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■ Case report ■

Postnatal cytomegalovirus infection in an extremely premature infant transmitted via breast milk: A case report

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= Abstract =

Cytomegalovirus (CMV) is one of the most commonly encountered viral pathogens in newborn infants and is found in 0.3-2.4% of all live births. It has been demonstrated that 40-96% of seropositive mothers shed the virus via their breast milk. Breast milk containing CMV can cause almost one-third of CMV infections occurring in infants. A case of postnatal CMV infection in an extremely premature infant (gestational age 24^{+5} weeks, birth weight 750 g) transmitted via breast milk is presented. For neonatal intensive care unit (NICU) management of severe thrombocytopenia, anemia, and sepsis syndrome, the infant received repeated transfusions of platelets; intravenous (IV) immunoglobulins; and gamma-irradiated, filtrated packed red cells and was fed her mother's breast milk since the second week of life. CMV infection was diagnosed with positive CMV immunoglobulin M (IgM) and positive urine CMV culture at the second month of life. Considering the negative CMV IgM and urine CMV culture at birth, postnatally-acquired CMV infection was suspected and confirmed with completely identical nucleotide sequence alignments of the infantile blood isolate and the maternal breast milk isolate. To our knowledge, this is the first case of proven postnatal CMV infection transmitted via breast milk in an extremely premature infant in Korea. (Korean J Pediatr 2009;52:1053-1058)

Key Words : Cytomegalovirus, Glycoprotein B, Breast milk, Infant, Very low birth weight

Introduction

Cytomegalovirus (CMV) is the most common cause of intrauterine and perinatal infections worldwide[1]. CMV can be transmitted in three distinct modes; transplacentally (congenital CMV) or through ingestion of infected body fluids such as maternal genital secretions during the intrapartum (perinatal CMV), or through ingestion of infected breast milk (postnatal CMV)[2]. Acquisition of CMV through breast milk has been identified as the most important route of postnatal transmission[3, 4]. Transmission of CMV to infants who have been fed with CMV-positive breast milk had been reported to be as high as 38-70%[5, 6]. Approximately 87 percent of women are reported to have CMV antibodies by child bearing age in South Korea[7]. In another study, 96 percent of pregnant women had CMV antibodies by the end of the third trimester[8]. Postnatal CMV infection via breast milk in a community with such high prevalence of maternal CMV immunity as South Korea would be an important issue in determining the best feeding practices for very low birth weight infants. We report a case of an extremely low birth weight infant in whom, by nucleotide sequencing, CMV infection was confirmed to have been acquired through maternal breast milk.

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Case Report

A 31-year-old gravida 1 para 0 woman was referred to the emergency department due to preterm premature rupture of membranes and threatened labor. After admission, an extremely low birth weight female infant (750 g) was delivered at 24^{+5} weeks of gestation by Cesarean section. The infant had Apgar scores of one at 1 minute

and 3 at 5 minutes. Her general appearance was lethargic and cyanotic without spontaneous respiratory effort. Morphologic features showed no abnormalities except a single umbilical artery. Soon after delivery, the baby was intubated. The infant required resuscitation and was immediately transferred to the neonatal intensive care unit. Her laboratory tests showed a total leukocyte count of 8,300/μL with 50% neutrophils and 23% lymphocytes, hemoglobin 14.7 g/dL, hematocrit 44.9%, and platelet count 114,000/μL. C-reactive protein (CRP) was less than 0.3 mg/dL. Initial chest radiograph showed total hazziness of both lungs which was characteristic of hyaline membrane disease. Surfactant replacement therapy was started soon along with mechanical ventilation. Ampicillin and gentamicin were started empirically due to maternal premature rupture of membranes. On echocardiographical examination, the infant had a patent ductus arteriosus with left to right shunting, which required intravenous indomethacin treatment. Placental biopsy obtained on birth was reported as acute chorioamnionitis. Tests for serum Chlamydia and Mycoplasma polymerase chain reaction (PCR), serum CMV IgM, and urine CMV culture were all negative. The plasma value of immunoglobulin M was 43.1 mg/dL at birth, which was in the normal range.

