

Review Paper

Cytomegalovirus in Human Breast Milk: Risk to the Premature Infant

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ABSTRACT

Human cytomegalovirus (HCMV) can be transmitted through breast milk to neonates. Although healthy full-term infants rarely develop symptoms of CMV infection; premature or low-birth-weight infants can experience symptomatic infection that is occasionally severe. There is limited information on the long-term effects of postnatal CMV infection in premature infants, suggesting that these infants do not develop cognitive function delays or hearing loss, although those with intrapartum infection do. Readily available methods of treating breast milk to inactivate the CMV either diminish the immunologic and nutritive benefits of breast milk or incompletely inactivate the virus.

This review considers the data on measuring CMV in breast milk, the recent clinical studies on CMV transmission via breast milk, reported methods of inactivation of CMV in breast milk, and immunologic factors that may play a role in transmission. CMV-IVIG treatment needs further evaluation but appears promising. Recommendations are made to help address the issue of CMV transmission to premature infants in clinical practice in the neonatal intensive care unit (NICU).

INTRODUCTION

HUMAN CYTOMEGALOVIRUS (HCMV) is ubiquitous. In the normal immune host HCMV infection is usually asymptomatic. Individuals with an altered immune system because of infection (HIV), or immunosuppressive therapy (cancer, transplantation) often experience severe disease with CMV infection because of primary infection, reactivation disease, or new infection. Congenital CMV infection in the fetus causes significant morbidity and mortality, with approximately 7% of cases being clinically symptomatic at birth.¹ Approximately 15% of

the infants who are asymptomatic at birth with congenital CMV infection manifest developmental delay or sensorineural hearing loss later in life.² Postnatal infection in the full-term, well infant via breast milk containing HCMV has been described as “natural immunization” with virtually no risk of illness or sequelae.³

Perinatal CMV infection in premature or low-birth-weight (LBW) infants resulting from transmission of the virus from maternal cervical secretions, breast milk, or blood products can result in severe acute illness.⁴⁻⁸ In the last 10 years, the use of new techniques (separation of milk [cells and cell-free whey] for testing)

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and technology (detection of HCMV DNA and RNA via polymerase chain reaction [PCR] testing) has been utilized in a number of studies to elucidate the high rates of HCMV excretion in breast milk of seropositive women and trying to correlate that with clinical disease and laboratory abnormalities related to CMV infection in premature, LBW, and very-low-birth-weight (VLBW) infants.⁹⁻²¹ In light of this and other new data concerning CMV and human breast milk; this review discusses various issues and considerations relative to the use of breast milk in premature infants and the risk of symptomatic CMV infection.

CMV IN BREAST MILK

Early studies of the presence of CMV in human breast milk using cell culture technique on colostrum or unseparated milk samples demonstrated a prevalence of CMV in breast milk of 13% in women without consideration of their CMV serostatus. On the other hand, 68% was reported in lactating mothers of infants with known congenital CMV infection.^{3,6,22} More recent studies utilizing samples separated into cell-associated and milk whey fractions and newer techniques to identify CMV (PCR detection of DNA and RNA, high-speed centrifugation with a microculturing process) have demonstrated a much higher percentage of women excreting CMV in breast milk. In CMV-seropositive lactating women, the prevalence of CMV in their milk varied between 67% and 97%, whereas CMV-seronegative women never had CMV detected in their breast milk either by culture or the detection of CMV DNA.^{9,12,14,16,17,23,24} The pattern of CMV excretion in human breast milk has been reported to be "unimodal"²⁴ with low levels of virus in the first week postpartum increasing to a maximum between 4 to 8 weeks and declining through 10 to 12 weeks or longer postpartum. Although the pattern is similar in most lactating women, the amount of measured CMV DNA and CMV infectivity varies from woman to woman over the course of the excretion of CMV in breast milk. There is evidence to suggest that the peak DNA levels (DNA_{lactia}) in breast milk correlate with CMV infectivity

and transmission of CMV to the infant. No lower-limit of DNA_{lactia}, below which transmission does not occur, has been identified.^{13,24}

