

Full Length Research paper

Detection of cytomegalovirus (CMV) antibodies or DNA sequences from ostensibly healthy Iranian mothers and their neonates

Seyed Hamidreza Monavari^{1*}, Hossein Keyvani², Bahman Abedi Kiasari³, Hamidreza Mollaei², Mehdi Fazlalipour², Mostafa Salehi Vaziri² and Fatemeh O. Fallah⁴

¹Department of Virology and Antimicrobial Resistance Research Center, Tehran University of Medical Sciences, Tehran, Iran.

²Department of Virology, Tehran University of Medical Sciences, Tehran, Iran.

³Human Viral Vaccine Department, Razi Vaccine and Serum Research Institute, Hesarak, Karaj, Iran.

⁴Paediatric Disease Department, Valiasr Hospital, Imam Khomeini Hospital, Tehran University of Medical Sciences, Tehran, Iran.

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Cytomegalovirus (CMV) remains the most common cause of viral intrauterine infection. The objective of this research was to determine the prevalence of at-risk pregnancies for congenital cytomegalovirus transmission in a randomly selected pregnant women and their newborns. Enzyme Link Immunosorbent Assay (ELISA) and real-time polymerase chain reaction (PCR) were utilized to screen the sera of mothers (n = 100) and consecutive umbilical cord blood samples from their newborn (n = 100). Of the 100 mother's sera analyzed, 100 (100%) and 3 (3%) were positive for cytomegalovirus IgG and IgM antibodies, respectively. Of the 100 cord serum specimens analyzed, 99 (99%) and 2 (2%) were positive for cytomegalovirus IgG and IgM antibodies, respectively. Cytomegalovirus DNA was detected in 4 out of 100 (4%) cord blood samples of newborns. From four CMV DNA positive cases, Case 1 had no IgM in cord serum, but had IgM in mother's sera. Cases 2 and 4 were positive for IgM in both mother's sera and cord serum. Case 3 had no detectable CMV IgM in sera and cord serum. As many as 66 and 100% of CMV IgM-positive women in this study also had CMV IgM and CMV DNA in their delivery cord blood samples, respectively suggesting an increased risk of congenital CMV infection in those pregnancies. A paired women sera/cord blood CMV IgM-negative was found to be positive for CMV DNA. The data may also suggest the utility of PCR in place of CMV IgM as a diagnostic method for congenital CMV infection.

Key words: Cytomegalovirus, cord blood, congenital disease, real-time polymerase chain reaction (PCR), antibodies.

INTRODUCTION

Human cytomegalovirus (HCMV) remains the most common cause of viral intrauterine infection and the major cause of congenital defects in developed countries for over 20 years (Munro et al., 2005; Boppana et al., 2001). Involvement of CNS is the most common and

important clinical manifestations of congenitally infected infants (Schleiss and Choo, 2006; Dollard et al., 2007).

Transmission can occur during pregnancy or after birth, from breast milk, cord blood, saliva, urine, fomites and other sources (Lazzarotto et al., 2004). Some of infants are infected during delivery, through breast milk or following the transfusion of CMV-contaminated cord blood to a sero-negative newborn that is not protected by maternal antibodies. Congenital CMV infection is identified by viral isolation from the infant at birth or within

*Corresponding author. E-mail: hmonavari@yahoo.com.
Tel/Fax: +98(021)88602205.

3 weeks of birth and diagnosis beyond that age may indicate acquired infection from exposure to virus in the birth canal or breast milk (Daiminger et al., 2005; Revello and Gerna, 2002). Cord blood is a sample of blood collected from the umbilical cord at the time of birth and may help mother to child transmission of CMV and causes congenital CMV infection. Classically, the gold standard diagnostic test for congenital CMV infection in cord blood has been viral isolation but the cell culture of CMV takes long time and expensive. Alternative approach, polymerase chain reaction (PCR) is especially promising for its high sensitivity, specificity and relative simplicity (Demler et al., 1998; Xu et al., 1993).