Enteral feeding of breast milk from her mother was started 8 days after birth. For the management of thrombocytopenia and anemia of prematurity, the infant required repeated transfusions of gamma-irradiated, filtrated platelets and packed red cells from the third day after birth.

On the 17th day after birth, her respiratory condition, which deteriorated with direct hyperbilirubinemia (2.1 mg/dL), improved with critical care including empirical antibiotics (cefamezin and tobramycin). Her blood cultures, urine culture, and cerebrospinal fluid culture were all negative and on day 23 she was started on a one-week course of dexamethasone expecting to wean from the ventilator.

However, since day 30 her thrombocytopenia aggravated and neutropenia was observed, which required repeated transfusions of platelets and intravenous immunoglobulins, and recombinant human granulocyte-colony stimulating factors. Even on vancomycin and amikacin, she continued to be aggravated with decreased general activity, generalized petechiae, hepatosplenomegaly, and persistent direct hyperbilirubinemia (6.3 mg/dL), and amphotericin B was started for 'suspected' fungal infection. CMV IgM was positive on day 47 and urine CMV culture was positive on day 52. Negative CMV IgM and urine CMV culture at birth suggested that CMV infection was acquired postnatally, and breast milk feeding and amphotericin B were discontinued. Although congenital CMV was unlikely, since her clinical manifestations were worsening, ganciclovir therapy (6 mg/kg/dose every 12 hour) was started. During antiviral treatment, thrombocytopenia improved immediately and the viral shedding in urine disappeared. Ganciclovir was discontinued due to severe neutropenia. Cessation of ganciclovir rendered urine culture to be positive for CMV again, but the leukocyte count, platelets, CRP values, and bilirubin levels remained normal.

To clarify the mode of CMV infection acquisition, genotyping and nucleotide sequencing were performed on blood, urine and breast milk specimens isolated from infant and mother as previous described manner[9]. Approximately 296 bp size human CMV gB gene amplification products were detected from infantile blood, infantile urine, and maternal breast milk (Fig. 1). Restriction patterns on subsequent analysis of the viral UL55 region with restriction enzyme *Hinf* I and *Rsa* I revealed CMV gene products on both infant and maternal samples to be identical with Towne strain which is gB type 1 (Fig. 2). The alignments of the sequences were carried out on the gB PCR products of infantile blood, infantile urine, and maternal breast milk and the sequence data were compared with each other as well as with the published sequences of the GenBank database using the BLAST software. The nucleotide sequence alignments of the infantile blood and urine resulted to be completely identical with the maternal breast milk, and the sequences of the infantile blood (GenBank

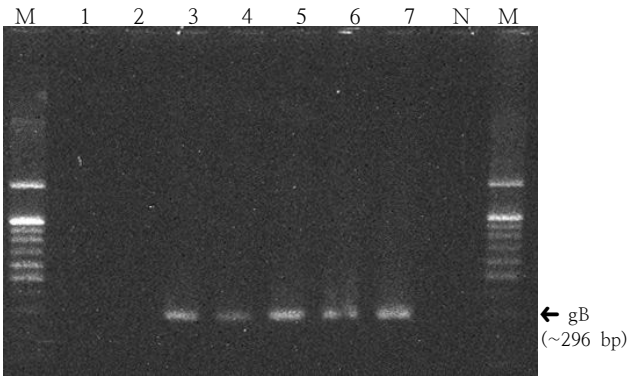

Fig. 1. Human CMV gB gene amplification products were detected from maternal blood, urine, breast milk, infantile blood, urine, and the CMV laboratory-adapted strains, Towne, and AD 169.