CLINICAL EVIDENCE OF HCMV TRANSMISSION VIA BREAST MILK IN PREMATURE INFANTS

Over 20 years ago, studies documented clinically significant CMV infection in premature infants because of exposure to maternal cervical secretions, breast milk, or blood products.^{6,7,25} Studies performed in the last 10 years have used methods to exclude potential horizontal transmission of CMV from cervical secretions (because of high rates of Cesarean section) or from blood products by leukocyte filtration of blood products. Congenital CMV infection was ruled out by screening infants for viruria in the first 3 weeks of life as confounding variables. These studies have reinforced the importance of fresh breast milk as a source of symptomatic CMV infection in premature or LBW infants.

An early study of premature infants reported 85% of seropositive mothers excreting CMV in their breast milk with transmission occurring only in the breastfed infants of the seropositive "CMV-excreting" mothers. Infection occurred in 59% (17/29) of the exposed breastfed infants and five of the VLBW infants (mean gestational age, 24.4 ± 0.5 weeks) developed severe symptoms. The symptoms included acute deterioration with a "sepsis-like" picture in four of the five infants, with apnea and bradycardia as well as leucopenia and thrombocytopenia. The VLBW infants developed symptoms at an earlier age (4 to 7 weeks) compared with the other 12 infants, who developed symptoms after 8 weeks postpartum.¹¹

A larger prospective study by the same investigators, utilizing DNA PCR demonstrated CMV in fresh breast milk (DNA_{lactia}) from 96% (73/76) of the seropositive women with virolactia (CMV positive culture from milk cells, whey, or both) in 76% (58/73). CMV infection occurred in 33 of 87 (38%) infants born to mothers with DNA_{lactia} but only one infant of a mother with DNA_{lactia} but no virolactia (detectable positive CMV culture of her breast

milk). DNA lactia and infectious virus were detected earlier in milk whey and milk cell cultures (a mean of 5 days postpartum) from mothers who transmitted CMV to their infants. Infected children had CMV detected in plasma or leukocytes before the detection of virus or DNA in urine or IgM seroconversion. Clinical or laboratory evidence of CMV infection occurred in 16 of 17 infected children (hepatopathy, neutropenia, thrombocytopenia) and four children (12%, 4/33) developed a "sepsis-like" illness. The risk of CMV transmission to these premature infants was related to the early onset of viral DNA and infectious virus in the breast milk. The average "incubation period" from birth to the onset of symptomatic CMV infection was 47 days.¹²

Other studies using fresh breast milk have documented varying rates of CMV transmission to premature infants. Mosca et al.¹⁴ documented 25% (5/20) postnatal CMV transmission to premature infants less than 34 weeks gestational age after exposure to CMV positive milk. None of these infants demonstrated clinical evidence of infection. CMV infection was demonstrated by the detection of viral DNA or virus in the infant's urine or saliva a median of 34 days after CMV was detected in the mother's milk. The authors proposed that the lower rate of infection and the lack of symptomatic infection could be related to lower levels of infectious virus in the breast milk or the administration of intravenous immunoglobulins commonly given in their neonatal intensive care unit (NICU).¹⁴ Miron et al.²¹ demonstrated CMV transmission in breastfed premature infants of seropositive mothers. Four of 70 neonates (5.7%) were infected in the first 3 to 7 weeks of life. One infant developed severe disease with acute respiratory changes, hemodynamic instability, and hepatosplenomegaly accompanying the identification of CMV in the urine. One of the children was asymptomatic, whereas the other two had transaminase elevations. All four infants were reported to have normal hearing and development at 24 months of age.²¹ Meier et al.²⁰ reported HCMV transmission in 55% (21/55) of infants exposed to DNA positive (DNA lactia) breast milk. One infant became CMV infected after exposure to breast milk without CMV DNA detected de-

spite the exclusion of congenital CMV infection or exposure to CMV positive blood products or donor milk. Only two infants had symptoms suggestive of CMV disease; however, the authors reported more frequent respiratory distress syndrome, bronchopulmonary dysplasia, and retinopathy of prematurity in the CMV infected children, which was not statistically significant from their occurrence in CMV negative infants. The two symptomatic children were treated with ganciclovir with clinical improvement.²⁰