The presence of CMV DNA in cord blood of infected pregnant women could be a risk marker for transmission of the virus to the fetus. Screening for congenital CMV may lead to more accurate diagnosis of infant mortality and newborn screening may help identify both asymptomatic and misdiagnosed cases of congenital CMV. The study determined the prevalence of at-risk pregnancies for congenital cytomegalovirus transmission in a randomly selected population by detection of cytomegalovirus specific antibodies (IgG and IgM) in the sera of the mothers and cytomegalovirus specific antibodies (IgG and IgM) and DNA in cord bloods from their newborns.

MATERIALS AND METHODS

Specimen collection and processing

Two groups of specimens were included in the study: Group I consisted of serum specimens taken from 100 pregnant women who visited the Clinic of Rasoul Akram Hospital, Tehran, Iran, immediately after delivery in the period of April, 2010 and May, 2011 were included in the study. Group II consisted of consecutive cord blood specimens (n = 100) taken from their newborn cord in the period of April, 2010 and May, 2011. The method of collection and processing of cord blood has been described (Kogler et al., 1996). Patients gave their full informed consent at the time the samples were taken. Tehran University of Medical Science (TUMS) ethics committee approval was granted in March, 2010 to test these samples for the presence of congenital infections. The specimens were stored in the cord blood and serum bank of the Tehran University of Medical Science (TUMS) separately.

Real-time polymerase chain reaction (PCR) assay

Nucleic acid extractions were performed manually with a High pure viral nucleic acid kit (Roche, Germany), according to manufacturer's instructions and stored at -20°C until use. The real-time PCR was carried out using SYBR Green Master Mix reagents containing 5 units Taq polymerase, 0.01% gelatin, 0.6 µM of each primer, 200 µM of each deoxynucleotid triphosphate, 5 µl of reaction buffer (50 mM KCl, 10 mM tris-HCl, pH = 8.3) and 1.5 mM MgCl₂. Samples were subjected to 1 cycle at 50°C for 2 min and at 95°C for 10 min, followed by 40 cycles at 95°C for 20 s, 60°C for 40 s using a thermal cycler (Rotor Gene 6000, Corbett Research). SYBR Green I fluorescence was detected and plotted using Rotor-gene 6000 series software (Corbett Research) during the 60°C extension phase for each cycle. Threshold cycles and log starting quantities

for patient samples were calculated. Forward and reverse primers were as follows: GTGTGGGACATAGGCCAGAG and GCGACATCC CCGCCTACTAC (201-bp product). Standard precautions were taken to avoid sample-to-sample contamination and PCR product carry-over. Sterile water, normal serum control and a negative serum (non reactive on multiple assays) were used as negative controls. CMV plasmid containing gpB region was used as positive control.

Serological assay

Paired sera and cord serum samples collected from 100 pregnant women and their newborn were examined for the presence of CMV IgG and IgM. Enzyme link immunosorbent assay (ELISA) was carried out using commercial ELISA kit (Third generation ELISA kit; RADIM, Italy).

RESULTS

Patient characteristics

The median age of the 100 pregnant women was 34 years. The age distribution of the study population was: < 29 years with one birth; 29.1%; > 29 years with more than one birth; 70.9%. The male to female ratio of babies was 1.31:1. There were no signs of primary infections during pregnancy in pregnant women. Their pregnancies were uncomplicated. All babies were delivered at term and were apparently healthy. They had a normal birth weight, height and head circumference. Thirty percent of these infants were born by cesarean section. All mothers and their children were discharged from the hospital 4 days after delivery. Clinical data of CMV positive patients are shown in Table 1.

Cytomegalovirus polymerase chain reaction (PCR) and antibody assays

The results of the cytomegalovirus PCR and antibodies tests in women sera and cord blood are presented in Table 2. Consecutive umbilical cord blood samples from newborn (n = 100) were analysed for cytomegalovirus DNA using real-time PCR. Cytomegalovirus DNA was detected in 4 out of 100 (4%) cord blood samples from newborns. Paired women sera and cord serum samples (n = 100) were analysed for IgG antibody levels against CMV using ELISA. Of the 100 mother's serum samples and 100 cord serum samples analysed, 100 (100%) and 99 (99%) have detectable levels of CMV IgG antibodies respectively. Sera samples of pregnant mothers (n = 100) and cord serum (n = 100) were analysed for IgM antibody levels against CMV using ELISA. Of the 100 sera and 100 cord serum samples analysed, 3 (3%) and 2 (2%) have detectable levels of CMV IgM antibodies respectively. In total, 4 cases had CMV IgM or CMV DNA identified in women sera or cord blood specimens of their neonates. Case 1 had no IgM in cord serum, but had IgM

