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Original Article

Frequency of *Toxoplasma gondii*, Rubella, and Cytomegalovirus Antibodies in Pregnant Women in Yazd Province, Iran

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Abstract

Background: TRC (*Toxoplasma gondii*, rubella virus, and cytomegalovirus [CMV]) infections during pregnancy can lead to serious sequelae in the uterine fetus. Prenatal testing, which includes screening and diagnosis of antibodies to TRC infections, is one of the most important aspects of prenatal care recommended during pregnancy. The aim of the present study was to determine the seroprevalence of TRC infections in pregnant women who attended the antenatal clinic in Yazd province in central Iran.

Methods: For this purpose, 8355 sera samples were obtained from pregnant women attending the central laboratory of Yazd province in central Iran and subjected to screening for immunoglobulin G (IgG) and IgM antibodies against TRC using the enzyme-linked immunosorbent assay method. Of these, 4,245, 2,190, and 1,920 pregnant women were screened for anti-*Toxoplasma*, rubella, and CMV, IgG, and IgM antibodies, respectively.

Results: The results revealed that 77.4% (1695/2190) and 78.1% (1500/1920) were positive for anti-rubella and anti-CMV IgG antibodies, and n.o seropositive was detected for anti-rubella and -CMV IgM antibodies. For anti-*Toxoplasma* antibodies, 20.85% (885/4245), 9.54% (405/4245), and 12.01% (510/4245) were positive for IgG, IgM, and both IgG-IgM antibodies, respectively. In addition, our findings showed the high prevalence of chronic TRC infections and a low recently acquired *Toxoplasma* infection in pregnant women.

Conclusion: Our findings confirmed the high prevalence of chronic TRC infections and a low recently acquired *Toxoplasma* infection in pregnant women. Hence, observing personal behavioral practices (the source of infection and hygienic measures) are recommended to women at reproductive-age in general and seronegative pregnant women in particular.

Keywords: *Toxoplasma gondii*, Rubella, Cytomegalovirus, Screening, Pregnant women

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Introduction

Congenital infections caused by *Toxoplasma gondii*, rubella, and cytomegalovirus (CMV), best abbreviated as TRC, during pregnancy are the major causes of serious complications, which can involve the baby's health (1,2). In fact, these pathogenic organisms generally cause only symptom-free or mild infections, especially in healthy immunocompetent people (3). *T. gondii* is a cosmopolitan and protozoan intracellular parasite and the cause of a zoonotic disease called toxoplasmosis. *Toxoplasma* infection is commonly found among the world population, and the seroprevalence in pregnant women is between 21.8% and 85% (4,5).

Risk factors for toxoplasmosis include partially cooked food and infected meat, eating food contaminated with utensils, knives, cutting boards, or other foods that have been in contact with raw and drinking unpasteurized milk, contact with cats, and accidental ingestion of

oocysts. The other risk factors are contaminated soil when gardening or consuming unwashed vegetables or fruits, drinking water contaminated with *Toxoplasma* parasites, congenital infection, organ transplant recipients, receiving contaminated blood through blood transfusions, and immunocompromised patients (6). During pregnancy, *Toxoplasma* infection can be primarily acquired through vertical transmission, which may cause damage to the fetus (7). On the other hand, congenital toxoplasmosis, a consequence of transplacental Toxoplasma infection, can lead to a high morbidity. Congenital toxoplasmosis is composed of the typical clinical trials of chorioretinitis, hydrocephalus (microcephaly and mental retardation), and encephalitis (seizures) that can lead to miscarriage in the developing fetus (8,9).

Similarly, rubella is a proven teratogenic agent that is capable of triggering congenital malformation in humans and affects more than 90% of the cases caused by vertical transmission. Risk factors for rubella infection include contact with infected patients, failure to get vaccinated, overcrowding, a poor immune response to the vaccine, and medical personnel (10). In this context, congenital rubella syndrome is defined as a group of physical abnormalities that have developed in an infant because of a maternal infection and subsequent fetal infection with the rubella virus. Some of the defects caused by rubella infection involve deafness, blindness, severe fetal malformations, mental retardation, and subsequently miscarriages.

