

Keywords: *Toxoplasma gondii,* rubella virus, cytomegalovirus, pregnancy loss, seroprevalence

INTRODUCTION

Pregnancy loss, defined as the loss of a pregnancy before fetal viability, is a common and complex reproductive health problem with multiple potential etiologies. Recurrent pregnancy loss, generally defined as the loss of two or more pregnancies, affects approximately 1-5% of women of reproductive

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age worldwide [1,2]. Despite advances in reproductive medicine, many cases remain unexplained, highlighting the importance of considering less evident etiological factors, including infectious agents [3,4].

Among TORCH pathogens, *Toxoplasma gondii,* rubella virus, and cytomegalovirus (CMV) are particularly important because of their ability to affect fetal development [5,6]. These pathogens are associated with congenital abnormalities, intrauterine fetal death, and miscarriage, particularly when primary infection occurs during pregnancy [7,8].

Toxoplasma gondii is a globally distributed intracellular protozoan parasite. Undercooked meat, contaminated food or water, and contact with soil contaminated with oocysts are important sources of human infection [9]. Climate and socioeconomic conditions contribute to substantial variation in global seroprevalence, ranging from less than 10% in some high-income countries to over 80% in some low-resource settings [10,11]. Although persistent infection is usually asymptomatic, primary infection during pregnancy poses a significant risk of vertical transmission and adverse fetal outcomes [12].

Rubella virus infection remains an important concern in areas with low immunization coverage. Maternal infection during early pregnancy is associated with congenital rubella syndrome (CRS), which may include severe fetal abnormalities such as cardiac defects, cataracts, and hearing impairment [13,14]. Vaccination programs have reduced its incidence worldwide, but many populations, particularly in developing countries, remain susceptible [15].

Cytomegalovirus (CMV) is the most common congenital viral infection worldwide and is a major cause of neurological impairment in children [16,17]. CMV infection during pregnancy may occur as a primary infection, reactivation, or reinfection. The risk to the fetus is greatest following primary maternal infection [18]. Due to widespread exposure, CMV seroprevalence is generally high in developing countries, frequently exceeding 70% [19].

Recent epidemiological research has highlighted the importance of examining associations between serological markers and clinical characteristics, such as age and a history of previous spontaneous miscarriage, in addition to determining seroprevalence [20]. While IgM seropositivity may suggest recent infection, IgG seropositivity generally reflects previous exposure and has been reported to increase with age [21,22].

Data on TORCH infections among women with a history of spontaneous miscarriage in Iraq are limited and inconsistent, particularly in Basrah [23,24]. Furthermore, few studies have investigated these associations using complementary statistical approaches to explore the relationships between serological markers and clinical characteristics.

Thus, the aim of this study was to determine the seroprevalence of *Toxoplasma gondii,* rubella virus, and cytomegalovirus among pregnant women with previous pregnancy loss in Basrah, Iraq, and to evaluate their associations with selected clinical characteristics, including age and history of previous pregnancy loss, using appropriate statistical methods.

MATERIALS AND METHODS

Study design

This cross-sectional analytical study was conducted at Al-Shifaa Investment Hospital in Basrah, Iraq, from October 2024 to October 2025. The goals of the study were to determine the seroprevalence of *Toxoplasma gondii,* rubella virus, and cytomegalovirus (CMV) in pregnant women with a history of previous pregnancy loss and to evaluate their associations with selected clinical characteristics.

Sample collection

The study included a total of 256 pregnant women. The case group comprised 156 pregnant women who had experienced one or more previous spontaneous miscarriages, whereas the control group comprised 100 pregnant women with no history of spontaneous miscarriage. No specific gestational-age threshold for pregnancy loss was prespecified or consistently documented in the study records. Therefore, eligibility was based on the recorded history of spontaneous miscarriage, without retrospectively applying a gestational-age cutoff.

Controls were selected from the same hospital during the same study period as the case group. Frequency matching was used to obtain a comparable distribution of participants across the predefined age groups (15-19, 20-29, 30-39, and 40-50 years). Matching was performed at the group level rather than through individual case-control pairs. Data regarding socioeconomic status, gestational trimester, parity, and rubella vaccination history were not systematically collected and therefore were not included in the analysis.

