Seroprevalence of Toxoplasma gondii, Rubella and Cytomegalovirus Among Women of Reproductive Age in Mashhad, Northeast of Iran

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Abstract

**Purpose:** TORCH syndrome is responsible for 2-3% of all congenital anomalies caused by *Toxoplasma gondii* (T. gondii), *Rubella virus* (RV), *Cytomegalovirus* (CMV), and *Herpes simplex virus* (HSV). The current study aimed to determine the prevalence of TORCH infection in women in Mashhad.

**Method:** This cross-sectional study was conducted on 417 patients who were referred to three laboratories in Mashhad, Iran. Laboratory data were collected from April 2016 to March 2020 to detect the specific IgG and IgM against TORCH syndrome.

**Result:** The specific IgG antibodies were found to be positive in for CMV in 402 cases (96.4%), for RV in 394 cases (94.5%), and for T. gondii in 80 cases (19.2%). Moreover, 7 (1.6%) of them were found to be positive for anti-CMV IgM, 6 (1.4%) for anti-IgM RV, and 8 (1.9%) for anti-IgM T. gondii. In addition, the relationship between age and anti- T. gondii IgG in the age group of 37-47 years was 6.44 times higher than the age group of 17-27 years. Also, The relationship between age and anti-CMV IgG in the age group of 27-37 years was 4.13 times higher than the age group of 37-47 years.

**Conclusion:** All women of reproductive age should be screened for the TORCH complex regularly to prevent congenital TORCH syndrome.

1. **Introduction**

Congenital defects are a worldwide concern. It is also the primary cause of mortality in infants of both developing and developed countries. A variety of abnormalities are due to genetic inheritance and environmental factors. Infection during pregnancy plays a significant role in the development of major birth defects. One of these infections is TORCH that will not harm the fetus if detected and treated early on[1-3]. The term TORCH refers to infectious agents that can be transmitted to a child by vertical infection, either intrauterine or post-natal. Primary infections associated with some TORCH pathogens during pregnancy, especially in the first trimester, are associated with an increased risk of miscarriage, stillbirth, sterilization, preterm delivery, congenital anomalies, and transient or chronic fatal disease. The risk of this is dependent on gestational age and pathogens[4]. Primary infections are more harmful than secondary infections during pregnancy[5]. TORCH syndrome can lead to spontaneous abortion or severe birth defects in the fetus[2, 3]. This infection is responsible for 2-3% of all congenital anomalies. Mental retardation is one of the major complications that will impose a significant economic burden on society and the family[6, 7].

This infection is associated with several factors and occurs at various stages of pregnancy. *Toxoplasma gondii, Rubella virus* (RV), *Cytomegalovirus* (CMV), and *Herpes simplex virus* (HSV) types 1 and 2 are known as TORCH syndrome that leads to neonatal mortality worldwide [8-10]. The majority of these pathogens do not affect the mother but have disastrous consequences for the fetus[10, 11]. Therefore, it will be very difficult to diagnose this syndrome in pregnant mothers. Measuring IgM antibodies against TORCH is a way to detect an early infection[12].
If a growing fetus becomes infected with a TORCH agent, it may cause a miscarriage, stillbirth, fetal growth retardation (intrauterine growth retardation), or congenital malformation. Lethargy, fever, nutritional problems, hepatosplenomegaly, and Low hemoglobin levels are symptoms and clinical findings in newborns infected with any of the TORCH agents. Moreover, petechiae or purpura may develop on the skin in infected infants. Yellow skin, whites of the eyes hemolytic anemia, thrombocytopenia, jaundice, and chorioretinitis can be observed in infected infants. Other abnormalities may occur, depending on the fetal development stage at the time of infection and the severity of the infection[13, 14].

According to different results obtained from screening experiments, the prevalence of TORCH factors are dependent on geographical area, economic and cultural [15, 16].

Toxoplasma gondii (T. gondii) is a parasitic infection and zoonotic disease that cause TORCH syndrome. TORCH syndrome symptoms range from asymptomatic findings to chorioretinitis [17], hearing impairment, hydrocephalus, and fetal psychomotor disorders[18]. Toxoplasma infection is prevalent in China due to its special diet [19]. Because of the adverse effects of this infection on the fetus, it is critical to diagnose infected women before pregnancy to prevent fetal disorders.

RV is a viral disease with mild or asymptomatic and even catastrophic consequences in the fetus [14]. RV can transmit through respiration and mother-fetal vertical transmission [20]. This is one of the most important causes of TORCH syndrome. In the first 12 weeks, the fetal infection rate exceeds 80%. To be reduced to 25% by the end of the second quarter. At 27 to 30 weeks, it may be 35%[14].