Table 1. Clinical presentation and demographics of CMV positive cases.

| Pair no. | Mothers | | | | | Infants | | | | | |
|----------|---------|------------------------|-------------------|------------------|-----------------|-------------------|--------|------------------|-------------|--------------------|-------------------|
| | Age | Gestational age (week) | Mode of pregnancy | Mode of delivery | Prior pregnancy | Sign of infection | Sex | Birth weight (g) | Height (cm) | Head circumference | Sign of infection |
| 1 | 33 | 38 | Uncomplicated | Spontaneous | 1 | None | Male | 2900 | 50 | 33 | None |
| 2 | 35 | 38 | Uncomplicated | Spontaneous | 1 | None | Female | 3200 | 55 | 35 | None |
| 3 | 27 | 38 | Uncomplicated | Caesarean | 0 | None | Female | 3000 | 49 | 35 | None |
| 4 | 29 | 40 | Uncomplicated | Spontaneous | 0 | None | Male | 3300 | 52 | 36 | None |

Table 2. Comparison of the ELISA and PCR results for CMV positive patients.

| Pair no. | Mothers | | Infants | | |
|----------|----------|----------|----------------|----------------|----------------|
| | Sera IgG | Sera IgM | Cord serum IgG | Cord blood IgM | Cord blood PCR |
| 1 | + | + | + | - | + |
| 2 | + | + | + | + | + |
| 3 | + | - | + | - | + |
| 4 | + | + | + | + | + |

in sera and CMV DNA in cord blood. Cases 2 and 4 were identified as positive for IgM in both sera and cord serum and CMV DNA in cord blood. Case 3 had no detectable CMV IgM in sera and cord serum; however she had CMV DNA in cord blood.

DISCUSSION

The seroprevalence of CMV varies according to studies conducted in different parts of the world. But, it has been reported that 0.2 to 2% of live birth have congenital CMV infection, considering as the leading cause of congenital infections worldwide (Demmler, 1991; Brown and Abernathy, 1998; Kumar et al., 1973). The fetus is at risk of acquiring CMV infection either

intrauterine or delivery. Intrauterine transmission of CMV infection may occur following either primary or recurrent infection (Ahlfors et al., 1988; Fowler et al., 1992). Involvement of central nervous system (CNS), including late central nervous system sequelae, primarily sensory-neuronal deafness is the most important clinical manifestation in 10 to 20% of such CMV congenital infected infants. Screening of mothers for CMV and early diagnosis play an important role to minimize CMV congenital infection and its serious consequences.

Molecular and serological assays were utilized to screen CMV congenital infection in pregnant women sera and cord blood of their newborns at the time of delivery and analyzed the distribution of CMV infection in a group of population with no history of CMV infection. The study showed a

frequency of 4% for CMV infection determined by PCR in cord blood samples from newborns compared with 2% CMV infection identified by IgM assay and 99% IgG positivity; 100% IgG positivity and 3% IgM positivity in their mothers as tested by ELISA.

The seroprevalence of CMV IgG antibody in pregnant women (100%) and the frequency of maternal infection (3%) obtained in this study is similar to the previous study in Iran (Tabatabaee and Tayyebi, 2009). They studied the prevalence of CMV antibody in 1472 pregnant women in the period of January, 2007 and July, 2007. The study reported a frequency of 97.69% for the seroprevalence of CMV IgG antibody and an active maternal CMV infection in 4.3% of the population. In our neighbour country, Turkey, the rate of CMV seropositivity was reported to be

98.5% and the prevalence of maternal CMV infection was reported to be 1.2% (Satilmis et al., 2007). A study in Cuba (Kourí et al., 2010) screened 1131 pregnant women for the presence of CMV IgG and IgM in the period of 2007 to 2008. They found that most women (92.7%) were CMV IgG seropositive. Primary and active non-primary infection was reported in 20 (1.77%) and 7(0.62%) of pregnant women.

