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Evaluation of rubella, *Toxoplasma gondii*, and cytomegalovirus seroprevalences among pregnant women in Denizli province

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Aim: There is no national screening program for rubella, *Toxoplasma gondii*, and cytomegalovirus (CMV) infections for pregnant women in Turkey. The purpose of this study was to determine the prevalence of these infections among pregnant women in Denizli province, and to review seroprevalences reported from different regions of Turkey.

Materials and methods: Between April 2008 and April 2009, 1268 women were investigated for rubella, 1102 for toxoplasma, and 1000 women for CMV seroprevalences in the first trimester of pregnancy. Toxoplasma and rubella IgM and IgG antibodies were assayed by the automated Vitros ECiQ system based on an immunometric technique. Cytomegalovirus IgM and G antibodies were tested using the Chorus enzyme immune assay. We also reviewed the relevant literature reporting seroprevalences from different regions of Turkey.

Results: Rubella IgG seropositivity was found in 1206 patients (95.1%) with no IgM seropositivity. CMV IgG and IgM positivities were detected in 987 (98.7%) and 12 (1.2%) subjects, respectively. Of 1102 women evaluated for toxoplasmosis, 408 (37%) were positive for IgG and 15 (1.4%) were positive for IgM. The review of the relevant literature reported from Turkey showed seroprevalence rates in the range of 92.6%-97.3% for rubella, 93.5%-96.1% for CMV, and 30.7%-69.5% for toxoplasma.

Conclusion: High seropositivity rates for rubella and CMV in Turkey indicate that most of the women were exposed to these viruses before child-bearing age. Although rubella and CMV seroprevalences show a relatively homogeneous distribution throughout Turkey, toxoplasma prevalence varies greatly according to the geographic region.

Key words: Pregnancy, rubella, cytomegalovirus, *Toxoplasma gondii*, seroprevalence

Denizli bölgesinde gebe kadınlarda rubella, *Toxoplasma gondii* ve sitomegalovirus seroprevalansının değerlendirilmesi

Amaç: Türkiye'de gebe kadınlarda rubella, *Toxoplasma gondii* ve sitomegalovirus (CMV) enfeksiyonları için, ulusal bir tarama programı yoktur. Bu çalışmanın amacı, bölgemizde gebe kadınlarda rubella, *Toxoplasma gondii* ve CMV seroprevalansını belirlemek ve ülkemizin farklı bölgelerinden seroprevalans değerlerini bildiren ilgili literatürü gözden geçirmektir.

Yöntem ve gereç: Nisan 2008 ve Nisan 2009 tarihleri arasında, gebeliğin ilk trimesterindeki 1268 kadın rubella, 1102 kadın toxoplasma, 1000 kadın da CMV seroprevalansı açısından incelendi. Toxoplasma ve rubella IgM ve IgG antikorları immünoimetrik tekniğe dayalı otomatik Vitros ECiQ sistemi ile, CMV IgM ve IgG antikorları ise Chorus enzim immünoassay yöntemi ile değerlendirildi. Türkiye'nin farklı bölgelerinden seroprevalans değerlerini bildiren ilgili literatür gözden geçirildi.

Bulgular: Bin-ikiyüz-altı (% 95,1) kadında rubella IgG pozitif saptanırken hiçbir kadında IgM pozitifliği saptanmadı. CMV IgG ve IgM pozitifliği sırasıyla 987 (% 98,7) ve 12 (% 1,2) hastada saptandı. Toksoplazma için incelenen 1102 kadından, 408'inde (% 37) toksoplazma IgG, 15'inde (% 1,4) toksoplazma IgM pozitifliği. Türkiye'den bildirilen literatür

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gözden geçirildiğinde rubella seropozitifliğinin % 92,6 - 97,3 arasında, CMV seropozitifliğinin %93,5 - 96,1 arasında ve toxoplasma seropozitifliğinin % 30,7 - 69,5 arasında değiştiği gözlemlendi.