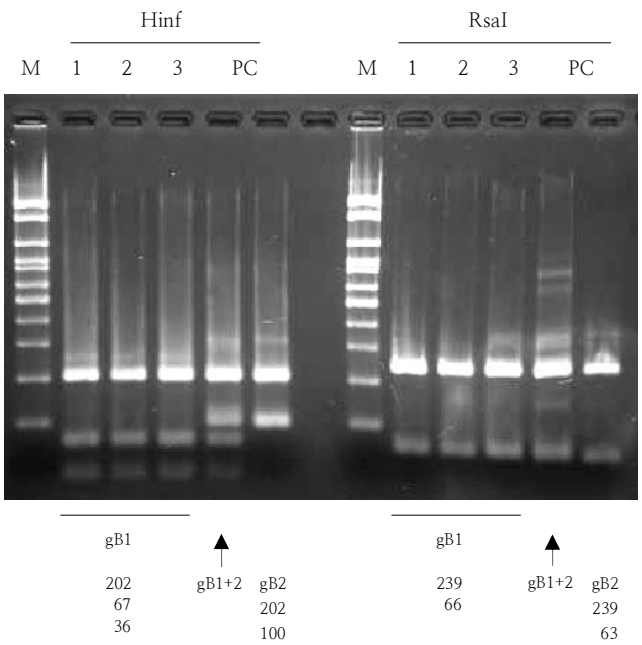

Fig. 2. Human CMV gB restriction patterns of the maternal blood, urine, breast milk, infantile blood, urine, and the CMV laboratory-adapted strains, Towne, and AD 169 with restriction enzyme *Hinf*I and *Rsa*I revealed CMV gene products on both infant and maternal samples to be identical with the Towne strain (gB type 1).

accession number FJ485728), infantile urine, and maternal breast milk were 96% and 100% identical to the corresponding portion of Towne strain (GenBank accession number M22343) and wild type Merlin strain (GenBank accession number AY446894), respectively (Fig. 3).

Cranial ultrasonography revealed germinal matrix hemorrhage on the left side, but no cerebral calcifications were seen. Ophthalmologic examinations revealed no abnormalities. The infant survived with no other long-term problems of prematurity such as chronic lung disease and retinopathy of prematurity. She was discharged home after clinical stabilization with a close follow-up. Hearing and neurological developments were normal at the age of 6 months. On growth evaluation, no microcephaly was seen.

Discussion

Distinguishing between congenital from postnatal CMV infection is important because of significant differences in their outcomes. Congenital CMV infection is characterized by sequelae such as intrauterine growth retardation, mi-

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CMV-TOWNE       GGAACTCGAACGTTTGGCCAACCGCTCCAGTCTGAATCTTACTCATAATAGAACCAAAAG 60
CMV-Merlin      ******G*****************************************G********** 60
Infant blood    ******G*****************************************G********** 60
Infant urine    ******G*****************************************G********** 60
Breast milk     ******G*****************************************G********** 60

CMV-TOWNE       AAGTACAGATGGCAACAATGCAACTCATTTATCCAACATGGAGTCGGTGCACAATCTGGT 120
CMV-Merlin      ***********************************G*******A****************** 120
Infant blood    ***********************************G*******A****************** 120
Infant urine    ***********************************G*******A****************** 120
Breast milk     ***********************************G*******A****************** 120

CMV-TOWNE       CTACGCCCAGCTGCAGTTCACCTATGACACGTTGCGCGGTTACATCAACCGGGCGCTGGC 180
CMV-Merlin      ************************************************************ 180
Infant blood    ************************************************************ 180
Infant urine    ************************************************************ 180
Breast milk     ************************************************************ 180

CMV-TOWNE       GCAAATCGCAGAAGCCTGGTGTGTGGATCAACGGCGCACCCTAGAGGTCTTCAAGGAACT 240
CMV-Merlin      *********G**************************G************T*G****** 240
Infant blood    *********G**************************G************T*G****** 240
Infant urine    *********G**************************G************T*G****** 240
Breast milk     *********G**************************G************T*G****** 240

CMV-TOWNE       TAGCAAGATCAACCCGTCAGCTATTCTCTCGGCCATCTACAACAAACCGATTGCCGCGCG 300
CMV-Merlin      C*******************C**************T********************** 300
Infant blood    C*******************C**************T********************** 300
Infant urine    C*******************C**************T********************** 300
Breast milk     C*******************C**************T********************** 300