The primary CMV infection is almost always asymptomatic in adults and healthy children, including women during pregnancy. In this case, infection is triggered by the inoculation of the virus onto a mucosal site by hand contact or intimate and sexual contact (10,11). Immunodeficiency important risk factors for CMV infection include transplantation and immunocompromised patients (12). The seroprevalence rate of CMV infection in women of reproductive age ranges from 40% to 83% (12), and it prevails in women of lower socioeconomic status who have had previous CMV infection (12-14). This infection is one of the important causes of neonatal morbidity in addition to major long-term consequences such as hearing impairment, cerebral palsy, and mental retardation (15).

Infection with one of the TRC pathogenic agents contracted during pregnancy may be passed through the placenta to the fetus and can affect the fetus and newborn in several ways, potentially causing serious birth defects, where asymptomatic infants may develop abnormalities later in life (16). These infections can be prevented if detected early; therefore, a primary screening program must be implemented, especially in areas of high endemicity. This program should be introduced before or in early pregnancy to determine the mother's exposure to TRC infections (3). Similarly, primary prevention of congenital rubella syndrome is possible through preconception vaccination (17,18). During pregnancy, the main sources of infection are infected young children and close personal contacts. In this respect, prevention of maternal and congenital CMV infections depends on counseling women regarding the sources of infection and personal hygiene that might prevent the infection (11). Pregnant women with no history of previous *Toxoplasma* infection could be contaminated by either the ingestion of tissue cysts from not properly cooked meat or the ingestion of infective oocysts deposited by a cat with a recently acquired infection and contact with soil via gardening or eating raw or unwashed vegetables and fruits. Therefore, these women should avoid eating undercooked meat, contact with soil, unwashed fruits and vegetables, and contact with stray cats (8). Some techniques have been consistently used for the serology diagnosis of acute infections of toxoplasmosis, such as the BioPlex 2200 immunoglobulin G (IgG) assay, among others (19-23).

In Iran, previous studies have reported the incidence and prevalence of congenital infections among pregnant women (24-28). Thus, it is imperative for TRC screening among women during pregnancy in the region, which is useful for early detection and proper management (diagnosis, treatment, and follow-up); this can further prevent complications among newborns. Accordingly, the aim of the present investigation was to determine the seroprevalence of TRC infections in pregnant women who attended the antenatal clinic in Yazd province in central Iran.

Materials and Methods

Study Area

This retrospective study was performed at the central laboratory of Yazd province in central Iran (31°2′N, 53°45′E to 31°34′N, 54°33′E, Figure 1). In this study, 8355 pregnant women were referred to this laboratory from March 2015 to December 2019. Of these, 4245, 2190, and 1920 pregnant women were screened for anti-*Toxoplasma*, rubella, and CMV, IgG, and IgM antibodies, respectively. The inclusion criterion was the participation of all pregnant women in the first trimester of pregnancy between the ages of 15 and 45. General sampling was used (with informed consent) to get direct information from the study participants. The central laboratory provides obstetric and gynecological services to the local population of this province. All recruited women were of childbearing age, in the first trimester of pregnancy, and in healthy conditions.

Sample Collection

Approximately 10 mL of venous blood sample was drawn from every participating pregnant woman by venipuncture into a sterile test tube. Sera were obtained after separation by centrifugation at 2500 × g for 8 minutes and subsequently kept at –20 ˚C until further use. In Iran, it is mandatory for all women in their first trimester of pregnancy to be subjected to screening for TRC infections.