Several other studies using treated or stored breast milk as well as fresh milk have demonstrated lower rates of CMV transmission to premature infants. Sharland et al.¹⁵ examined CMV transmission in infants less than 32 weeks gestational age, who received either banked expressed breast milk pasteurized and frozen at -20°C or maternal expressed breast milk that was frozen at -20°C before use for an unidentified period of time, "whenever possible." Only one of 18 infants (5.55%) receiving breast milk was asymptotically infected with CMV.¹⁵ Yasuda et al.¹⁶ reported 10% CMV transmission in 3/30 premature infants. The infants received raw breast milk sometime in the first 6 to 11 days of life with about half of the infants receiving fresh breast milk at least once. After that they received expressed breast milk frozen at -20°C with supplemental formula if needed. Breast milk from 87.5% of the seropositive mothers (26/30) was positive for DNA (DNA lactia). CMV DNA was detected in the breast milk within the first week, with most samples being positive by 2 weeks postpartum. The DNA levels peaked at 6 to 8 weeks postpartum. None of the three CMV infected infants were symptomatic.¹⁶ Jim et al.¹⁷ studied 42 premature infants (<35 weeks gestational age and <1500 g birth weight) in Taiwan born to 38 mothers, of whom 97.3% (36/38) were seropositive for CMV. CMV DNA was detected in 35 of 36 seropositive mothers, but only six had virus cultured from breast milk whey. The infection rate was 15% (6/40) exposed infants and infection occurred only in infants who received CMV culture-positive breast milk. Infected infants and their mothers showed significantly elevated CMV IgG levels at 5 days and 30 days postpartum compared with the uninfected in-

infants and mother pairs. The authors postulated that elevated CMV IgG concentration early on in lactating mothers or high CMV DNA PCR levels might be used as surrogate markers for risk of CMV transmission. They explained that their practice of freezing maternal breast milk at -18°C overnight or for several days before feeding the infants might be the reason for the lower transmission rate.¹⁷ Researchers from Brazil also reported a relatively lower rate of CMV transmission (22%, 21/95) to premature infants (<34 weeks gestational age and <1500 g birth weight) fed either pasteurized banked breast milk or refrigerated expressed breast milk (natural) (4°C for 8 hours maximum).¹⁸ In this population of 98% seropositive mothers, the authors also demonstrated that the CMV infection rate was higher in infants who received natural breast milk before or at 30 days of life (OR = 4.5 [95% CI = 1.14 to 17.6]) and in infants receiving natural breast milk for longer than 30 days (OR = 7.9 [95% CI = 1.5 to 41.3]). A clinical picture consistent with CMV infection was noted in 4.8% (1/21) of the CMV-infected children. Anti-CMV neutralizing antibodies were measured in the infants at birth, demonstrating a median titer of 128 with 85% of infants having titers greater than 64. Doctor et al.¹⁹ reported on CMV transmission to premature infants with birth weights <1000 g receiving fresh, refrigerated (4°C for ≤ 24 hours) or frozen (-20°C for up to 3 months) breast milk. The transmission rate was relatively low at 6% (4/65) with only one of the four infected infants demonstrating symptoms (liver abnormalities and a "sepsis-like" picture). The breast milk was not tested for the presence of CMV by culture or DNA PCR and of the 119 infants originally enrolled in the study only 92 infants had urine collections through 49 days of life for the detection of CMV. The CMV-infected infants were exposed to an average of 15.3% fresh breast milk of their total oral intake in the first 4 weeks of life compared to only 3.2% in the uninfected infants. The authors considered the amount of fresh breast milk received and the relatively short observation/testing period for CMV infection as possible explanations of the low transmission rate.¹⁹

