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 Kani Taraması

Keziban Dogan¹, Ayse Kafkasli², Cihan Kaya¹, Huseyin Cengiz¹

¹Department of Obstetrics and Gynecology, Bakirkoy Dr Sadi Konuk Teaching and Research Hospital, Istanbul, Turkey
²Department of Obstetrics and Gynecology, Özel Erdem Hastanesi, Istanbul, Turkey

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

Bulgular: Çalışmaya katılan gebelerin ortalaması 28±5.3 olup hiçbir olguda serokonversiyon görülmemiştir. 212 gebede (%97.7) IgG pozitif bulunmuşken, 29 gebede (%13.4) ise IgM pozitif tespit edilmiştir. Gebelerin %2.3'ünde sadece (%2.3) IgM pozitif iken, 24'ü (%11.3) ise hem IgM hem IgG pozitif olarak bulunmuştur. Ig M pozitif olan 29 gebenden tümü, polimeraz zincir reaksiyonu ve antijenemi yöntemiyle yeniden taramış ve seropozitif tespit edilmiştir. Alınan örnekler ELISA yöntemi ile lüksotilde insan Sitomegalovirüs protein pp65 antijeni ve plasmasında insan Sitomegalovirüs DNA tespiti yapılmıştır.

Bulgular: Çalışmada, gebe ve yenidoğan kordon kanında insan Sitomegalovirüs enfeksiyonunun seropozitiflik, serokonversiyon ve fetusa geçiş oranı tespit edilecek, rutin taramanın gerektürü arasındaki ayrica, anne yaş ve doğum sayısı ile insan Sitomegalovirüs serolojisi ve polimeraz zincir reaksiyonu bulguları arasındaki ilişki araştırılacaktır.

Amaç: Çalışmada, gebe ve yenidoğan kordon kaninda insan Sitomegalovirüs enfeksiyonunun seropozitiflik, serokonversiyon ve fetusa geçiş oranları tespit edilecek, rutin taramanın gerektüğünü tartışılan programa ait gebe ve yenidoğan kordon kanı taraması ve bunun bu bölgede en fazla verimi olduğu değerlendirilmiştir. Çalışmada, gebe ve yenidoğan kordon kanında insan Sitomegalovirüs protein pp65 antijeni ve plasma İnsan Sitomegalovirüs DNA tespiti yapılmıştır.

Amaç: Çalışmaya katılan gebelerin ortalaması 28±5.3 olup hiçbir olguda serokonversiyon görülmemiştir. 212 gebede (%97.7) IgG pozitif bulunmuşken, 29 gebede (%13.4) ise IgM pozitif tespit edilmiştir. Gebelerin S%inde sadece (%2.3) IgM pozitif iken, 24’si (%11.3) ise hem IgM hem IgG pozitif olarak bulunmuştur. Ig M pozitif olan 29 gebenden tümü, polimeraz zincir reaksiyonu ve antijenemi yöntemleriyle yeniden taramış ve seropozitif tespit edilmiştir. Alınan kordon kanlarında ise IgG pozitif olarak tespit edilmiştir. Seropozitif oranlara, doğum sayısı artışında istatistiksel anlamlılık bulunmamış (p=0.487). İnsan Sitomegalovirüs serozitifliği ile yaş ilişkisi araştırılmış, gebelerimiz 35 yaş üstü ve altı olmak üzere iki grupa ayrılmış, her iki grup karışımlarında seropozitiflik artış oranında istatistiksel anlamlılık tespit edilmiştir (p=0.045).

Sonuç: Malatya bölgesinde gebelik ve yenidoğan taraması gerekliliği göze alınma olgunun bulunduğu görülmüştür. Çalışmada, gebe ve yenidoğan kordon kanında insan Sitomegalovirüs enfeksiyonunun seropozitiflik, serokonversiyon ve fetusa geçiş oranı tespit edilecek, rutin taramanın gerektüğü değerlendirilmiştir. Çalışmada, gebe ve yenidoğan kordon kanında insan Sitomegalovirüs protein pp65 antijeni ve plasmasında insan Sitomegalovirüs DNA tespiti yapılmıştır.
Introduction

Human cytomegalovirus (HCMV), which consists of double-stranded 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 İvonu 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 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 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 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.

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...
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 Ornroy, 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 accidently 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.

References
1. Collinet P, Subtil D, Houfflin-Debarge V, Kacet N, Dewilde A, Puech F. Routine CMV screening during pregnancy. Eur J Obstet Gynecol Reprod Biol 2004; 114: 3-11.
2. Revello MG, Gerna G. Diagnosis and management of human cytomegalovirus infection in the mother, fetus, and newborn infant. Clin Microbiol Rev 2002; 15: 680-715.
3. Revello MG, Gerna G. Pathogenesis and prenatal diagnosis of human cytomegalovirus infection. J Clin Virol 2004; 29: 71-83.
4. Liesnard C, Donner C, Brancart F, Gosselin F, Delforge ML, Rodesch F. Prenatal diagnosis of congenital cytomegalovirus infection: prospective study of 237 pregnancies at risk. Obstet Gynecol 2000; 95: 881-8.
5. Lazzarotto T, Varani S, Guerra B, Nicolosi A, Lanari M, Landini MP. Prenatal indicators of congenital cytomegalovirus infection. J Pediatr 2000; 137: 90-5.
6. Azam AZ, Vial Y, Fawer CL, Zufferey J, Hohlfeld P. Prenatal diagnosis of congenital cytomegalovirus infection. Obstet Gynecol 2001; 97: 443-8.
7. Nigro G, Anceschi MM, Cosmi EV; Congenital Cytomegalic Disease Collaborating Group. Clinical manifestations and abnormal laboratory findings in pregnant women with primary cytomegalovirus infection. BJOG 2003; 110: 572-7.
8. Kenneson A, Cannon MJ. Review and meta-analysis of the epidemiology of congenital cytomegalovirus (CMV) infection. Rev Med Virol 2007; 17: 253-76.
9. Ornoy A, Diav-Citrin O. Fetal effects of primary and secondary cytomegalovirus infection in pregnancy. Reprod Toxicol 2006; 21: 399-409.
10. Hyde TB, Schmid DS, Cannon MJ. Cytomegalovirus seroconversion rates and risk factors: implications for congenital CMV. Rev Med Virol 2010; 20: 311-26.
11. Krech U. Complement fixing antibodies against cytomegalovirus in different parts of the world. Bull World Health Organization 1973; 49: 103-6.
12. Tookey PA, Ades AE, Peckham CS. Cytomegalovirus prevalence in pregnant women: the influence of parity. Arch Dis Child 1992; 67: 779-83.
13. Gratacap-Cavallier B, Bosson JL, Morand P, et al. Cytomegalovirus seroprevalence in French pregnant women: parity and place of birth as major predictive factors. Eur J Epidemiol 1998; 14: 147-52.