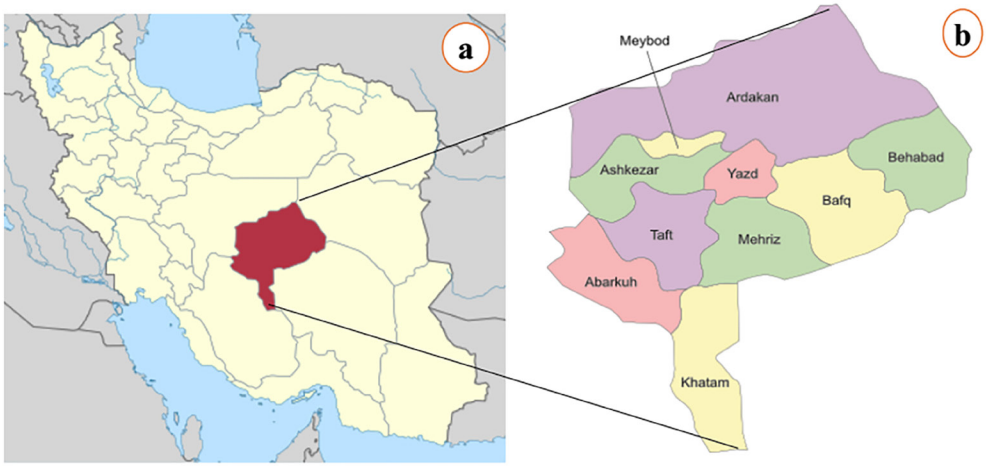

Figure 1. Yazd Province in Central Iran

Detection of Anti-Toxoplasma gondii, Rubella, and Cytomegalovirus Antibodies

The sera were tested for anti-TRC IgG- and IgM-specific antibodies using the enzyme-linked immunosorbent assay kits, which are standard commercial indirect enzyme-linked immunosorbent assays (PishtazTeb Zaman Diagnostics, Tehran, Iran) based on the manufacturer's instructions. Data were then classified as seropositive and seronegative for specific antibodies tested, and the equivalent results were excluded from this study.

Statistical Analysis

Statistical analysis was performed with the aid of the statistical package for social sciences (SPSS Inc., Chicago, IL, USA) software, version 23. This software was employed to calculate variables such as frequencies and ranges. Central indicators such as means and standard deviations were computed for overall IgG and IgM levels against CMV, rubella virus (RuV), and *Toxoplasma gondii*.

Results

The age range of pregnant women was 15–45 years. Of the 8,355 pregnant women, 4,245 were screened for anti-*Toxoplasma*, 2,190 for anti-rubella, and 1,920 for anti-CMV IgG and IgM antibodies. The results showed that 77.4% (1695/2190) and 78.1% (1500/1920) were positive for anti-rubella and anti-CMV IgG antibodies, respectively. In contrast, there were no seropositives for anti-rubella and anti-CMV IgM antibodies. For anti-*Toxoplasma* antibodies, the results revealed that 20.85% (885/4245), 9.54% (405/4245), and 12.01% (510/4245) were positive for IgG, IgM, and both IgG–IgM antibodies, respectively. The prevalence proportions of seropositivity for *Toxoplasma*, rubella, and CMV IgG and IgM are presented in Table 1. During the time of this study, there was no evidence of clinically confirmed congenital diseases reported in the newborns.

Table 1. Number of Seropositive Samples (%) of Antibodies Against *Toxoplasma gondii*, Rubella, and *Cytomegalovirus* Among 8,355 Pregnant Women

Variables	Number of Seropositive Samples [% (95% CI)]	Total
Anti-Toxoplasma IgG	885 [20.8 (CI: 20.7–20.9)]	4245
Anti-Toxoplasma IgM	405 [9.5 (CI: 9.35–9.65)]	4245
Anti-Toxoplasma IgG + IgM	510 [12.01 (CI: 11.9–12.2)]	4245
Anti-rubella IgG	1965 [89.7 (CI: 89.5–89.9)]	2190
Anti-rubella IgM	0 (0.0)	2190
Anti-CMV IgG	1500 [78.1(CI: 77.9–78.3)]	1920
Anti-CMV IgM	0 (0.0)	1920
People with antibodies	5265	5265
Total	-	8355

Note. CI: Confidence interval; Ig: Immunoglobulin; CMV: Cytomegalovirus.