It was reported that the rate of CMV transmission in seropositive mothers undergoing recurrent infection and pregnant women with primary infection is 0.2 to 2% and 20 to 40%, respectively (Boppana et al., 1999; Stagno and Whitley, 1985). As the seroconversion of pregnant women studied here was not previously monitored, the detection of CMV IgG does not lead to suspicion of primary infection. The study did not perform IgG avidity assay and the differentiation of primary and non primary infection is difficult. IgM assay was performed and 3% of pregnant women and 2% of cord serum of their newborns showed IgM positivity. Only two cord serum samples have detectable CMV IgM antibodies that is less than the IgM positivity in their corresponding mothers (n = 3). With the fact that IgM does not cross the placental barrier, these two babies may have intrauterine CMV infection. Detection of specific CMV IgM antibodies is a serological based method for diagnosis of primary CMV infection (Mace et al., 2004). However, different situations can be associated with the presence of IgM antibodies including the recurrent infection, the convalescent phase of a primary infection, the persistence of IgM or IgM crossreactivity due to herpes viruses other than CMV (Hodinka, 2003). Detection of IgM may be related to a primary infection occurring during pregnancy, although IgM can also be detected during reactivations. It has been previously proved that the main risk of intrauterine infection is associated with a primary infection during pregnancy (Fowler et al., 1992). However, with the fact that none of pregnant women in this study showed clear history of CMV infection during pregnancy and no IgM-positive pregnant mother or positive baby gave symptoms and signs suggestive of CMV infections suggesting that intrauterine infection had occurred from a reactivated, asymptomatic infection with CMV.

The study revealed a variation in ELISA and PCR results between mother sera and cord blood. Due to placental transfer of IgG, all mother's serum and cord serum samples revealed identical results for IgG antibodies against CMV. Paired mother sera/cord serum in 2 cases (case 2 and 4) showed IgM positivity in both sera and cord serum and DNA positivity in cord blood suggesting an active CMV infection in both mother and her baby. As the IgM does not cross the placental barrier, the IgM obtained in cord serum samples could originated from an active CMV infection occurred in babies during pregnancy or delivery. Detection of CMV DNA in cord blood by PCR may support this hypothesis. Case 3 had no detectable CMV IgM in sera and cord serum; however

she had CMV DNA in cord blood suggesting an active CMV infection diagnosed only by PCR. Case 1 had no IgM in cord serum, but had IgM in sera and CMV DNA in cord blood suggesting an active CMV infection in both mother and baby. The most interesting finding in this study was that in ceases 1 and 3, PCR could detect CMV DNA in cord blood samples, while there was no detectable IgM in sera of mother (case 3) and cord serum (cases 1 and 3). Although, IgM assay is still considered as a reasonable tool for congenital CMV infection diagnosis (Melish and Hanshaw, 1973) it was reported that only 45 to 80% of babies congenitally infected with CMV could be recognized by detection of IgM (Griffiths et al., 1982). These data are further supported by the present study with the finding that in two cases of the 4 congenital infected cases identified by PCR could IgM be found. The data show that the rate of congenital CMV infection detected by PCR (4%) is higher than that detected by IgM assay (2%). The utility of PCR in place of CMV IgM as a diagnostic method for congenital CMV infection is the most important finding of this study. PCR has been previously recognized as an important screening tool for congenital CMV infection. Also, to differentiate an active IgM case from non-active or false positive IgM case, PCR and virus detection has been suggested (Demler et al., 1998; Kourí et al., 2010). CMV DNA detected in specimens from 9 out of the 27 pregnant women by PCR. They suggested that mothers with active infection are at risk to have congenitally infected children. Also, no specific IgM could be in the fetal sera, the serological methods are unable to show this transmission. Thus, PCR method used here is the most suitable technique to screen CMV infection in newborns.

Rapid screening and accurate diagnosis of active cytomegalovirus (CMV) infection are needed for complication prediction, treatment, infection control, and reducing the severity of the disease. PCR and an antibody assay are common methods for cytomegalovirus (CMV) infection detection, however, PCR may lead to more accurate diagnosis of maternal and congenital CMV infection. Future studies with larger number of samples and follow up the positive cases are required to determine the clinical impact of congenital CMV infections.

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