Sonuç: Türkiye'deki yüksek rubella ve CMV seropozitifliği, kadınların çoğunun doğurganlık yaşından önce bu virüslere maruz kaldığını göstermektedir. Rubella ve CMV seroprevalansı rölatif olarak homojen bir dağılım gösterirken, toxoplasma seroprevalansı bölgesel farklılıklar göstermektedir.

Anahtar sözcükler: Gebelik, rubella, sitomegalovirus, *Toxoplasma gondii*, seroprevalans

Introduction

Prenatal infections are thought to be responsible from 2%-3% of all congenital anomalies (1). Primary infections caused by *Toxoplasma gondii*, rubella, and cytomegalovirus (CMV) in pregnancy can bring about serious problems in the developing fetus. Rubella is a viral infection with a tiny red skin rash and usually affects children. However, when the primary infection occurs in the first trimester of pregnancy, the virus may cause congenital rubella syndrome (CRS) associated with multiple developmental anomalies (2,3). It is difficult to decide about rubella seropositivity by means of the patient's history because many illnesses with rashes may simulate rubella and up to 50% of rubella infections may pass without any symptom (3,4). Therefore, serologic testing is necessary for detection of susceptible patients.

Cytomegalovirus (CMV) may affect 0.5% to 1.0% of all live births (5). It is the most frequent cause of congenital viral infections and may lead to sensorineural deafness and mental retardation (5,6). Intrauterine transmission of CMV occurs as a result of maternal infection. However, unlike rubella and toxoplasmosis, fetal infection can occur following both primary and recurrent infection (reactivation of endogenous virus or reinfection with a new strain). Transmission rate varies between 30% and 40% after primary and 1% following secondary infection (7,8). The time of infection during pregnancy is also important in the transmission rate; it is lowest at the time of conception, the same in the second and first trimester, and highest in the third trimester (9). Furthermore, 5% to 10% of infected newborns are symptomatic at birth and among asymptomatic newborns 10% to 15% will eventually show some developmental disorders, mainly sensorineural-hearing loss (10).

Toxoplasma gondii is an obligate intracellular protozoan with worldwide distribution influencing both animals and humans (11). Sexual forms of the parasite are found in the intestinal epithelium of definitive hosts such as domestic cats where they transform into oocysts subsequently shed into the environment. Oocysts are transmitted to other hosts through ingestion of undercooked meat or contact with feline feces or from soil (12). In immunocompetent humans, 90% of *T. gondii* infections are asymptomatic, or may mimic a minor viral illness (13). Nevertheless, primary infection during pregnancy may cause spontaneous abortion, major ocular and neurological problems, or stillbirth depending on the virulence of the parasite, the immune response of the mother, and stage of the pregnancy (14). Effective prevention of congenital disease depends on avoidance of infection during pregnancy (15). Due to its long-term complications and the fact that *T. gondii* is omnipresent, epidemiological studies on its seroprevalence help to shape health policies in individual countries.

The aims of this study were to assess the seroprevalence of rubella, *Toxoplasma gondii*, and CMV infections through antenatal screening in Denizli province, and compare our findings with those reported in other studies from Turkey. We assume that the results of this study would provide further information to estimate the susceptibility of pregnant women to congenital infections, and enforce appropriate preventive measures.

Materials and methods

Study population

The study subjects were recruited from pregnant women undergoing routine obstetrical evaluation between April 2008 and April 2009 in Denizli State

Hospital, Denizli, Turkey. A total of 1268 women with a mean age of 28.2 years (range 18-40 years) were tested for rubella seroprevalence, 1102 women with a mean age of 29.8 years (range, 18-40 years) were evaluated for *Toxoplasma gondii* seropositivity, and 1000 women with a mean age of 29.4 years (range, 18-40 years) were screened for CMV seroprevalence in the first trimester of pregnancy. The study protocol was approved by the local research ethics committee and informed consent was obtained from all participants.