CMV is one of the major causes of congenital anomalies in newborns and is associated with severe fetal anomalies such as chorioretinitis, sensorineural deafness, and cerebral palsy[21, 22].

The prevalence of this syndrome varies based on the geographical area. But Southeast Asia and sub-Saharan Africa have the highest mortality rates from this infections [23]. A general national study that involves TORCH effects on pregnant women and whether they are associated with adverse pregnancy outcomes is not available[19]. Since our country is also in a high-risk region, research in this field seems necessary. The present study was performed to determine the prevalence of TORCH infection in Mashhadian women of reproductive age.

2. Materials And Methods

2.1. Study Population

This study evaluated the serological records of the 417 women of reproductive age who admitted to three laboratories in Mashhad from April 2016 to March 2020. The subjects were women of reproductive age with a mean age of 30.8 ± 5.77 years, a minimum of 17 years, and a maximum of 47 years. Informed consent was obtained from the subjects and the Ethics Committee of Gonabad University of Medical
Sciences approved the study protocol. Moreover, all the personal information such as names and addresses of the patients were kept confidential, and samples were numbered based on the given codes.

2.2. Determination of CMV Infection

IgG and IgM antibodies against T. gondii, CMV, HSV and RV were assayed by (the enzyme-linked immunosorbent assay (ELISA) kit (Pishtazteb, Tehran, Iran).

2.3. Data Analysis

Statistical data were analyzed by SPSS software version 23.0 and the Chi-square test. A 95% confidence interval (CI) was estimated for each of the TORCH agents in the positive patients.

3. Results

Figure 1 shows the serological prevalence of various factors of TORCH syndrome in women of reproductive age in three private laboratories in Mashhad. As shown in Fig 1, CMV is the most common infection (96.4%) and followed by rubella and Toxoplasma infections with 94.5% and 19.2% respectively. Table 1 shows the serum prevalence of TORCH IgG and IgM in women of reproductive age.

|            | CMV IgG | CMV IgM | Rubella IgG | Rubella IgM | TOXO IgG | TOXO IgM |
|------------|---------|---------|-------------|-------------|----------|----------|
| Positive (n) | 402     | 7       | 394         | 6           | 80       | 8        |
| Negative (n) | 15      | 410     | 23          | 411         | 337      | 410      |
| Total:      | 417     | 417     | 417         | 417         | 417      | 417      |
| Positive (%)| 96.4    | 1.6     | 94.5        | 1.4         | 19.2     | 1.9      |
| Negative (%)| 3.6     | 98.4    | 5.5         | 98.6        | 80.8     | 98.1     |

*CMV: Cytomegalovirus; TOXO: Toxoplasma

In our study, 402 cases (96.4%), 394 cases (94.5%), and 80 cases (19.2%) have IgG antibodies against CMV, rubella, T. gondii, respectively.

IgM was positive for CMV 7 (1.6%), for rubella 6 (1.4%), and T. gondii 8 (1.9%)

In this study, the age of women was between 17 to 47 years with a mean age of 30.8 ± 5.77 years.

Data were evaluated for IgM and IgG antibodies by chi-square test. Figure 2.1-2.6 demonstrate the frequency of positive and negative cases of serological tests of TORCH syndrome in three age groups.
The frequency of positive serological tests in TORCH syndrome with Toxoplasma factor and cytomegalovirus increases with age. TORCH syndrome with T. gondii agent and CMV increased 1.8 and 4.13 times with age, respectively. However, the results were not significant for RV.

According to the presented results, the relationship between age and anti-T. gondii IgG was significant (P-value = 0.00, P<0.05), as it was 6.44 times higher in the age group of 37-47 years compared with the age group of 17-27 years (Table 2).

Table 2

**TOXO IgG seroprevalence in various age groups and the result of Chi-square test**

| TOXO IgG | Positive | Negative | Total | Chi-square test |
|----------|----------|----------|-------|-----------------|
|          | Number (%) | Number (%) | Number (%) | |
| 17-27 years | 15 (18.8%) | 123 (36.5%) | 138 (33.1%) | \( \chi^2=26.3 \) |
| 27-37 years | 43 (53.8%) | 186 (55.2%) | 229 (54.9%) | df=2 |
| 37-47 years | 22 (27.5%) | 28 (8.3%) | 50 (12.0%) | p=0.00 |

Also, the relationship between age and CMV IgG was significant (P-value = 0.03, P<0.05), as CMV IgG in the age group of 27-37 years is 4.13 times higher than the age group of 37-47 years (Table 3). These results were not obtained for RV antibodies. These results were also evaluated by the Benjamini-Hochberg test, which was consistent with the results obtained from the Chi-square test. Finally, the rate of T. gondii and CMV infections increase with age, that the both of them are important factors for TORCH syndrome.

