

Investigation of Seroprevalence of *Toxoplasma Gondii*, Rubella and Cytomegalovirus in Pregnant Population in Istanbul

İstanbul'da Gebe Popülasyonda *Toksoplazma*, Rubella ve Sitomegalovirus Seroprevalansının Araştırılması

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ABSTRACT

Aim: *Toxoplasma gondii*, rubella and cytomegalovirus (CMV) are among the most common intrauterine infection agents associated with congenital anomalies in the world. In this study, it was aimed to determine the seroprevalence of *Toxoplasma gondii*, rubella and CMV in pregnant women in Istanbul by ELISA screening tests, to evaluate them according to three different age groups and to compare them with the literature.

Material and Methods: In 5485 serum samples of pregnant women, IgM and IgG antibodies against *Toxoplasma gondii*, rubella and CMV were investigated and total of 16496 tests were evaluated retrospectively. Samples were tested on the Architect i2000SR (Abbott Diagnostics, USA) using kits based on the chemiluminescence microparticle enzyme immunoassay principle (Architect, USA).

Results: *Toxoplasma gondii*, rubella and CMV IgM positivity rates were 0.6%, 0.3% and 2.7%, respectively, and 23%, 89.1% and 98.7% for IgG, respectively. The *Toxoplasma gondii* IgG seropositivity rate was the highest in the 36-49 age group (p=0.012). The *Toxoplasma gondii* IgM seropositivity rate was the lowest in the 26-35 age group (0.5%). The lowest rubella and CMV IgG seropositivity were found in 36-49 (87.8%) and 26-35 (98.4%) age groups.

Conclusion: In pregnant women, serological screening tests seem to be important for monitoring and informing high risk groups, especially seronegative, due to the low seroprevalence of *Toxoplasma gondii*. However, routine screening of rubella and CMV in the pregnant population should be questioned due to the high immunity rates.

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

ÖZ

Amaç: Toksoplazma, rubella ve sitomegalovirus (CMV), dünyada konjenital anomalilerle ilişkili en yaygın intrauterin enfeksiyon etkenlerindedir. Bu çalışmada, İstanbul'da gebe kadınlarda ELISA tarama testleri ile toksoplazma, rubella ve CMV seroprevalansının belirlenmesi, üç farklı yaş gruplarına göre değerlendirilmesi ve literatür ile karşılaştırılması amaçlandı.

Gereç ve Yöntemler: Gebe kadınlara ait 5485 serum örneğinde, toksoplazma, rubella ve CMV'ye karşı oluşan IgM ve IgG antikorları araştırıldı ve toplam 16496 test retrospektif olarak değerlendirildi. Örnekler kemilüminesans mikropartikül enzim immünasay prensibine dayanan kitler (Architect, ABD) ile Architect i2000SR (Abbott Diagnostics, ABD) cihazında çalışıldı.

Bulgular: Toksoplazma, rubella ve CMV IgM pozitiflik oranları sırasıyla %0.6, %0.3 ve %2.7, IgG için ise %23, %89.1 ve %98.7 bulundu. Toksoplazma IgG seropozitiflik oranı en yüksek 36-49 yaş grubunda saptandı (p=0.012). Toksoplazma IgM seropozitiflik oranı en düşük (%0.5) 26-35 yaş grubunda belirlendi. En düşük rubella ve CMV IgG seropozitifliği 36-49 (%87.8) ve 26-35 (%98.4) yaş grubunda saptandı.

Sonuç: Gebe kadınlarda toksoplazma seroprevalansının düşük bulunması sebebiyle serolojik tarama testleri özellikle seronegatif olan yüksek riskli grupların izlenmesi ve bilgilendirilmesi için önemli görünmektedir. Bununla birlikte, rubella ve CMV'nin yüksek bulaşıcılık oranlarından dolayı gebe popülasyonda rutin olarak taranması sorgulanmalıdır.

Anahtar Kelimeler: Toksoplazma, rubella, sitomegalovirus, gebelik, seroprevalans

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INTRODUCTION

Toxoplasma gondii, rubella and cytomegalovirus (CMV) are among the most common intrauterine infection factors associated with congenital anomalies in the world (1). These factors in the TORCH group constitute 2-3% of congenital anomalies (2). The severity of infection varies according to the gestational age of the fetus, virulence of the organism, and the degree of placental damage and maternal disease (3). *Toxoplasma gondii*, rubella and CMV infections can cause various clinical symptoms such as microcephaly, intracranial calcification, intrauterine growth retardation, jaundice, hepatosplenomegaly and thrombocytopenia (4,5). It is one of the most important reasons of perinatal morbidity and mortality especially in underdeveloped and developing countries (5).

