Original Article

A Two-Step Strategy for Detecting Intrauterine Cytomegalovirus Infection with Clinical Manifestations in the Mother, Fetus, and Newborn

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SUMMARY: We selected a high-risk group based on clinical manifestations and performed virological tests to detect intrauterine cytomegalovirus (CMV) infection. We tested the efficacy of this detection protocol in this study. We analyzed 2,309 newborns at 22 weeks or more of gestation from January 1992 to December 2000. Clinical manifestations of the mother, fetus, and newborn were used in the initial step to identify the high-risk group. For the high-risk group, if the causes of clinical manifestation remained unclear, we assayed for CMV DNA in the amniotic fluid, umbilical cord blood, or newborn urine using polymerase chain reaction (PCR) as a second step. Positive PCR results were confirmed by isolating CMV. The initial step detected 287 high-risk cases from 2,309 deliveries. In this group, 100 cases did not have reasonable explanations for the clinical manifestation. In the second-step PCR, intrauterine CMV infection was diagnosed in 10 of 100 cases (10%). The initial clinical classification reduced the proportion of cases needing laboratory investigation to 4%. Thus, our strategy detected intrauterine CMV infection in as many as 10% of high-risk patients after the first step, which is much higher than the general screening rate.

INTRODUCTION

Intrauterine infection by cytomegalovirus (CMV), a neurotropic virus, is one of the leading causes of mental retardation and other neural defects in infants. Recent data suggest that only 10% of cases of cerebral palsy are caused by intrapartum asphyxia (1-3) and that most cases of cerebral palsy are caused by developmental defects, migrational defects, infections, toxins, and other causes. Intrauterine CMV infection is a major concern for obstetricians and pediatricians because of its long-term sequelae. We have recently demonstrated that 40% of CMV-infected fetuses showed ominous fetal heart rate patterns during labor and delivery, including prolonged decelerations or recurrent late decelerations without acidemia (4). Thus, it is important to distinguish neurological sequelae caused by CMV infection from perinatal asphyxia.

At present, there are no commonly accepted guidelines for screening for intrauterine CMV infection during pregnancy and the neonatal period. Checking maternal serum for the presence of CMV IgM would detect those women at risk of transmitting the virus to the fetus. However, the presence of IgM can vary among different clinical situations, such as during the acute and convalescent phases of a primary CMV infection, or in the persistence of IgM antibody (5). Detection of CMV in the neonatal urine in the first postnatal week is the standard method of diagnosing intrauterine CMV infection. However, this is not practical for universal screening because specimen collection and laboratory procedures are time-consuming and not cost effective. Screening the serum for CMV IgM from newborns is an alternative, but it does not identify about one-third of CMV-infected children (6).

In Japan, CMV-seronegatives have been increasing among the younger generations (7). Therefore, maternal primary infection and subsequent intrauterine CMV infection are a major concern in the care of pregnant women in Japan.

In this study, we developed a two-step protocol to identify a group of women at high risk of intrauterine CMV infection, and we assessed the validity of our protocol.

MATERIALS AND METHODS

Subjects and detection protocol: This study was performed prospectively at the Perinatal Center, University of Miyazaki, a tertiary center, Miyazaki, Japan, between January 1992 and December 2000. In this period, 2,309 pregnant women delivered their babies, and all these women were enrolled in this study. The fetal heart rate was monitored throughout labor and delivery, and the umbilical cord blood gases and pH were measured in all subjects. A cranial ultrasonography was performed routinely immediately after birth. The maternal, fetal, and neonatal abnormalities relating to CMV infection (Table 1) were carefully reviewed as the first step. The clinical manifestations were defined as follows. Maternal fever was defined as a temperature exceeding 38°C and lasting more than 24 h. Maternal liver dysfunction was defined as elevated liver enzyme concentrations (aspartate aminotransferase > 100 IU/L and alanine aminotransferase > 100 IU/L). Oligohydramnios was defined as an amniotic fluid index of ≤5 cm, and hydramnios was defined as an amniotic fluid index of ≥25 cm. Ventricular dilatation was defined as a ratio of the lateral ventricular width (LVW) to the hemispheric width (HW) of ≥40%. LVW and HW were measured according to the method described by Johnson et al. (8). Intrauterine growth restriction (or light for date)
was defined as birth weight below the 10th percentile for gestational age according to the Japanese standard birth weight curve (9). Those who had one or more of these abnormal findings were selected for further analysis.