CMV-TOWNE       TTTC 304
CMV-Merlin      **** 304
Infant blood    **** 304
Infant urine    **** 304
Breast milk     **** 304
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Fig. 3. Partial nucleotide sequences for glycoprotein B genes of cytomegalovirus isolated from blood and urine of a Korean newborn infant and her mother's breast milk were completely identical.

crocephaly, developmental delay, and deafness. Approximately 10% of congenitally infected infants have disease symptoms noted at birth, which may include hepatosplenomegaly, chorioretinitis, and skin manifestations including petechiae and purpura. In contrast, postnatal acquisition of CMV infection appears to be largely asymptomatic in term infants. However, it may have significant risk for low-birth-weight, premature infants[10]. The main reason for transmission of CMV from mother to postnatal child is breast milk feeding. Using highly sensitive methods like PCR to screen for viral DNA in breast milk or cell-free milk whey, it has been demonstrated that 40% to 96% of seropositive mothers shed the virus via their breast milk[11]. In different studies, postnatal transmission rates of CMV to term and preterm infants varied between 10% and 60%[4, 6, 12-14].

It has been indicated that CMV DNA in breast milk is the consequence of locally restricted virus reactivation in the breast rather than the consequence of a systemic virus reactivation[12]. CMV secretion into milk may begin in the first week postpartum with low viral load and reaches a maximum at about 4-8 weeks after birth with a decline from week 9-12 postpartum. The transmission event takes place close to the maximum of viral DNAlactia or virolactia, which normally coincide[15]. Significant DNA in breast milk is observed in breast milk up to day 40 postpartum, but the mechanism and site of human CMV reactivation during lactation are unknown at present.

Genotyping and nucleotide sequencing of maternal milk, infantile blood, and infantile urine samples could be a useful method to distinguish between congenital and postnatal CMV infections, as we confirmed postnatal CMV gB1 infection transmitted via maternal breast milk in our case.

Clinical studies suggest that gB genotypes of CMV strains may influence the clinical manifestations and outcome of acquired CMV infections. Studies of the glycoprotein genotypes distribution of CMV infections in premature babies, transplant recipients, or HIV-infected patients indicate that the gB1 genotype are predominant in different geographical regions of the world[16-18]. A study of distribution of CMV gB genotypes in hematopoietic stem cell transplant recipients in Korea showed gB1 predominance, but no significant association between the genotypes and diseases was found[9]. It has been demonstrated that the patients who survived CMV infections after bone marrow transplantation were more likely to excrete strains with the gB1 genotype than were patients who died[16]. The presence of gB2 in blood isolates was potentially associated with a greater risk of development of CMV retinitis in patients with AIDS[17]. Although the population in these studies were different, the favorable outcome of the infant in our case might not only be partially due to postnatal mode of CMV infection, but it can also be explained by the gB1 genotype. In recent studies, mixed infection with multiple gB genotypes has been shown to be associated with severe clinical manifestations[19], a hypothesis that needs additional confirmation. However, gB genotyping is only one of the multiple factors which may influence the outcome of the patient, and we should be cautious in predicting clinical outcomes on the basis of gB genotype alone.

In our case, intrapartum transmission in the birth canal as a mode of acquisition of CMV infection is less likely because the infant was born by Cesarean section. In addition, serum CMV IgM and urine CMV culture were negative on birth. The role of transfusion-acquired CMV infection could not be disregarded fully in our case because none of the blood products the patient received was examined for CMV. Because of high prevalence of CMV seropositivity, no screening of donor blood for CMV antibodies is performed routinely in Korea. With the increasing proportion of extremely premature infants in Korea, future strategies are needed to screen all blood products that are to be transfused to very low birth weight infants.

Based on previous studies, "inactivated" milk, which is treated by freeze-storing or pasteurization, reduces or destroys the viral load[20-22]. In these studies, it is suggested that various transmission rates despite high rate of CMV DNA in breast milk might be associated with the method of breast milk preservation. However, these studies demonstrate that despite virus inactivation, the transmission of CMV could not be prevented completely. Some other studies have proven that freeze-thawing of breast milk is not a safe intervention to prevent symptomatic CMV infection in preterm infants[23, 24]. More detailed data on the dynamics of CMV reactivation are needed to settle the debate on whether and how to inactivate CMV in breast milk.