Toxoplasma gondii infections usually progress with asymptomatic or mild symptoms (6). While the risk of transition to the fetus in the early period of pregnancy in toxoplasmosis is less than 6%, it rises to 60-80% in the third trimester. However, although the passage of *Toxoplasma gondii* into the fetus during embryogenesis is rare, it can lead to serious congenital malformations (7).

Rubella virus is a highly infectious, and teratogenic pathogen that can cross the placenta and cause fetal infection. In pregnant women who develop rubella infection in the first trimester of pregnancy, the risk of infection for the fetus is 10% to 54% (8). Its importance for public health stems from the teratogenic potential of the virus that causes congenital rubella syndrome (CRS). Rubella infection can cause multiple fetal anomalies in 90% of cases in the first 8-10 weeks of pregnancy and may result in miscarriage or stillbirth. The risk of CRS decreases after the 18th week of pregnancy (9). Rubella infection is a viral infection disease that can be prevented by vaccination, although it has no specific treatment (10). Though the vaccine was licensed in 1969, rubella vaccine was added to the childhood national vaccination calendar in 2006 and the application still continues (11).

CMV is one of the most common perinatal infectious agents with a frequency of 0.2-2.4% in all live births (12). The fetus can be affected by both primary and secondary infections of CMV. While vertical transition after primary infection is 30-50%, it is less than 1% after secondary infection (8). Perinatal infections caused by CMV can cause serious sequelae formation such as optic atrophy with 5% mortality, microcephaly, intracranial calcification, and sensorineural hearing loss in the following months (13).

In the diagnosis of *Toxoplasma gondii*, rubella and CMV in

fections, ELISA tests are used, which mainly identify agent-specific IgM and IgG antibodies (10,14). Aim of this study is to detect *Toxoplasma gondii*, rubella and CMV seroprevalence in pregnant women in Istanbul by prenatal screening tests, to evaluate according to different age groups, to compare the data obtained with the literature in different countries and Turkey.

MATERIAL AND METHODS

This retrospective cross-sectional study included pregnant women who applied to the Obstetrics and Gynecology outpatient clinics of Istanbul Education Research Hospital between July 2018 and July 2019. Consent was obtained from the Ethics committee of Istanbul Education Research Hospital, (Date:11.10.2019, Decision number:2011) and adhered to the Human Rights Declaration of Helsinki throughout the study.

A total of 16496 results were examined which belong to *Toxoplasma gondii* IgM (n=3141), IgG (n=1533) rubella IgM (n=5338), IgG (n=3047) and CMV IgM (n=2640), IgG (n=797) IgM antibody tests investigated in serum samples. In addition, seropositivity rates of pregnant women according to age groups of 18-25, 26-35 and 36-49 were determined. Serum samples were studied in the Medical Microbiology Laboratory of the hospital on the Architect i2000SR (Abbott Diagnostics, USA) with kits based on the chemiluminescence microparticle enzyme immunoassay principle (Architect, USA). Tests with intermediate values were rerun, and intermediate values were not taken into account because they were considered statistically insignificant.

Statistical analysis

The analysis of the data was done on the computer using the SPSS 25.0 (IBM Corp., Armonk, NY, USA) statistical software. The normal distribution suitability of the variables were evaluated by visual methods (histogram and probability graphs) and Kolmogorov-Smirnov test. Continuous variables were given as median value, categorical variables as frequency and percentage. Pearson Chi-Square or Fisher's exact tests were used to compare categorical variables. In the study, the confidence intervals (CI) of seropositivities were determined using the related formulas. Results with a P value below 0.05 were considered statistically significant.

RESULTS

In our study, a total of 16496 test results, including anti-*Toxoplasma gondii*, anti-rubella and anti-CMV IgM and IgG, of 5485 pregnant women aged 18 to 48 years (median age 28 years) were analyzed. In serum samples, 0.6%

(n=19/3141; CI: 0.4%-0.9%) positivity is found in *Toxoplasma gondii* IgM antibodies, while found seropositivity in IgG antibodies is 23% (n=352/1533; CI: 20.9%-25.2%). The positivity rates of Rubella and CMV IgM antibodies are 0.3% (n=16/5338; CI: 0.2%-0.5%) and 2.7% (n=72/2640; CI: 2.1%-3.4%), the positivity rates of IgG antibodies are 89.1% (n=2715/3047; CI: 87.9%-90.2%) and 98.7% (n=787/797; CI: 97.7%-99.4%), respectively (Table 1).