There are limited data available on the long-term clinical outcomes of premature infants

with CMV infection owing to breastfeeding. Preterm and sick term infants with postnatally acquired CMV infection from any maternal source, cervical secretions, or breast milk were evaluated using head circumference, electroencephalogram, psychometric, audiology, neurology, and ophthalmology evaluation at 3 years of age and compared with matched controls for late sequelae of CMV infection.²⁵ The full-term infants (>2000 g birth weight) did not have an increase in long-term sequelae in association with postnatal CMV infection, as noted in other studies.^{6,8,26} They did not document an increased occurrence of sensorineural hearing loss or late/chronic chorioretinitis with postnatal CMV infection. In the 34 CMV-infected infants with birth weights less than 2001 g, those with early urinary excretion of CMV (before 8 weeks of age or <36 weeks corrected gestational age) or with severe neonatal cardiopulmonary disease were more likely to have severe handicaps (developmental quotient <70, severe neuromuscular impairment, or limitations of vision or hearing). Jim et al.¹⁷ observed CMV infected ($n = 6$) and noninfected ($n = 34$) premature infants at a corrected age of 6 months and found no increased sensorineural hearing loss or neuromotor developmental delay in the infected children.¹⁷ Vollmer et al.²⁷ reevaluated 22 postnatally CMV infected premature infants with 22 CMV negative controls at 2 to 4.5 years of age and found no difference between the two groups in terms of growth, sensorineural hearing, neurologic, and neurodevelopmental status. Miron et al.²¹ reported normal hearing and development evaluated at 24 months of age during routine follow-up in four premature infants with breast milk-acquired CMV infection.²¹

CYTOMEGALOVIRUS INACTIVATION IN BREAST MILK

The eradication of viable, infectious CMV from human breast milk without decreasing its immunologic or nutritional benefits would be a first step to protecting premature infants from the CMV infection through breast milk.²⁸

Holder pasteurization of milk (heating to 62.5°C for 30 minutes) effectively eliminates vi-

able CMV, but clearly decreases the immunologic components in breast milk including: sIgA, lactoferrin, lysozyme, and the number and function of cells.^{29–32} Rapid high-temperature treatment of human milk (72°C for 5 or 15 seconds) inactivated CMV inoculated into pooled milk samples without decreasing the levels of sIgA, lactoferrin, or lysozyme.³³ Hamprecht et al.³⁴ demonstrated the elimination of CMV infectivity using a special heating and cooling device to treat the milk at 72°C for 5 seconds in one sample of CMV-inoculated breast milk and four samples of wild-type, naturally infected breast milk. There was no decrease in the levels of lysozyme or sIgA, but the lipase and alkaline phosphatase levels were decreased by this treatment. Microwave treatment of breast milk clearly decreases the immunologic components of breast milk.³⁵

Cold treatment or freeze-storing of breast milk at –20°C does not appear to decrease sIgA, lysozyme, lactoferrin, C3 complement, or the number or function of cells in breast milk, or adversely affect its nutrient composition.³² The possibility of transmission of CMV to premature infants through previously frozen breast milk remains a possibility based on clinical studies (see the preceding).^{15–19} Although freezing at –20°C for variable time periods does decrease the viability of CMV in inoculated or naturally infected milk samples, there remain concerns about what might happen with high viral loads and what period of freezing is necessary to achieve 100% inactivation.^{29,34,36,37} Storage at –20°C for 3 days decreased infectivity in culture by 99%.²⁹ Storage for 4 to 10 days did not completely eliminate infectivity in samples with higher viral loads.³⁴ Storage for 7 days did not eliminate evidence of infectivity in 5/12 samples.³⁶ Curtis et al.³⁷ reported that storage for 10 days did not eliminate CMV detection in 3/3 samples, but storage for 20 days did eliminate the virus in 2/2 samples.