Serology

Peripheral blood samples were obtained from pregnant patients in the first trimester for laboratory tests. Toxoplasma and rubella IgM and IgG antibodies were assayed by the automated Vitros ECiQ system based on an immunometric technique (Vitros ECIQB system Johnson & Johnson, New Brunswick, NJ, USA). Cytomegalovirus IgM and G antibodies were tested using the Chorus enzyme immune assay (Chorus Elisa system, Disease Diag., Siena, Italy). The assays were performed according to the manufacturer's instructions. CMV IgM antibody titers greater than 1.1 ratio and CMV IgG antibody titers greater than 1.2 IU/mL were regarded as positive. Rubella IgG antibody titers above 10.0 ratio and IgM titers above 1.0 ratio were considered positive. Toxoplasma IgG antibody titers greater than 3.0 IU/mL and IgM greater than 1.0 ratio were considered positive. All positive results were double-checked. We

also reviewed the relevant literature reporting seroprevalences from different regions of Turkey.

Results

Rubella IgG seropositivity was detected in 1206 (95.1%) of 1268 subjects with no patient with IgM seropositivity. CMV IgG and IgM seroprevalences were detected in 987 (98.7%) and 12 (1.2%) of 1000 pregnant individuals, respectively.

Of the 1102 women evaluated for toxoplasmosis, 408 (37%) were positive for toxoplasma IgG, and 15 (1.4%) were positive for IgM. The rates of seropositivity for rubella, CMV and toxoplasma were shown on Table 1.

Four of 12 patients with IgM seropositivity for CMV and 9 of 15 patients with IgM seropositivity for toxoplasma showed high avidity. These patients had uneventful perinatal follow up and healthy babies. On the other hand, 8 patients with IgM seropositivity for CMV and 6 patients with IgM seropositivity for toxoplasma showed low avidity, and they were referred to institutions with well-equipped perinatology units.

The review of the pertinent literature reported from Turkey showed a 95.9% (range 92.6%-98.7%) seroprevalence rate for rubella, 94.9% (range 93.5%-96.1%) seropositivity for CMV, and 47.2% (range 30.7%-69.5%) seroprevalence rate for toxoplasma (Table 2).

Table 1. The rates of seropositivity for rubella *Toxoplasma gondii* and cytomegalovirus (CMV) infections.

Test	Negative (n)	%	Positive (n)	%	Total
Anti-Rubella IgM	1268	100	0	0	1268
Anti-Rubella IgG	62	4.9	1206	95.1	1268
Anti-Toxoplasma IgM	1087	98.6	15	1.4	1102
Anti-Toxoplasma IgG	694	63	408	37	1102
Anti-CMV IgG	13	1.3	987	98.7	1000
Anti-CMV IgM	988	98.8	12	1.2	1000

Table 2. Comparison of studies evaluating rubella, toxoplasma, and CMV seroprevalences from different regions of Turkey.

Study	District seropositivity (%)	Rubella seropositivity (%)	Toxoplasma seropositivity (%)	CMV
Present study	Denizli	95.1%	37%	98.7%
Kaleli et al. (23)	Denizli	93.5%	-	93.1%
Aksakal et al. (19)	Ankara	94.5%	-	-
Yilmazer et al. (20)	Afyon	95.1%	30.7%	92.6%
Uyar et al. (21)	Samsun	94.3%	-	97.3%
Tamer et al. (22)	Kocaeli	96.1%	-	96.4%
Ocak et al. (29)	Hatay	95.5%	52.1%	97.3%
Harma et al. (30)	Urfa	-	60.4%	-
Tekay et al. (27)	Urfa	-	69.5%	-
İnci et al. (31)	Kayseri	-	33.4%	-

Discussion

Toxoplasma, rubella, and CMV are important microbiological agents causing perinatal infections. These infections often lead to mild or asymptomatic infection in the mother. However, they may result in serious congenital abnormalities, intra-uterine growth retardation, and even death of the fetus (16-18).