Pregnancy was confirmed for each participant using a blood pregnancy test performed in the hospital laboratory at the time of enrollment.

Inclusion and exclusion criteria

Pregnant women who had experienced at least one previous spontaneous miscarriage, attended Al-Shifaa Investment Hospital during the study period, and consented to participate in the study met the inclusion criteria for the case group. Pregnant

women who attended the same hospital during the same study period and had no history of previous spontaneous miscarriage comprised the control group.

Women who refused to participate or had insufficient laboratory or clinical data were excluded from the study.

Data collection

Clinical and demographic data were collected using a standardized questionnaire. Data included age, pregnancy status, and the number of previous spontaneous miscarriages. Participants were classified into four age groups (15-19, 20-29, 30-39, and 40-50 years). The number of previous spontaneous miscarriages was recorded as an ordinal variable ranging from one to five.

Sample processing and laboratory analysis

A 5 mL venous blood sample was aseptically collected from each participant. Blood samples were allowed to clot and were then centrifuged to obtain serum for serological testing.

Serological testing for anti-*Toxoplasma gondii* IgG/IgM, anti-rubella virus IgG/IgM, and anti-cytomegalovirus (CMV) IgG/IgM antibodies was performed using the enzyme-linked fluorescent assay (ELFA) technique on the VIDAS automated system (bioMérieux, Marcy-l'Étoile, France). The VIDAS® ToRC Solution was used, including VIDAS TOXO IgG II, VIDAS TOXO IgM, VIDAS RUB IgG II, VIDAS RUB IgM, VIDAS CMV IgG, and VIDAS CMV IgM assays. All tests were performed and interpreted according to the manufacturer's instructions.

For *T. gondii*, IgG concentrations <4 IU/mL were classified as negative and ≥8 IU/mL as positive, whereas IgM index values <0.55 and ≥0.65 were considered negative and positive, respectively. For rubella virus, IgG values <10 IU/mL were considered negative and values ≥10 IU/mL were considered positive, while rubella IgM index values <0.8 and ≥1.2 were interpreted as negative and positive, respectively. For CMV IgG and CMV IgM, values <0.5 IU/mL were considered non-reactive, values between 0.5 and 1.0 IU/mL were considered indeterminate, and values >1.0 IU/mL were considered reactive. No equivocal or indeterminate results were observed during the study period.

Interpretation of results

The manufacturer's recommendations were followed when interpreting the serological data. Using predetermined cutoff values, the results were classified as positive or negative for each marker. Variable coding was standardized prior to statistical analysis to ensure consistency and accuracy.

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics version 23.0. Categorical variables were summarized as frequencies and percentages.

Associations between TORCH seropositivity and history of spontaneous miscarriage were evaluated using the chi-square test or Fisher's exact test, as appropriate. Spearman correlation analysis was used to evaluate the relationship between serological markers and ordinal variables such as age and number of previous spontaneous miscarriages. The strength of associations between categorical variables was assessed using Cramér's V statistic. A p-value of less than 0.05 was considered statistically significant.

RESULTS

This study included 100 control pregnant women with no history of spontaneous miscarriage and 156 pregnant women with a history of previous spontaneous miscarriage. At the time of enrollment, pregnancy was confirmed in all participants using blood pregnancy testing.

The case group showed a high prevalence of IgG antibodies against the investigated TORCH pathogens. *Toxoplasma gondii* IgG seroprevalence was 79.5%, suggesting extensive previous exposure. No *T. gondii* IgM-positive cases were detected, suggesting the absence of serological evidence of recent infection at the time of testing.