Discussion

Infections transmitted from the mother to the fetus or congenital infections are often the cause of numerous abnormalities or malformations and, sometimes, fetal abortion, resulting in both economic burden and social concerns (29-31). To the best of our knowledge, the current study is the first to provide seroprevalences of antibodies against multiple pathogens of the TRC in women residing in Yazd province, central Iran. The discussion of the investigation presented in this study is largely based on comparison to previous research studying one of the TRC pathogens and is therefore well-organized by pathogen.

Toxoplasma gondii

Our findings from this investigation revealed that 20.8% of pregnant women in the study area were seropositive for anti-*Toxoplasma* IgG antibodies. Along this line, published data indicated varying prevalence rates in various geographical areas around the world, including France (51%) (32), Poland (43.7%) (29), Germany (20%–77%) (30), Norway (9.3%) (31), China (less than 10%) (33), the United States (22.5%) (34), Turkey (85%) (35, 36), and Spain (18.8%) (37). These rates were 45%, 51.8%/26.9%, 38.7%, 71.6%, 55.95%, 51.5%, 39.6%, and 14%–25.7% in other geographical areas, such as India (8),

Iran (38)/(39), Pakistan (40), Guatemala (41), Ghana (42), Sub-Himalayan Region (43), Western Romania (44), and Sweden (45), respectively. These differences in prevalence proportions could be due to the variations in subtropical environments, the education of physicians, polluted water supplies, food-related factors (inadequate culinary practices or malnutrition), a lack of hygienic measures, and the occurrence of larrikin cats spreading the parasite (46). Though chronic infections are usually shown as asymptomatic conditions in immunocompetent persons, this should be taken seriously in immunocompromised individuals without chemoprophylaxis to prevent the secondary reactivation of chronic *Toxoplasma* infection (toxoplasmic encephalitis), which can lead to life-threatening or even fatal conditions. Therefore, toxoplasmosis is a preventable and treatable disease (38). In this respect, our findings demonstrated that 9.54% of pregnant women were seropositive for only anti-*Toxoplasma* IgM antibodies, indicating a recently acquired infection. Considering that there is no evidence of clinical toxoplasmosis, the serological interpretation should be taken with caution due to false positive results. Different serological panels should also be introduced for a clinically confirmed diagnosis. Interestingly, our results indicated that 12.01% of pregnant women in the study area showed *Toxoplasma* seropositivity for both IgG and IgM antibodies. Hence, it is mandatory to perform IgG avidity test measurements to differentiate between past and recently acquired infections, particularly in a single serum sample of pregnant women, as they are at high risk for *Toxoplasma* infection, which is congenitally transmitted to their fetuses (38).

Rubella

Rubella infection typically occurs without any clinical symptoms; therefore, the diagnosis must be measured by serological evidence. This infection leads to insignificant complications, but if contracted during the first trimester of pregnancy, it can cause devastating effects on fetal development (47). Based on our findings, the *Rubella* seropositive rate of IgG antibodies was 89.7%, and no evidence of *Rubella*-IgM seropositivity was found in pregnant women. In this context, the seroprevalence of *Rubella* infection was reported to be 100%, 98%, 95–96.2%, 93.3–94%, 91.2%, 90%, 83%, and 75–96% in the USA (48), Mozambique (49), Turkey (50–52), Saudi Arabia (53), Hodeidah (54), El-Beida (55), Sub-Himalayan Region (43), and Iran (39), respectively. The prevalence rate of CMV, rubella, and *Toxoplasma* in miscarriage women was 17.6%, 15.1%, and 2.6%, respectively (56). Preconception care vaccinations and rubella screening should be introduced to both women of childbearing age and pregnant women who test seronegative, as recommended by the findings of this study.