Rubella is an important agent for the first trimester maternal infections. In Turkey, rubella vaccine was incorporated into the national immunization program in 2006. However, no vaccination program is available for rubella after the age of 18. Previous studies from different regions of Turkey reported high rubella seropositivity ranging between 93.5% and 96.1% in first trimester pregnancies (Table 2) (19-23). Concordantly, rubella IgG positivity was found as 93.5% in a previous study conducted in 302 pregnant patients in Denizli (23). The seroprevalence rate of 95.1% in our study indicates a small increase, which was probably due to the rubella vaccination program that commenced in 2006 for subjects under 18 years old. However, because we did not obtain data with respect to rubella vaccination status in our study, the degree of influence of the vaccination program cannot be determined definitively. Since the susceptibility of pregnant women to rubella was found to be low in our

study, the necessity of rubella vaccination in the child-bearing age group remains controversial. Larger studies from different age groups from urban and rural settings are needed to determine the necessity of such a national vaccination program for this country.

The high rate of seropositivity detected in this study was also similar to the rates reported in different countries without national vaccination programs in adults. Rubella seroprevalence was reported to be 96.2% in Iran (24), 91.1% in Saudi Arabia (25), and 92.2% in Egypt (26), among non-vaccinated women of reproductive age.

Our results showed that toxoplasma IgM and IgG seropositivities in pregnant women were 1.4% and 37% respectively. There was no patient with both toxoplasma IgM and IgG seropositivity. *Toxoplasma gondii* prevalence is higher in places consuming undercooked meat. In Turkey, the seroprevalence of *Toxoplasma gondii* varies greatly among different regions ranging from 30.7% to 69.5% (Table 2). The seroprevalence is higher in eastern and central Anatolia probably due to consumption of undercooked meat and raw vegetables (20,27-31). The increased number of stray cats especially in the rural regions may also contribute to the high rates.

Toxoplasma seropositivity was reported as 43.8% in France (32), and 62.8% in Brazil (33). Lower seroprevalence rates were reported in the United Kingdom (34) and Norway (14) (7.7%-9.1% and 10.9% respectively). The likelihood of acquiring primary toxoplasma infection during pregnancy is high in subjects without previous exposure to infection, particularly for those migrating to areas with higher rates of seroprevalence. As a result, it is important to detect seronegative pregnant women and educate them about simple protective measures such as hand washing, flushing all vegetables and fruits, and avoidance of raw or undercooked meat consumption.

In our study, the seropositivity rate of pregnant women for CMV IgM and IgG antibodies were 1.2% and 98.7% respectively. Previous studies from different locations of Turkey showed CMV seroprevalence rate in the range of 92.6%-97.3% (Table 2) (20,21,23). The rate was reported to be 46.8% in France (35), 56.3% in Finland (36), 78% in Russia (37), 84% in Spain (38), and 92.1% Saudi Arabia (39). CMV infections are highly associated with poor hygienic conditions, communal life style, and close contact with day care units (35). Therefore, hygiene information has a positive impact and could significantly reduce the incidence of maternal CMV infection during pregnancy for seronegative patients (24). Whether the CMV testing has to be proposed to all pregnant women or restricted to high risk populations is still under debate in the scientific community.

Our study has a few limitations. First, we investigated the seroprevalences only in the first trimester of pregnancy. Because we did not have longitudinal follow up in all patients during the later stages of pregnancy, we do not have the data regarding the outcome of pregnancy and the well-being of the newborns. It would have been valuable to provide information whether or not the pregnant women without immunity to rubella or toxoplasma acquired infections. Second, we did not obtain information about the rubella vaccination status of pregnant women. Because all women under the age of 18 have been vaccinated since July 2006, we can assume that women under the age of 21 have probably been vaccinated previously. Third, we also did not collect information about the living conditions, eating habits, or socioeconomic and educational status, which could have been more explanatory for toxoplasma and CMV seropositivity rates.

In conclusion, results for rubella and CMV seropositivity in Denizli district were similar to those of other regions of Turkey. However, seroprevalence for *Toxoplasma gondii* was lower compared to other regions. Although most of the women were exposed to rubella and CMV before child-bearing age in our district, the significant population was susceptible to *Toxoplasma gondii*. The necessity of a rubella vaccination program in adults is a subject of debate in this country due to the high rate of rubella seropositivity. The education of pregnant women about transmission routes and measures seems to be the only effective way to protect seronegative women from toxoplasma and CMV infections.

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