TABLE 1. Seroprevalence of *Toxoplasma gondii,* cytomegalovirus, and rubella in the case group

Infection	IgG positive, n (%)	IgM positive, n (%)	Controls IgM positive, n (%)
Toxoplasma gondii	124 (79.5%)	0 (0%)	0 (0%)
Rubella	132 (84.6%)	2 (1.3%)	0 (0%)
Cytomegalovirus	116 (74.4%)	8 (5.1%)	0 (0%)

Rubella IgG antibodies were detected in 84.6% of the patients, indicating a high level of immunity that may have resulted from previous vaccination or natural infection. Rubella IgM positivity was uncommon, being detected in two patients (1.3%).

CMV IgG antibodies were detected in 74.4% of women in the case group, indicating a high frequency of previous exposure. CMV IgM seropositivity was observed in 8 women (5.1%) with a history of previous pregnancy loss and in none of the controls. Because of the zero cell count in the control group, Fisher's exact test was used and showed a statistically significant association ($p = 0.024$) (Figure 1).

Toxoplasma gondii IgG and CMV IgG seroprevalence differed statistically between cases and controls ($p < 0.001$), with higher seroprevalence in the

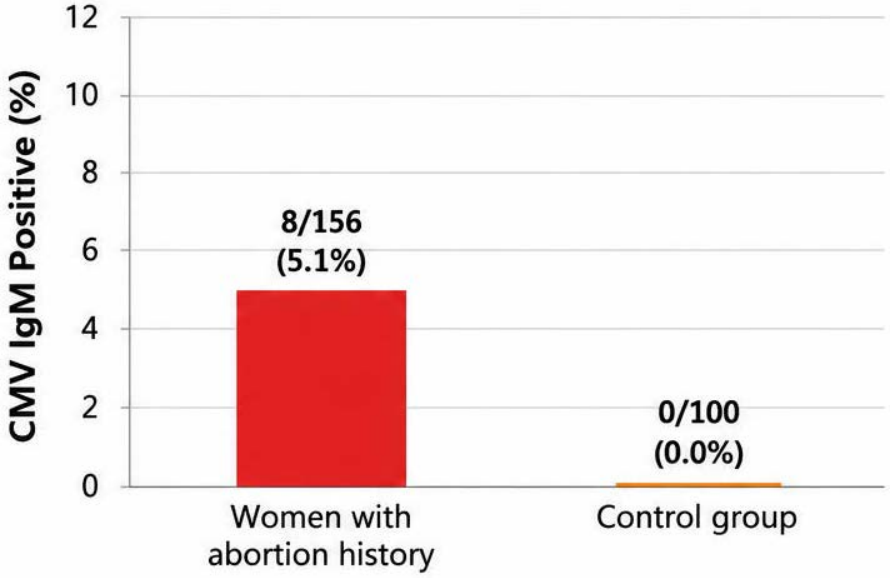


FIGURE 1. Relationship between history of previous spontaneous miscarriage and CMV IgM positivity

case group. Rubella IgG seroprevalence also differed significantly between the two groups ($p < 0.001$) (Table 2).

TABLE 2. Comparing of seropositivity between cases and controls for *Toxoplasma gondii,* cytomegalovirus, and rubella

Infection	Cases positive, (%)	Controls positive, (%)	p-value
Toxo IgG	79.5	13.0	<0.001
Rubella IgG	84.6	100	< 0.001
CMV IgG	74.4	0	<0.001

Spearman correlation analysis showed statistically significant positive correlations between age and IgG seropositivity. Moderate positive correlations were observed between age and CMV IgG seropositivity ($\rho = 0.38$, $p = 0.003$) and between age and *T. gondii* IgG seropositivity ($\rho = 0.42$, $p = 0.001$), suggesting greater cumulative exposure with increasing age (Figure 2).

Furthermore, a weak but statistically significant positive correlation ($\rho = 0.29$, $p = 0.02$) was observed between the number of previous spontaneous miscarriages and CMV IgM positivity, suggesting a possible association that warrants further investigation. The number of previous spontaneous miscarriages was not significantly correlated with *T. gondii* IgG seropositivity ($p > 0.05$) (Table 3).