Seropositivity ratios in *Toxoplasma gondii*, rubella, CMV IgM and IgG antibodies according to 16-25, 26-35 and 36-49 age groups in pregnant women are given in Table 2. The

Toxoplasma gondii IgM seropositivity rate was the lowest in the 26-35 age group (0.5%) and the same (0.8%) in the other age groups (p=0.714). *Toxoplasma gondii* IgG seropositivity rate was found the lowest in 18-25 age group (19.4%) and the highest 36-49 age group (26.3%) (p=0.012). Rubella IgM seropositivity was found 0.4% in the 36-49 age group. The lowest rubella and CMV IgG seropositivity was found in 36-49 (87.8%) and 26-35 (98.4%) age groups, respectively. No significant difference was found when rubella IgM/IgG and CMV IgM/IgG seropositivity rates were compared by age groups (p=0.184, p=0.205, p=0.499 and p=0.473, respectively).

Table 1. Seroprevalance rates of *Toxoplasma gondii*, rubella and CMV in pregnant women

	Negative n (%)	Intermediate n (%)	Positive n (%)	95% CI	Total n
Toxoplasma gondii IgM	3119 (99.3)	3 (0.1)	19 (0.6)	0.4 - 0.9	3141
Toxoplasma gondii IgG	1136 (74.1)	45 (2.9)	352 (23.0)	20.9 - 25.2	1533
Rubella IgM	5317 (99.6)	5 (0.1)	16 (0.3)	0.2 - 0.5	5338
Rubella IgG	162 (5.3)	170 (5.6)	2715 (89.1)	87.9 - 90.2	3047
Cmv IgM	2553 (96.7)	15 (0.6)	72 (2.7)	2.1 - 3.4	2640
Cmv IgG	10 (1.3)	0 (0.1)	787(98.7)	97.7 - 99.4	797
Total					16496

CI: Confidence Interval, CMV: Cytomegalovirus

Table 2. Distribution of seropositivity rates of *Toxoplasma gondii*, rubella and CMV IgM and IgG antibodies in pregnant women by age groups

		Age groups (year)			p
		18-25 n (%)	26-35 n (%)	36-49 n (%)	
Toxoplasma gondii	IgM positive	4 (0.8)	9 (0.5)	6 (0.8)	0.714
	IgM negative	521 (99.2)	1858 (99.4)	740 (99.1)	
	Intermediate	0 (0)	2 (0.1)	1 (0.1)	
	IgG positive	44 (19.4)	206 (22.4)	102 (26.3)	0.012
	IgG negative	181 (79.7)	687 (74.8)	268 (69.1)	
	Intermediate	2 (0.9)	25 (2.7)	18 (4.6)	
Rubella	IgM positive	1 (0.1)	10 (0.3)	5 (0.4)	0.184
	IgM negative	954 (99.8)	3104 (99.6)	1259 (99.4)	
	Intermediate	1 (0.1)	1 (0.03)	3 (0.2)	
	IgG positive	445 (89.6)	1587 (89.6)	683 (87.8)	0.205
	IgG negative	32 (6.4)	84 (4.7)	46 (5.9)	
	Intermediate	20 (4.0)	101 (5.7)	49 (6.3)	
Cytomegalovirus	IgM positive	11 (2.7)	38 (2.4)	23 (3.4)	0.499
	IgM negative	386 (96.3)	1516 (97.1)	651 (96.0)	
	Intermediate	4 (1.0)	7 (0.4)	4 (0.6)	
	IgG positive	105 (100)	482 (98.4)	200 (99.0)	0.473
	IgG negative	0 (0)	8 (1.6)	2 (1.0)	
	Intermediate	0	0	0	

DISCUSSION

Toxoplasma gondii, rubella and CMV are among the important public health problems, which can be seen in all age groups and cause prenatal and perinatal infections in pregnant women in the world and in our country (14). Infections of these pathogens in pregnant women can cause the fetus to become infected, thereby causing abortion, preterm birth and congenital malformations. The determination of these infectious agents in the TORCH group is very useful in terms of early diagnosis and treatment (15). The seroprevalence of *Toxoplasma gondii*, rubella and CMV infections differs according to geographic regions (16,17). Therefore, the monitoring of seroprevalence rates in women of childbearing age in the region is important in determining prenatal screening strategies in our country and in countries without screening programs (16). In this study, in order to contribute to the creation of national screening programs, *Toxoplasma gondii*, rubella and CMV seropositivity rates in pregnant women in Istanbul region and their sensitivity to these pathogens were determined.

