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Seroprevalence and Seroconversion Rates of Cytomegalovirus pp65 Antigen and Cord Blood Screening of Pregnant Women in Malatya, Turkey

Malatya İlindeki Gebelerde Sitomegalovirüs pp65 Antijeninin Seropozitiflik-Serokonversiyon Oranları ve Kord Kanı Taraması

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Abstract

Objective: The rates of seropositivity, seroconversion and fetal infection with human cytomegalovirus were analyzed in pregnant women and newborn cord blood in this study. The relationships between maternal age, parity, cytomegalovirus serology and polymerase chain reaction results were evaluated.

Materials and Methods: A total of 217 pregnant women attended our pregnancy clinic between April 2004 and October 2005. During each trimester, 5 cc of maternal blood was obtained and 5 cc of cord blood was collected after birth. An enzyme-linked immunosorbent assay (ELISA) was used to assess these samples for the presence of human cytomegalovirus protein pp65 antigen (in leukocytes) and cytomegalovirus DNA (in plasma).

Results: The mean age of the pregnant women in our study was 28.1±5.3 years. No seroconversion was observed. Among the pregnant women, 212 (97.7%) were IgG positive, and 29 (13.4%) were IgM positive. Five of the pregnant women were positive for IgM alone (2.3%), whereas 24 (11.3%) were positive for both IgM and IgG. The 29 IgM-positive patients were reevaluated using the polymerase chain reaction, and no seropositivity was found. None of the cord blood samples were IgM positive, whereas 211 (97.3%) were IgG positive. There was no significant correlation between parity and seropositivity (p=0.487). The relationship between human cytomegalovirus seropositivity and maternal age was evaluated by dividing the pregnant women into two groups, with a cut-off age of 35 years. There was a significant difference in seropositivity between these two groups (p=0.045).

Conclusion: Clearly, there is no need to screen pregnant women for Human cytomegalovirus (HCMV) in the Malatya region. Confirming serology results using the polymerase chain reaction and antigenemia testing to detect false positive results offers the advantage of avoiding unnecessary invasive interventions.

Key Words: Cord blood, cytomegalovirus, cytomegalovirus pp65 antigen, polymerase chain reaction, pregnancy

Özet

Amaç: Çalışmada, gebe ve yenidoğan kordon kanında insan Sitomegalovirüs enfeksiyonunun seropozitiflik, serokonversiyon ve fetusa geçiş oranı tespit edilecek, rutin taramanın gerekliliği tartışılacak ayrıca, anne yaşı ve doğum sayısı ile insan Sitomegalovirüs serolojisi ve polimeraz zincir reaksiyonu bulguları arasındaki ilişki araştırılacaktır.

Gereç ve Yöntem: Çalışmaya Nisan 2004-Ekim 2005 tarihleri arasında fakültemiz gebe polikliniğine başvuran 217 gebe dahil edilmiştir. Gebelerden her üç trimestırde 5'er cc kan alınmış ve doğum sırasında ayrıca 5 cc fetal kord kanı toplanmıştır. Alınan örneklerin serumları ayrılarak -20°C de saklanmıştır. Alınan örneklerde ELISA yöntemi ile lökositlerde insan Sitomegalovirüs protein pp65 antijeni ve plasmada insan Sitomegalovirüs DNA tespiti yapılmıştır.

Bulgular: Çalışmaya katılan gebelerin ortalama yaşı 28.1±5.3 olup hiçbir olguda serokonversiyon görülmemiştir. 212 gebede (%97.7) IgG pozitif bulunmuşken, 29 gebede (%13.4) ise IgM pozitif tespit edilmiştir. Gebelerin 5'inde sadece (%2.3) IgM pozitif iken, 24'ü (%11.3) ise hem IgM hem IgG pozitif olarak bulunmuştur. Ig M pozitif olan 29 gebenin tümü, polimeraz zincir reaksiyonu ve antijenemi yöntemiyle yeniden taranmış ve seropoziflik tespit edilmemiştir. Alınan kordon kanlarında IgM pozitifliğine rastlanmazken, 211 (%97.3) kordon kanında ise IgG pozitif olarak tespit edilmiştir. Seropozitiflik oranlarıyla, doğum sayısı arasında istatistiksel anlamlılık bulunamamıştır (p=0.487). İnsan Sitomegalovirüs seropozitifliği ile yaş ilişkisi araştırılmış, gebelerimiz 35 yaş üstü ve altı olmak üzere iki gruba ayrılmış, her iki grup karşılaştırıldığında seropozitiflik açısından istatistiksel anlamlılık tespit edilmiştir (p=0.045).