IMMUNITY TO CMV IN NEONATES AND PREMATURE INFANTS

Humoral immunity is not the key component of the host's defense against HCMV infection. Neutralizing antibody against CMV does seem

to decrease the likelihood of congenital CMV infection and the severity of the infection.^{38,39} The fact that more preterm infants than full-term infants develop symptomatic CMV infection when infected postnatally via breast milk might be explained by the differences in serum IgG levels in these infants. Acquisition of passively acquired IgG occurs primarily throughout the third trimester; with infants of 25 to 28 weeks gestational age having less serum IgG than infants of 29 to 32 weeks gestational age and significantly less than full-term infants.⁴⁰ The serum levels of IgG all drop significantly in the first 3 months of life for all gestational ages. CMV specific antibody declines in the first 8 weeks of life.⁴¹

Cell-mediated immunity is believed to be important in host defense against CMV. Cytotoxic T cells against specific CMV early antigens are probably most important in protecting against severe CMV disease.⁴² Young children, under 4 years of age, have a "selective deficiency" of CD4 T cell immunity against CMV with decreased CMV-specific IL-2 and CD154 expression and decreased interferon- γ (IFN- γ) response to CMV.⁴³ It has been postulated that CD8 T cells function to prevent symptomatic disease due to CMV in early childhood,⁴⁴ but that CMV replication and viremia persist for long periods of time because of deficient CD4 T-cell functioning. Premature infants and fetuses have poor T-cell proliferation responses to antigens, fewer CD45RO+ (mature CD4 T cells), and limited production of IFN- γ and tumor necrosis factor.^{45,46}

Lactoferrin from breast milk has a demonstrated antiviral effect on CMV probably at the level of virus adsorption or penetration.^{47,48} Lactoferrin levels correlated with CMV viral load in breast milk, but not with transmission of infection to preterm infants.¹³ Vitamin A, monolaurin, and secretory IgA in breast milk also limit the growth of CMV.⁴⁸ A population of antigen-specific CD8+ T cells against HCMV are present in human breast milk. A large percentage of these cells are CD45RO+HLADR+, consistent with an effector memory cell, and express the intestinal homing receptor, CD103 and the mucosal homing receptor CCR9, suggesting activation at an intestinal site and migration to the breast.⁴⁹ The role of these cells in

protection against CMV transmission via breast milk is uncertain.

ANTIVIRAL THERAPY IN INFANTS

Ganciclovir has been used to treat symptomatic CMV disease in immunocompromised patients (HIV infected, solid organ and bone marrow transplant recipients). It has been used also in individual cases of symptomatic congenital CMV or primary postnatally acquired CMV disease in premature infants. Phase II and III trials of ganciclovir to treat symptomatic congenital CMV infection have been published.^{50,51} Ganciclovir produces a decrease in viruria during therapy that rebounds after stopping the medication, seems to prevent hearing deterioration at 6 months in congenitally infected infants with symptoms of central nervous system disease, and causes significant neutropenia in more than two-thirds of infants receiving ganciclovir for 6 weeks of therapy.

Intravenous immune globulin infusions have been tried to prevent or modify symptomatic CMV disease. Mosca et al.¹⁴ suggested that even though they had a 20% transmission rate of CMV to premature infants via breast milk, there was an absence of symptomatic infection. They ascribed that to the routine administration of IVIG to premature infants in their nursery.¹⁴ A randomized placebo-controlled, double-blind trial of cytomegalovirus specific immune globulin (CMVIG) was performed to prevent symptomatic CMV infection in premature infants receiving multiple transfusions.⁵² Symptomatic disease was equally frequent in the treated and placebo groups, but there was a tendency toward less disease in infants of CMV-seropositive mothers who received CMVIG compared with placebo recipients. Recently in a nonrandomized trial, CMV-specific hyperimmune globulin was given to pregnant women with recent primary CMV infection to prevent or treat congenital CMV infection. The findings showed a significantly lower risk of congenital CMV disease in infants whose mothers received the immune globulin compared with those infants whose mothers did not receive it. Sixty-eight women received the CMV-IVIG without noted side-effects.⁵³

CONCLUSION

There continues to be considerable debate about what to do concerning the use of human breast milk in premature infants.^{28,54-58}

It is clear that CMV is shed in breast milk in the majority of CMV-seropositive women in varying amounts during the first 8 to 12 weeks or longer of lactation. Although well full-term infants rarely develop symptomatic disease, premature infants do experience acute symptoms that are occasionally severe and often lead to extensive evaluations and therapeutic interventions. The actual frequency of symptomatic CMV disease in premature infants related to breast milk appears to vary widely depending on handling of the breast milk (fresh, frozen, or pasteurized) and maternal seropositive rates in different nurseries. There is an apparent absence of long-term sequelae (delayed or abnormal development, sensorineural hearing loss) in the small number of infants with postnatal CMV infection who have been adequately evaluated at 2 to 4.5 years of age. Large prospective studies are needed to determine the frequency and magnitude of breast milk-related CMV infection and disease in premature babies because postnatal disease is less severe than perinatally acquired disease.

