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...
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.
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 20.8% (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.

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),

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

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

Note: CI: Confidence interval; Ig: Immunoglobulin; CMV: 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 Kazeroun (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 Syiadatpanah.
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 Syiadatpanah.
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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References