The association between CMV IgM seropositivity and history of previous spontaneous miscarriage showed moderate strength according to Cramér's V

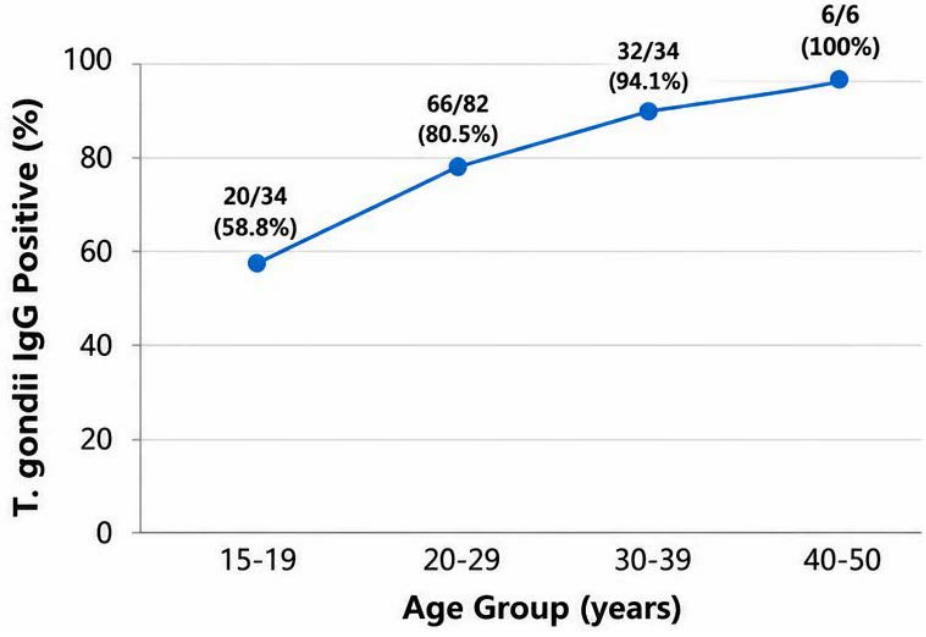


FIGURE 2. Trends in *Toxoplasma gondii* IgG seropositivity across age groups

(V = 0.31). Age and IgG seropositivity for CMV and *T. gondii* also showed moderate associations (Table 4).

TABLE 3. Spearman correlation analysis between TORCH serological markers and clinical factors

Variables	Correlation coefficient (ρ)	p-value	Interpretation
Age vs Toxo IgG	0.42	0.001	Moderate positive
Age vs CMV IgG	0.38	0.003	Moderate positive
Previous miscarriage vs CMV IgM	0.29	0.02	Weak positive
Previous miscarriage vs Toxo IgG	0.21	0.08	Not significant

TABLE 4. Strength of associations according to Cramér's V

Association	Cramér's V	Strength
CMV IgM vs previous miscarriage	0.31	Moderate
Age vs Toxo IgG	0.36	Moderate
Age vs CMV IgG	0.33	Moderate

DISCUSSION

By demonstrating a high seroprevalence of IgG antibodies against *Toxoplasma gondii,* rubella virus, and CMV, the current study provides epidemiological insight into TORCH infections among women in Basrah. These findings are consistent with studies conducted in developing countries, where high seroprevalence reflects substantial previous exposure [20,22].

The 79.5% T. *gondii* IgG seroprevalence is comparable to values reported in Iraq and neighboring countries, which range from 60% to 85% [23,24]. This high prevalence may be related to dietary habits, exposure to contaminated soil and water, and climatic conditions [9]. Previous studies have shown that the absence of IgM positivity suggests that most infections represent previous rather than recent exposure [25].

The 84.6% rubella IgG seropositivity observed in the study population indicates a high level of immunity. Similar findings have been reported in Middle Eastern countries, reflecting the effects of both vaccination programs and natural exposure [26,27]. The low frequency of rubella IgM positivity suggests that recent infection was uncommon in the study population.