Toxoplasmosis is one of the most common parasitic infections in the world and congenital toxoplasmosis is frequently seen in the early stages of pregnancy (16,18). Seroprevalence of toxoplasmosis in women of reproductive age or pregnant women has been reported at rates ranging from 10-75% in different regions of the world (5,19). Seronegative pregnant women in the middle and high regions of prevalence have a high risk of primary infection and its transition to the fetus (20). Although the risk of fetus exposure from maternal toxoplasmosis is 75% in the first trimester, the risk decreases to about 0% in the last trimester (10).

The rates of toxoplasmosis seroprevalence may vary depending on various factors such as lifestyle, socioeconomic conditions or nutritional habits in different regions of the world (16). European countries show great differences in seroconversion rates, 7-10% in Norway and England, 44% in France and 50% in Germany (21). Seropositivity in pregnant women varies between 11-83.6% in African countries, and these rates are higher than Arab countries (7-67.5%) (22). According to studies in different regions of Turkey, *Toxoplasma gondii* seroprevalence varies between 17.2% and 69.5% (23). In studies conducted in various cities in Turkey, anti-*Toxoplasma gondii* IgM and IgG seropositivity was found as 1.6% and 22.7% in Afyon (14), 2.26% and 17.5% in Kahramanmaraş (10), 1.3% and 30.3% in Artvin (15), 1.02% and 23.39% in Amasya (6), 0.4% and 23.1% in a study conducted in 2014 in Istanbul, respectively (24). In our study, the rate of seropositivity was found to be 0.6% in 3141 patients and 23% in 1533 patients for IgG, in accordance with the stu-

dies conducted in our region and other provinces. When evaluated by age groups, *Toxoplasma gondii* IgG seropositivity rates were found in the lowest 18-25 age group (19.4%) and the highest 36-49 age group (26.3%), similar to the various study results ($p=0.012$) (10,25,26). The detection of high seronegativity rates shows us that serological screening is necessary for *Toxoplasma gondii*.

Despite vaccination programs, Rubella seropositivity is reported in 71.6-98% of women of reproductive age in different countries (8). These rates were 83.4% in India (27), 53% in Nigeria (28), 88.1% in Taiwan (29), and 95.4% in Brazil (30). In the serological studies conducted in Isparta, Zonguldak, Kahramanmaraş and Van provinces of our country, Rubella IgM positivity is reported as 4.9%, 1.5%, 0.2% and 0.5%, while Rubella IgG positivity is reported as 97.5%, 93.8%, 93.2% and 86.5%, respectively (2,10,17,31). In our study, seropositivity rates were 0.3% for Rubella IgM and 89.1% for Rubella IgG. Seropositivity rate was higher in younger age groups compared to other studies (10,32,33). According to these results, it can be said that the vaccination studies carried out in our country are successful. However, it should not be overlooked that 10.9% of pregnant women in Istanbul are susceptible to rubella virus and are at risk of giving birth to children with CRS.

CMV affects 60% of women of childbearing age in developed countries and 90% of so in developing countries. Seroprevalence increases with age and varies according to geographical regions and socioeconomic status (34). Primary maternal infection can be reactivated in 1-4% of susceptible women and in approximately 10% of seropositive women (35). In a study conducted in pregnant women in Palestine (36) the CMV seropositivity rate was 96.6%, 92% in Saudi Arabia (37), 98.7% in China (38), and 87.5% in Singapore (39). In studies conducted in various regions in our country, CMV IgM and CMV IgG seropositivity rates are 1.2% and 98.6% in Artvin (15), 1.5% and 98.9% in Izmir (16), %3.2 and %99.3 in Kahramanmaraş (10) 1.30% and 97.98% in the results of meta-analysis of Cetinkaya's (40) consisting of 22 studies. In this study, CMV IgM values were 2.7% and CMV IgG values were 98.7%, in accordance with the literature in Turkey. In a study conducted according to age groups, seropositivity rates were found to be over 98%, similar to our study (10). In our study, the high detection of CMV seropositivity suggests that screening is not necessary for this pathogen, but the possibility of reinfection and reactivation should be considered.

Limitations of this study; since avidity test was not perfor-

med in the majority of pregnant women who were positive for IgG and IgM, infections in these pregnant women could not be evaluated.

CONCLUSION

We think that screening for *Toxoplasma gondii* is necessary especially in high-risk groups due to the high rates of seronegativity in a significant portion of pregnant women. Considering the problems that may arise with the primary infection of rubella in pregnant women, screening test should be performed before pregnancy, and if seronegative vaccination should be recommended. Due to the high rates of seropositivity of CMV, it can be discussed that it should be involved in routine screening. Since there is no proven treatment for congenital CMV infection, it is important for women of childbearing age to be informed about the prevention of CMV transmission during pregnancy and to determine their immune status.

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