If the causes of the above-mentioned clinical manifestations could not be specified, the second step was performed after obtaining informed consent from the pregnant woman or the newborn’s parents. Amniotic fluid or umbilical cord blood was used to detect CMV infection when available. All newborn urine was used to detect CMV infection. Amniotic fluid was collected by transabdominal amniocentesis under ultrasound guidance. Umbilical cord blood was collected soon after birth. A newborn urine sample was collected using a collector bag within the first 2 days of life. These three different specimens were examined for CMV DNA by polymerase chain reaction (PCR) with CMV-specific oligonucleotides (5,10), and CMV was isolated to confirm a positive PCR result.

**Detection of CMV-DNA by PCR:** DNA extraction: CMV-DNA was extracted from all samples using EXTRAGEN manufacturer’s instructions.

PCR: The oligonucleotides were located within exon 4 of the CMV major immediate-early gene. The oligonucleotide IE-S was: (5’-TATAACCCAGAGGGAGAAAAGTTCA-3’). The oligonucleotide IE-AS was: (5’-ATAAGCCATAATCTCAATCGGGAGGAG-3’). This resulted in a 426-bp PCR product.

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Amplification of a 426-bp product in the PCR indicated the presence of CMV-specific DNA in the DNA sample. The reaction mixture was incubated in a RoboCycler® (Stratagene, La Jolla, Calif., USA) as follows: 94°C for 5 min; 30 cycles of 94°C for 1 min, 60°C for 1 min, and 72°C for 1 min; and 72°C for 5 min. Five microliters of the amplified DNA product was resolved by electrophoresis on 1.3% agarose gels and visualized by ethidium bromide staining. Amplification of a 426-bp PCR product in the PCR indicated the presence of CMV-specific DNA in the DNA sample examined. The CMV DNA was detected by PCR and agarose gel electrophoresis with a detection limit of 5,100 copies of target DNA per reaction.

**Virus isolation:** The specimens were filtered through filters with a pore size of 0.45 μm and then inoculated onto human embryonic lung fibroblast (HEL) monolayers in a 24-well plastic plate. The cultures were replenished with maintenance medium twice a week and monitored over 2 months for the appearance of the cytopathic effects characteristic of CMV. CMV isolates were identified by the direct immunoperoxidase technique using a horse radish peroxidase (HRP)-conjugated F(ab’)2 fragment of human monoclonal antibody (humab C7), designated HRP-C7 (11).

**RESULTS**

As shown in Figure 1, 287 cases were identified as being in the high-risk group based on clinical manifestations in the first step; 187 of these had plausible causes (Table 2). The remaining 100 cases proceeded to the second step, and 10 of these were positive for CMV DNA by PCR and confirmed to have intrauterine CMV infection by virus isolation using the newborn urine sample. This resulted in a detection rate of 0.4% in all of the 2,309 deliveries and 10% (10/100) of the cases evaluated by laboratory tests (Figure 1).

Table 3 shows the abnormal manifestations of the 10 intrauterine CMV-infected cases in the mother, fetus, and neonate. Clinical signs leading to CMV detection were maternal (Cases 3 and 5), fetal (Cases 1 and 6), or neonatal (Cases 2, 4, 7, 8, 9, and 10). The neurological development was followed in 10 PCR-positive children (age range, 2 - 10 years). Three showed neurological sequelae such as mental retardation, hearing loss,