Cytomegalovirus

Our results demonstrated that 78.1% of pregnant women were seropositive for anti-CMV IgG antibodies, but none were seropositive for anti-CMV IgM antibodies. Numerous studies from different countries showed varying seroprevalence rates from 40% to 83% (12), but a higher seropositive for anti-CMV antibodies was found in Iran and other neighboring countries such as Turkey (95%–96.2%) (50-52), northern Kosovo and Metohija (96.2%) (57), Saudi Arabia (93.3–94%) (53), and the Sub-Himalayan Region (79.8%) (43). A high seroprevalence rate was mentioned in the other parts of Iran, including 97.6%, 88.5%, and 72.1% in Kazeroon (58), Azerbaijan (59), and Gonabad (60), respectively. It is suggested that the socioeconomic level might play a role in this variation (52). It should be noted that the seroprevalence of CMV infection in developing and low-income countries is generally higher when compared to their developed counterparts. Although no clinically confirmed cases were reported, congenital CMV infection can have devastating effects on the fetus, in spite of the frequent absence of symptoms at birth (61). To prevent congenital CMV infection, pregnant women should receive education on primary behavioral practices, including personal hygienic measures, especially hand washing. Currently, the screening program during the prenatal period for TRC infections is routinely performed during the first trimester of pregnancy. This is because pregnant women with seronegativity who are vulnerable to recently acquired infections can vertically transmit them to the fetus (62). The current status of the seroprevalence of TRC infections and the incidence of clinically confirmed congenital diseases in pregnant women can be assessed for the cost burden of the screening plan, early detection, and proper treatment, which can prevent any complications in newborns. A previous history of pregnancy loss and the immunological interpretation of TRC infections during early pregnancy must be considered to reduce fetal complications (63–65).

The screening of serological status prior to pregnancy is mandatory to reduce incidences of infections by *T. gondii*, rubella, and CMV infections. Nonetheless, serological screening for TRC infections in women during pregnancy (follow-up to delivery) is a routine practice in Iran; however, this prenatal care cannot be given to all pregnant women due to the different standards of living. There was no report of clinically confirmed cases caused by TRC infections in pregnant women; therefore, it is feasible that there will be a low disease burden from this high-risk population in this part of Iran. However, there is no reference that shows that anti-rubella testing is mandatory during pregnancy.

One of the limitations of this article is the financial restrictions for performing diagnostic tests for pathogens such as herpes simplex viruses (HSV-1 and HSV-2), *Bordetella pertussis*, *Chlamydia trachomatis*, *parvovirus B19*, *Treponema pallidum*, and *Varicella zoster* virus. Screening this pathogen at the beginning of gestation allows for the assessment of both the maternal immune

status and the risks of an adverse effect on the fetus in pregnancy. Unfortunately, demographic data were not collected in this study. On the other hand, the number of people who had two or three infections at the same time has not been investigated yet.

Conclusion

In summary, the findings of this investigation suggested a high prevalence of TORCH (*Toxoplasmosis*, Other [syphilis, varicella-zoster, and parvovirus B19], rubella, CMV, and herpes) infections (compared to other studies conducted in other parts of the world) and a low recently acquired *Toxoplasma* infection in pregnant women. Based on our findings, we recommend that primary healthcare systems (providers/facilities) should undergo changes and adopt preventive measures to minimize the occurrence of these infections. In addition, personal behavioral practices (source of infection and hygienic measures) should be highlighted among reproductive-age women in general, and seronegative pregnant women in particular. This will prevent, or at least lessen, the incidence of primary acquired infections through vertical transmission, congenital infections, and disease burden in pregnant women during their pregnancy in the future.

Authors' Contribution

Conceptualization: Roghayeh Norouzi.
Data curation: Roghayeh Norouzi, Reza Ahmadi, Abolghasem Siyadatpanah.
Formal analysis: Roghayeh Norouzi.
Investigation: Bahman Aghcheli, Farzaneh Mirzaei, Javad Sadeghinasab.
Methodology: Roghayeh Norouzi.
Project administration: Roghayeh Norouzi.
Supervision: Roghayeh Norouzi, Reza Ahmadi.
Writing–original draft: Roghayeh Norouzi, Reza Ahmadi, Abolghasem Siyadatpanah.
Writing–review & editing: Bahman Aghcheli, Farzaneh Mirzaei.

Competing Interests

None.

Data Availability Statement

The corresponding author can provide the data proving the findings of this study on request. Privacy or ethical restrictions prevent us from sharing the data publicly.

Ethical Approval

This study was approved by the Ethics Committee of the Shahid Sadoughi University of Medical Sciences (Reference No. 1396.493).

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