Breast milk feeding is beneficial for the infant, especially in the extremely low birth weight infants. However, as most immature preterm infants are at greater risk than term infants to acquire a symptomatic CMV infection, a

procedure to prevent CMV transmission through seropositive breast milk should be sought for preterm infants. Furthermore, studies to determine long-term outcome of postnatal CMV infection acquired via breast milk in premature infants are required.

To our knowledge, this is the first case of postnatal CMV infection through breast milk in Korea confirmed by DNA genotyping and sequencing. It demonstrated the usefulness of sequence-based molecular typing for investigation of the epidemiology of CMV infections in the clinical setting. Further studies, which can correlate individual CMV genotypes with clinical indicators of severity and viral load, are awaited and will elucidate our understandings of CMV infection. In addition, approaches to innovative methods for virus inactivation of seropositive breast milk are needed to provide better principles of breast milk feeding in extremely premature infants.

한글 요약

산모의 모유를 통하여 감염된 극소 저체중 출생아에서의 거대세포바이러스 감염

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거대세포바이러스(CMV)는 신생아에서 가장 흔한 선천성 감염의 원인 중 하나이며, 모든 정상 출산아 중 약 0.3-2.4%에서 감염되어 있다. 혈청에서 거대세포바이러스 양성인 모체 중 40-96%에서 모유를 통한 바이러스의 배출이 증명되었으며, 거대세포바이러스에 감염된 모유를 통한 감염은 전체 영아 거대세포바이러스 감염의 약 1/3을 차지한다. 본 증례에서는 극소 저체중 출생아에서 모유를 통하여 발생한 거대세포바이러스 감염을 기술하였다. 환아는 생후 7일 경부터 모유 수유를 하였으며, 심한 혈소판감소증, 빈혈, 그리고 패혈증과 유사한 임상 양상에 대하여 반복되는 혈소판 수혈, 적혈구 수혈, 면역 글로불린 요법을 시행받았다. 거대세포바이러스 감염은 생후 2개월 경 혈청 CMV IgM 양성 및 소변 CMV 배양 검사 양성 소견을 통하여 진단하였다. 출생 당시 혈청 CMV IgM 및 소변 CMV 배양 검사가 음성이었으므로 선천성 감염은 배제할 수 있었다. 환아의 혈청과 모유의 핵산 배열 순서 분석을 통하여 동일한 바이러스에 의한 감염임을 증명하였다. 저자들은 국내에서 최초로 nucleotide sequencing 방법을 이용, 모유 수유를 통한 거대세포바이러스 감염이 발생한 극소 저체중 출생아의 예를 보고하는 바이다.