Sonuç: Malatya yöresinde gebelikte rutin İSMV taramasına gerek olmadığı görülmüş olup, yanlış pozitiflikleri tespit edebilmek için, serolojik bulgularının polimeraz zincir reaksiyonu ve antijenemi ile teyid edilerek gereksiz invaziv girişimlerin önlenebileceği kanısına varılmıştır.

Anahtar Kelimeler: Gebelik, Kord kanı, Polimeraz zincir reaksiyonu, Sitomegalovirüs, Sitomegalovirüs pp65 antijeni

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Introduction

Human cytomegalovirus (HCMV), which consists of double-strained DNA and belongs to the herpes virus group, causes cytomegalic inclusion disease [1]. After the primary infection, the virus enters a latent period; similar to other herpes viruses, HCMV undergoes viral dissemination and periodic reactivation despite existing antibodies [2-4].

Although HCMV is common, it causes severe infections and sequelae only in adults with natural or drug-dependent immune deficiency or in the fetus [4, 5]. Viral contamination occurs via contact with infected body fluids through sexual intercourse, respiration, lactation or blood components or across the placenta. The prevalence of congenital infections is 0.5%-2.5% [4, 6, 7]. HCMV seroprevalence rates depend on age, socioeconomic status and environment [8, 9]. The rate of HCMV seroconversion during pregnancy varies between 1% and 7% [10].

In this study, seropositivity, seroconversion and the maternal-fetal transmission of HCMV were evaluated in maternal blood and newborn cord blood samples. These data were used to compare maternal age, parity and HCMV serologic findings with antigenemia and polymerase chain reaction (PCR) findings.

Materials and Methods

A cross-sectional study was performed in 217 pregnant women who attended the outpatient pregnancy clinic of the İnonu University Faculty of Medicine Department of Obstetrics and Gynecology during their first, second and third trimesters from April 2004 to October 2005. All of the participants were included in the study after obtaining ethical approval for the study and informed consent.

During each trimester, a 5 cc blood sample was obtained from each participant. Additionally, 5 cc of cord blood was obtained at birth and centrifuged. The sera from these blood samples were stored at -20°C until analysis. To exclude the risk of false positive results, blood samples from pregnant women identified as IgM (+) according to ELISA were reevaluated along with blood samples collected previously and over the following three weeks using the antigenemia and PCR methods of identifying HCMV. Furthermore, the fetuses of these patients were evaluated for anomalies by fetal ultrasound, and the cord blood samples were evaluated via the ELISA, antigenemia and PCR methods. These analyses were performed using an enzyme-based immunologic method (BioTek ELISA, VT, USA) to detect HCMV protein kinase, a kit (Argene-Biosoft, France) to detect the internal matrix phosphoprotein pp65 antigen in human peripheral blood leukocytes and a plasma HCMV monitoring system (the QIAamp DNA Mini Kit) to detect HCMV DNA. The statistical analyses were performed using Fisher's exact chi-square test and Student's t-test, and p values <0.05 were considered statistically significant.

Results

The mean age of the 217 participants was 28.1±5.3 years. There was no HCMV seroconversion during any trimester according to the ELISA results, whereas IgG positivity was found in 212 (97.7 %) of the pregnant women, and IgM positivity was found in 29 (13.4%). IgM positivity alone was observed in 5 (2.3%) of the pregnant women, whereas both IgM and IgG were positive in 24 (11.3%).

To detect false positive results, 29 (13.4%) of the IgM-positive pregnant women were reevaluated. Blood samples obtained previously and over the following three weeks were analyzed via the antigenemia and PCR methods of detecting HCMV, and no IgM positivity was observed. Furthermore, no fetal anomalies indicative of HCMV infection (ventriculomegaly; hydrocephaly; microcephaly; calcification of the thalamus, basal ganglia or periventricular space; posterior fossa cysts; hydrops fetalis; severe intrauterine growth restriction; hyperechogenic bowel; or hepatic calcification) were observed during fetal ultrasound screening of these patients. Following the reevaluation of blood samples and ultrasound findings, 29 (13.4%) of the pregnant women initially diagnosed as IgM positive were determined to be IgM negative.

HCMV IgM and IgG were evaluated in the cord blood samples. Although none of the 217 samples (0%) were IgM positive, 211 (97.3%) samples were IgG seropositive. The 29 pregnant women with IgM positivity also received evaluations of their newborns' cord blood via the antigenemia and PCR methods of HCMV detection; there were no positive results. There was no significant correlation between seropositivity and parity (Tables 1, 2). When the pregnant women were divided into two groups according to age (greater than or less than 35 years), there was a significant correlation between age and HCMV seropositivity (p=0.045) (Table 3).