The 74.4% CMV IgG seroprevalence is consistent with international studies reporting high levels of seropositivity in developing countries [19]. Fisher's exact test showed a significant association between CMV IgM seropositivity and history of previous pregnancy loss (p = 0.024). However, CMV IgM positivity may indicate primary infection, viral reactivation, reinfection, or persistent antibody responses rather than acute infection alone. Therefore, this finding should be interpreted with caution. Furthermore, causal inference is not possible because of the cross-sectional design and the relatively small number of positive cases. These findings should therefore be validated in larger prospective studies.

Previous studies have associated maternal CMV infection with adverse pregnancy outcomes, including congenital infection and miscarriage. However, additional diagnostic methods are generally required to confirm recent or active infection, including CMV IgG avidity testing, molecular assays, or serial serological testing. Therefore, the association observed in this study should be considered hypothesis-generating and warrants further investigation in larger prospective studies [18,28].

The significant positive correlations between age and IgG seropositivity support the concept of cumulative exposure over time [21]. This pattern has been widely observed in epidemiological studies and is consistent with increasing cumulative exposure throughout life.

Limitations

This study has several limitations. First, the relatively small sample size and single-center design may limit the generalizability of the findings.

Second, the cross-sectional design does not allow causal relationships to be established between TORCH seropositivity and history of spontaneous miscarriage. Therefore, the observed associations should be interpreted with caution.

Third, ELFA was used for serological testing without molecular confirmation methods such as polymerase chain reaction (PCR) or CMV IgG avidity testing. Consequently, differentiation between primary infection, reinfection, reactivation, and persistent IgM positivity was not possible.

In addition, no specific gestational-age threshold for previous spontaneous miscarriage was prespecified or consistently documented, which may have introduced heterogeneity in the definition of pregnancy loss.

Finally, several potential confounding factors were not investigated, including socioeconomic status, nutritional status, environmental exposure, and non-infectious causes of pregnancy loss, and these factors may have influenced the observed associations.

CONCLUSION

This study found a high seroprevalence of IgG antibodies against Toxoplasma gondii, rubella virus, and cytomegalovirus among pregnant women with a history of previous pregnancy loss in Basrah,

Iraq, indicating substantial previous exposure to these pathogens.

Compared with controls, women with a history of previous spontaneous miscarriage had a higher frequency of CMV IgM seropositivity. However, the cross-sectional design and the limited number of positive results prevent causal conclusions. Larger prospective studies are needed to determine whether this association is reproducible.

The observed increase in IgG seropositivity with age supports the concept of cumulative exposure over time. Further multicenter prospective studies incorporating molecular diagnostic approaches are needed to better understand the role of TORCH infections in adverse pregnancy outcomes.

Recommendations

1. Targeted evaluation for TORCH infections may be considered in pregnant women with a history of pregnancy loss when clinically indicated, together with appropriate confirmatory testing.
2. Larger multicenter studies with prospective designs are recommended to further investigate the relationship between TORCH infections and adverse pregnancy outcomes.
3. To differentiate between recent infection, reinfection, reactivation, and previous exposure, future research should incorporate molecular diagnostic methods such as PCR and IgG avidity testing.
4. Public health initiatives aimed at improving awareness of TORCH infections and preventive measures may help reduce infection-related pregnancy complications.

Conflict of interests

The authors declare no competing interests related to the publication of this manuscript.

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Ethical approval

The study protocol was reviewed and approved by the Ethics Committee of Al-Zahraa College of Medicine, University of Basrah, Iraq (Approval Code: E/T65; Approval Date: 29 April 2024). Verbal informed consent was obtained from all participants before enrollment. Participant confidentiality and anonymity were maintained throughout the study.

Authors' contribution

All authors contributed significantly to the conception, design, and development of this study. Wameedh Hashim Abbas Alqatrani led the study design and coordinated the research activities. Shant Antranik Sinbat, Taif Ibrahim Qasim, and Amna Shaker Mahdi were responsible for data collection and performed the initial analysis. Zainab Khalid Khaleel and Abeer Laily Mohammed contributed to the interpretation of the results and drafted the initial version of the manuscript.

All authors participated in the critical revision of the manuscript, approved the final version for publication, and agreed to be accountable for all aspects of the work.

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