There remains uncertainty about the contributory role of a number of factors (maternal, infant, and viral) in causing CMV infection in these infants. The viral load of CMV in breast milk, early postnatal exposure to CMV in breast milk, and infant "susceptibility" based on younger gestational age and lower birth weight seem to correlate with transmission and symptomatic disease. Maternally derived neutralizing antibody to HCMV, the immaturity of the premature infant's cell-mediated immunity (CD4 proliferation, cytotoxic T-cell function, and cytokine production [IFN- γ , tumor necrosis factor]), the presence of CMV-specific secretory IgA, lactoferrin, CD8+ T cells (CD45RO+ /HLADR+) in breast milk, and different infectivity and pathogenicity of genetically distinct strains of HCMV may all play as yet undetermined roles in CMV transmission via breast milk.

Clinically practical methodologies for the inactivation of CMV in breast milk without di-

minishing its immunologic and nutrient benefits need further evaluation, and their effectiveness in preventing CMV transmission needs to be tested in prospective clinical trials. Additional evaluation of the therapeutic benefit and risks of IVIG and antiviral medications and the preventive efficacy of newer CMV vaccines may be warranted.

At this juncture, when the current knowledge about CMV infection from breast milk in premature and LBW infants remains incomplete; universal recommendations can not address all the issues and considerations. The literature supports:

- The use of human breast milk in NICUs should be continued. Breastfeeding or providing mother's breast milk for all well, full-term infants and infants of CMV-seronegative mothers is recommended.
- The current policies in NICUs concerning breast milk use and breastfeeding of premature infants of CMV-seropositive mothers need ongoing reassessment.
- The existing methods of handling breast milk provided for premature infants in the facility (donor milk, mother-infant specific breast milk, collection, containers, labeling, storage, fortification, etc.) should be examined with CMV transmission in mind.
- The current frequency and nature of CMV infections in each neonatal unit (congenital, perinatal during delivery and transmission through breast milk) should continue to be evaluated.
- Established safe practices and guidelines for the prevention of CMV transmission in neonates via blood products should continue.

The following practices are recommended in neonatal intensive care units:

- The cord blood, or mother's blood should be screened for CMV serostatus of all premature and LBW infants admitted to the NICU units.
- Each infant should be evaluated for signs or symptoms of congenital CMV infection and tested for congenital infection if suspected.
- All infants of CMV-seropositive mothers

should be screened for congenital CMV by culture or DNA PCR detection in urine, if the infant is to receive his or her own mother's breast milk.

- The possible low risk of symptomatic CMV infection in the premature infant through breast milk with the CMV-seropositive mother should be discussed in a balanced consideration of the known risks and benefits.
- The feasibility and risk-benefit of various procedures intended to inactivate CMV in mother-specific breast milk (Holder pasteurization, high temperature rapid treatment [72 degrees C for 5 seconds] or freezing [-20°C for 10 to 20 days or longer]) prior to breast milk use in at-risk infants should be evaluated.

Current NICU care of all premature or LBW infants should include ongoing observation for clinical and laboratory evidence of congenital, perinatal, or acute postnatal CMV infection through 4 months of age. Routine ophthalmologic, neurologic, audiologic, and developmental assessment of all premature and LBW infants through 3 to 4 years of age, with a special emphasis on infants with suspected or proven congenital, perinatal, or postnatal CMV infection should be scheduled.

The evolving literature concerning CMV suggests that reevaluation of the approach to, management, and prevention of congenital CMV infection and postnatal infection in premature infants will need to continue. Policies should remain current with new findings.

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