Discussion

HCMV is a viral pathogen that represents the most common cause of intrauterine infections, with a global seropositivity rate of over 90%. In 1973, Krech [11] reported that HCMV antibodies were more common in developing countries and those with low socioeconomic levels compared with developed countries. A study by Tookey et al. [12] performed in London revealed that HCMV seropositivity varied between different races: The seropositivity rates were 45.9% in white women, 88.2% in Asian women and 77.2% in black

Table 1. HCMV seropositivity rates among pregnant women according to the ELISA method

		Negative		Positive		Total	
		n	%	n	%	n	%
Maternal blood	IgM	188	86.6	29	13.4	217	100
	IgG	5	2.3	212	97.7	217	100
Cord blood	IgM	217	100	0	0	217	100
	IgG	6	2.7	211	97.3	217	100

Table 2. HCMV seropositivity rates among pregnant women according to PCR and antigenemia methods

		Negative		Positive	
		n	%	n	%
Maternal blood	PCR	29	0	0	0
	Antigenemia	29	0	0	0
Cord blood	PCR	29	0	0	0
	Antigenemia	29	0	0	0

Table 3. HCMV seropositivity rates according to age

		Negative		Positive		Total	
		n	%	n	%	n	%
age<35	IgM	168	85.6	26	13.4	194	89.4
	IgG	5	2.6	189	97.4	194	89.4
age>35	IgM	20	86.9	3	13.04	23	10.6
	IgG	0	0	23	100	23	10.6

women (of African/Caribbean origin). Furthermore, the following international HCMV IgG seropositivity rates have been reported: 51.5% in French women, 68.3% in Italian women, 30.4% in Irish women, 63.5% in American women and 56.8% in Australian women [13-17]. In different regions of Turkey, HCMV seropositivity has been reported to be 84.3%-94.7% [18-20]. In our study, similar to other Turkish studies, a 97.7% seropositivity rate was found.

In a study by Akinbami et al. [21], there was no correlation between HCMV seropositivity and parity, marital status or educational level. However, Hamdan et al. [22] reported that low educational status and increased parity were strong risk factors for HCMV infection among women. In our study, there was also no association between seropositivity and parity. In studies by Kenneson and Ornoy, HCMV seroprevalence was found to increase by 50%-100% due to environmental factors, socioeconomic status and aging [8, 9]. In our study, there was a significance correlation between age greater than 35 years and HCMV seropositivity, in accordance with the literature.

The reported annual HCMV seroconversion rates vary between 1% and 7% [10]. The seroconversion rates of health care professionals who care for children and pregnant women are the same [23]. However, in our study, no seroconversion was detected due to the absence of infected pregnant women.

Leisnard et al. [4] reported that after a primary infection is confirmed via IgG avidity, antigenemia, PCR and the reevaluation of serologic tests, it is possible to diagnose fetal infection by a PCR analysis of amniotic fluid or fetal blood. Foulon et al. [24] evaluated 750,000 pregnancies in which 75 fetuses with severe sequelae were found. To detect these fetal anomalies, 6500 unnecessary amniocenteses were performed, resulting in 1755 healthy fetuses being accidentally aborted due to these unnecessary interventions. The same study reported a 91% rate of congenital HCMV infections based on an analysis of HCMV IgM in cord blood. In our study, pregnant women shown to be IgM positive using the ELISA method were reevaluated via PCR and antigenemia methods. The IgM results were demonstrated to be false positives; therefore, there was no need for further invasive interventions. Moreover, cord blood evaluations revealed the same serologic results as those obtained from the maternal blood samples.

In Turkey and throughout the world, the main purpose of detecting HCMV infection prenatally is the early identification of HCMV infection and its possible long-term sequelae to advise the patient about the termination or continuation of the pregnancy. The differential diagnosis between primary HCMV infection and the reactivation of a latent infection, as well as the determination of the time of infection, are difficult in seropositive pregnant women. Furthermore, no drugs have yet been investigated for treating HCMV infection or reducing fetal sequelae. When an infection is detected in a pregnant woman, the likelihood of fetal sequelae remains unclear [2, 25].

In light of the above information and considering the high 97.7% HCMV seropositivity rate, the use of invasive methods for diagnosis, cost-effectiveness issues and the absence of effective treatment options, routine HCMV screening for pregnant women is not appropriate in the Malatya region. However, young women with low socioeconomic status can be educated about HCMV. To determine a national health care policy, further studies with larger patient cohorts are needed in Turkey.

Conflict of interest statement: The authors declare that they have no conflict of interest to the publication of this article.

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