Table 3

**CMV IgG seroprevalence in various age groups and the result of Chi-square test**

| CMV IgG | Positive | Negative | Total | Chi-square test |
|---------|----------|----------|-------|-----------------|
|          | Number (%) | Number (%) | Number (%) | |
| 17-27 years | 134 (33.3%) | 4 (26.7%) | 138 (33.1%) | \( \chi^2=26.3 \) |
| 27-37 years | 223 (55.5%) | 6 (40.0%) | 229 (54.9%) | df=2 |
| 37-47 years | 45 (11.2%) | 5 (33.5%) | 50 (12.0%) | p=0.03 |

Discussion
Although TORCH syndrome can cause a mild illness in women, intrauterine infections during pregnancy can lead to serious complications in the fetus. Therefore during pregnancy and at childbearing age, serological tests (titers of both IgM and IgG antibodies) are recommended to screen and prevent congenital malformations[24].

There is little data on the prevalence of TORCH infections among pregnant women in different geographic areas. Because the primary infection with TORCH syndrome is asymptomatic, therefore it is vital to identify the suspected women and prevent congenital problems [21, 22, 25]. This study aimed to investigate the prevalence of TORCH syndrome in women of reproductive age in Mashhad and discuss whether the TORCH is related to age or not.

As shown in figure 2, these graphs have not changed significantly with age. Therefore, with age, no significant changes are seen in the prevalence of TORCH syndrome (with three factors: T. gondii, CMV, and RV).

In our study, the specific IgG antibodies were found to be positive in for CMV in 402 cases (96.4%), for RV in 394 cases (94.5%), and for T. gondii in 80 cases (19.2%). Moreover, 7 (1.6%) of them were found to be positive for anti-CMV IgM, 6 (1.4%) for anti-IgM RV, and 8 (1.9%) for anti-IgM T. gondii. Furthermore, all the patients (100%) were proven negative for anti-IgM and IgG HSV. The seropositive rates of IgMs and IgGs antibody in the TORCH syndrome are shown in Table1.

The risk of transmission with T. gondii in the first, second, and third Trimester of Pregnancy is 15%, 30%, and 60%, respectively. However, the disease severity seems higher is higher in early pregnancy [26].

The seroprevalence of T. gondii is prevalent in tropical countries and in areas where raw or semi-raw meat is consumed [6, 27, 28]. For example, in India, the prevalence of anti- T. gondii IgG in women of reproductive age has been reported between 25 to 28% [29, 30].

In our study, 1.9 % of cases were positive for the IgM T. gondii, and 19.2% for IgG T. gondii.

In a study, the prevalence of antibodies against T. gondii in Brazilian women 53.03% for IgG and 3.26% for IgM, indicating the relationship between infection during pregnancy and climate, geographic, and socio-economic characteristics of this country[31, 32]. After That, in China, the prevalence of this infection was high due to their specific dietary habits [33]. In women Turkish has been reported for IgG T. gondii (26%) [34] and 52.1% while 0.54% of the cases were positive for anti-T. gondii IgM[35]. Studies show the reduction of toxoplasmosis prevalence in Western countries[36].

Fortunately, in this current study and reports from other areas of Iran, the prevalence of this infection was reported low.

In our study, according to the results, the relationship between age and T. gondii IgG was significant (P-value = 0.00, P <0.05), T. gondii IgG in the age group of 37-47 years is 6.44 times higher than the age group of 17-27 years.
CMV is the leading cause of congenital infections all over the world [37]. In developed countries, the prevalence of CMV IgG in pregnant women ranges from 40 to 60 percent. However, it exceeds 95 percent in developing countries (40), indicating the high exposure of these people to CMV. Recent research suggests that the majority of CMV infants are born from women who have anti-CMV IgG. As a result, it is critical to diagnose cytomegalovirus in women of childbearing age to prevent fetal defects[38].

In one study, the prevalence of anti-CMV IgG among women of reproductive age was reported to be about 65% that increased with age [39]. In women Turkish of childbearing age, the serum prevalence for anti-CMV was reported (99%)[34, 35, 40]. Also, the high prevalence of anti-CMV has been reported from Mexico, Brazil, and China.