References

1) Numazaki K. Human cytomegalovirus infection of breast milk. FEMS Immunol Med Microbiol 1997;18:91-8.
2) Alford CA, Stagno S, Pass RF, Britt WJ. Congenital and perinatal cytomegalovirus infections. Rev Infect Dis 1990;12: S745-53.
3) Vochem M, Hamprecht K, Jahn G, Speer CP. Transmission of cytomegalovirus to preterm infants through breast milk. Pediatr Infect Dis J 1998;17:53-8.
4) Stagno S, Reynolds DW, Pass RF, Alford CA. Breast milk and the risk of cytomegalovirus infection. N Engl J Med 1980;302:1073-6.
5) Dworsky M, Yow M, Stagno S, Pass RF, Alford C. Cytomegalovirus infection of breast milk and transmission in infancy. Pediatrics 1983;72:295-9.
6) Maschmann J, Hamprecht K, Dietz K, Jahn G, Speet CP. Cytomegalovirus infection of extremely low-birth weight infants via breast milk. Clin Infect Dis 2001;33:1998-2003.
7) Shim YK. A serological survey for cytomegalovirus complement-fixing antibody among voluntary blood donors in Seoul area. Korean J Epidemiol 1981;3:99-104.
8) Sohn YM, Park KI, Lee C, Han DG. Congenital cytomegalovirus infection in Korean population with very high prevalence of maternal immunity. J Korean Med Sci 1992; 7:47-51.
9) Choi SM, Kim JH, Lee DG, Park SH, Choi JH, Yoo JH, et al. Distribution of human Cytomegalovirus gB genotypes and its association with diseases in hematopoietic stem cell transplant recipients in Korea; A preliminary report. Infect Chemother 2007;39:85-92.
10) Schleiss MR. Role of breast milk in acquisition of cytomegalovirus infection: recent advances. Curr Opin Pediatr 2006;18:48-52.
11) Meier J, Lienicke U, Tschirch E, Kruger DH, Wauer RR, Prosch S. Human cytomegalovirus reactivation during lactation and mother-to-child transmission in preterm infants. J Clin Microbiol 2005;43:1318-24.
12) Hamprecht K, Maschmann J, Vochem M, Dietz K, Speer CP, Jahn G. Epidemiology of transmission of cytomegalovirus from mother to preterm infant by breastfeeding. Lancet 2001; 357:513-8.
13) Asanuma H, Numazaki K, Nagata N, Hotsubo T, Horino K, Chiba S. Role of milk whey in the transmission of human cytomegalovirus infection by breast milk. Microbiol Immunol 1996;40:201-4.
14) Ballard RA, Drew WL, Hufnagle KG, Riedel PA. Acquired cytomegalovirus infection in preterm infants. Am J Dis Child 1979;133:482-5.
15) Hamprecht K, Maschmann J, Jahn G, Poets CF, Goelz R. Cytomegalovirus transmission to preterm infants during lactation. J Clin Virol 2008;41:198-205.
16) Fries BC, Chou S, Boeckh M, Torok-Storb B. Frequency distribution of cytomegalovirus envelope glycoprotein genotypes in bone marrow transplant recipients. J Infect Dis 1994;169:769-74.

17) Shepp DH, Match ME, Ashraf AB, Lipson SM, Millan C, Pergolizzi R. Cytomegalovirus glycoprotein B groups associated with retinitis in AIDS. J Infect Dis 1996;174:184-7.
18) Woo PC, Lo CY, Lo SK, Siau H, Peiris JS, Wong SS, et al. Distinct genotypic distributions of cytomegalovirus (CMV) envelope glycoprotein in bone marrow and renal transplant recipients with CMV disease. Clin Diagn Lab Immunol 1997; 4:515-8.
19) Coaquette A, Bourgeois A, Dirand C, Varin A, Chen W, Herbein G. Mixed cytomegalovirus glycoprotein B genotypes in immunocompromised patients. Clin Infect Dis 2004;39: 155-61.
20) Doctor S. Friedman S, Dunn MS, Asztalos EV, Wylie L, Mazzulli T, et al. Cytomegalovirus transmission to extremely low-birthweight infants through breast milk. Acta Paediatr 2005;94:53-8.
21) Mussi-Pinhata MM, Yamamoto AY, do Carmo Rego MA, Pinto PC, da Motta MS, Calixto C. Perinatal or early-postnatal cytomegalovirus infection in preterm infants under 34 weeks gestation born to CMV-seropositive mothers within a high-seroprevalence population. J Pediatr 2004;145:685-8.
22) Yasuda A, Kimura H, Hayakawa M, Ohshiro M, Kato Y, Matsuura O, et al. Evaluation of cytomegalovirus infections transmitted via breast milk in pretern infants with a real-time polymerase chain reaction assay. Pediatrics 2003;111: 1333-6.
23) Jim WT. Shu CH, Chiu NC, Kao HA, Hung HY, Chang JH, et al. Transmission of cytomegalovirus from mothers to preterm infants by breast milk. Pediatr Infect Dis J 2004;23: 848-51.
24) Lee HC, Enright A, Benitz WE, Madan A. Postnatal cytomegalovirus infection from frozen breast milk in preterm, low birth weight infants. Pediatr Infect Dis J 2007;26:276.