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<th>Mother</th>
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undiagnosed out of 2,309 deliveries. Thus, by using our strategy, only 11 cases remained without apparent diagnoses could be reasonably explained by one or more diagnostics were identified retrospectively by a precise history taking when intrauterine CMV infection was confirmed during the neonatal period. For example, in Case 1, mild maternal motor development at 2 years of age, but the neurological development gradually improved thereafter (Case 2). Some clinical manifestations were not noticed antenatally because they were mild, but these were detected during the neonatal period. For example, in Case 1, mild maternal symptoms were identified retrospectively by a precise history taking when intrauterine CMV infection was confirmed during the neonatal period. In Case 2, the fetal ventriculomegaly was mild. However, intrauterine CMV infection was diagnosed in the urine specimen during the neonatal period after a subependymal cyst was detected by routine neonatal cranial ultrasonography after birth.

In 79 of the 90 PCR-negative cases, the clinical manifestations could be reasonably explained by one or more diagnoses (Table 4), although 11 cases remained without apparent diagnosis. Thus, by using our strategy, only 11 cases remained undiagnosed out of 2,309 deliveries.

### DISCUSSION

The aim of this study was to develop a protocol for the effective detection of groups at risk for intrauterine CMV infection. We used clinical manifestations as the indicators for the first screening step. For the high-risk cases identified in the first step, CMV was identified in amniotic fluid, umbilical blood, or urine using PCR in the second step. This strategy enabled us to detect intrauterine CMV infection in as many as 10% of cases.

Universal maternal screening for CMV infection during pregnancy is controversial for the following reasons: (i) it is difficult to predict fetal damage; (ii) there is no approved effective treatment for the fetus; (iii) an effective vaccine for pregnant women is not yet available; and (iv) 90% of the affected infants are asymptomatic at birth and there are no absolute indications for treatment (12,13).

Intrauterine CMV infection is of great clinical importance because of the variety of clinical sequelae, particularly the neurological manifestations. Sameshima et al. reported three cases of cerebral palsy in 5,522 antepartum low-risk pregnancies; two of the three children with cerebral palsy had intrauterine CMV infection (14). The incidence of intrauterine CMV infection is estimated to be 30,000 - 40,000 newborns each year in the United States; about 9,000 of these children have permanent neurological sequelae (15). The death rate of symptomatic intrauterine CMV infection is about 30% (16). We have previously shown that CMV-infected fetuses are more likely to have abnormal intrapartum fetal heart rate patterns than noninfected fetuses. This suggests that the perinatal detection of CMV is necessary to distinguish CMV-related neurological sequelae from hypoxic-ischemic encephalopathy (4).

In our two-step procedure, the percentage of cases to be investigated is reduced to 4% of all newborns. Halwachs-Baumann et al. reported that CMV-IgM screening of maternal and umbilical blood at the delivery of high-risk neonates reduced the percentage of newborns needing further investigation to 4% (6). Our procedures are less time-consuming and more cost-effective.

One major limitation of our study is the risk of not identifying asymptomatic cases. However, in our study, no infants without clinical manifestations developed cerebral palsy, mental retardation, or sensorial neuronal hearing loss, except for premature babies or babies asphyxiated at birth. We also detected intrauterine CMV infection in 0.4% of all deliveries, a detection rate that corresponds to the general screening rate (5,17).

Intrauterine CMV infection that shows all the typical symptoms, such as hepatosplenomegaly, jaundice, petechiae, chorioretinitis, microcephaly, and intracranial calcification,
is extremely rare. Thus, we initially selected a high-risk group that had at least one of these clinical symptoms (Table 1). In our study, 30% of the infected cases had only one abnormal finding (e.g., intrauterine growth restriction, petechiae, or subependymal cyst). Because detecting clinical manifestations is difficult in some mild cases, we encourage clinicians to perform a routine cranial ultrasonography to detect abnormal findings immediately after birth.

In conclusion, our strategy is useful for identifying women at high risk of intrauterine CMV infection and is more efficient than universal screening of maternal serum IgM. Although the natural course of intrauterine CMV infection is not well understood, we believe our strategy is more effective for detecting intrauterine CMV infection for clinical study.

REFERENCES