In our study, the anti-CMV IgG was the highest. Also, the relationship between age and anti-CMV IgG was significant (P-value = 0.03, P<0.05); CMV IgG in the age group of 27-37 years was 4.13 times higher than the age group of 37-47 years. Similar studies in Iran have yielded the same results.

In a study in Kashan, the anti-CMV IgM was reported high, followed by anti-RV and -T. gondii [41]. In another study in Gorgan, 3.41% of mothers were negative or borderline for CMV antibodies, indicating that they can susceptible to CMV infection during pregnancy, as Intrauterine and prenatal infection with CMV can infect 9.5 % to 22% of newborns[42].

Interestingly, two studies conducted by health authorities in the United Kingdom and Japan concluded that only CMV antibodies testing was sufficient in maternal serum[43, 44].

Although, RV infection is a harmless dermatitis disease in childhood, during pregnancy (especially in the first 12 weeks) can result in congenital rubella syndrome (CRS), severe birth defects, and miscarriage[45]. One study found that the prevalence of RV antibodies increased from 93% to 98% in Brazilian women[32]. The prevalence of anti-RV IgG was also high in Turkish women (94%)[34, 35]. On the other hand, the anti-RV IgM in this study was 94.5%, suggesting vaccination in pregnant women to prevent RV infection.

A similar study in Bandar Abbas reported that 13% of women of childbearing age had anti-RV, therefore, vaccination was strongly suggested in premarital girls[46].

In our study, the rubella IgG rate was 94.5%, and no found a correlation between age and RV infection.

Studies show that there is a high prevalence of this infection in women of reproductive age worldwide. Therefore, the World Health Organization (WHO) recommends that countries introduce and use Rubella-containing vaccines (RCV) to reduce RV infection and congenital rubella syndrome (CRS). Analyzes show that the use of extensive vaccination will significantly reduce the amount of rubella.

Limitations:
Our study was conducted on a limited number of samples and only in the city of Mashhad. Also, unfortunately there were very little laboratory data about HSV infection as one of the pathogens of TORCH syndrome. Expanding this study throughout the country and understanding the main causes of this syndrome in Iran, will help to alleviate concerns about the percentage of congenital defects with infectious causes.

**Conclusion**

To avoid unfavorable fetal outcomes, all women of reproductive age should be checked for the TORCH complex. All studies in this field show that diagnosis TORCH infections during pregnancy and reproductive age is critical to prevent the development of congenital defects. It is also necessary to consider strategies for disseminating information and providing education in this area. According to our study the national screening program to diagnose TORCH infections and prevent fetal birth defects is critical, because of the high prevalence of TORCH syndrome among pregnant women.

**Declarations**

**Funding:**

No funding was received for conducting this study.

**Conflicts of interest/Competing interests:**

The authors have no conflicts of interest to declare that are relevant to the content of this article.

**Availability of data and material:**

The data that support the findings of this study are not openly available and are available from the corresponding author upon reasonable request.

**Code availability:**

Not applicable

**Ethics approval:**

Ethical approval was obtained by the Ethics Committee of Gonabad University of Medical Sciences in view of the retrospective nature of the study.
Authors' contributions:

Ali Nasimi: Conceived of the presented idea, Data Collection

Mitra Salehi: Conceived of the presented idea, Project development

Hamed Ghasemi: Data Collection

Hossein Nezami: Data analysis

Faria Hassanzadeh Haghighi: Manuscript writing

Consent to participate:

All the data we gathered was from anonymous patients, and we consider the aspect of confidentiality. Ethical approval was obtained by the Ethics Committee of Gonabad University of Medical Sciences in view of the retrospective nature of the study.

Consent for publication:

All the data we gathered was from anonymous patients, and we consider the aspect of confidentiality. Ethical approval was obtained by the Ethics Committee of Gonabad University of Medical Sciences in view of the retrospective nature of the study.

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**Figures**

![Bar graph showing TORCH IgG positive rate in women of reproductive age](image)

**Figure 1**

TORCH IgG positive rate in women of reproductive age
Figure 2

2.1: Rubella IgG seroprevalence in various age groups

2.2: Rubella IgM seroprevalence in various age groups

2.3: TOXO IgG seroprevalence in various age groups

2.4: TOXO IgM seroprevalence in various age groups

2.5: CMV IgG seroprevalence in various age groups

2.6: CMV IgM seroprevalence in various age groups
2.4: TOXO IgM seroprevalence in various age groups

2.5: CMV IgG seroprevalence in various age groups

2.6: CMV IgM seroprevalence in various age groups