

Minimal adverse outcomes of postnatal cytomegalovirus infection in term or late preterm infants

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Abstract

Background: To investigate at what extent breastfeeding and vaginal delivery can increase mother-to-child transmission of cytomegalovirus (CMV) and to observe the clinical outcomes of postnatal CMV infection in infants.

Methods: In this secondary study of prospectively collected clinical data and serum samples, April 2012 through March 2015, 380 pregnant women with CMV IgG positive/CMV IgM negative and their 384 infants (4 twin pairs) with gestational age ≥ 32 weeks were included. CMV IgG and IgM were measured with enzyme-linked immunosorbent assay.

Results: Of 384 infants followed up at 10.2 ± 2.3 months age, 177 (46.1%) were defined with CMV infection based on the presence of higher CMV IgG levels than in their mothers. The infection rate in 190 breastfed infants was higher than in 194 formula-fed infants (62.6% vs 29.9%, $P < 0.001$). Vaginally delivered infants (172) had higher CMV infection rate than 212 infants delivered by caesarean section (55.2% vs. 38.7%, $P = 0.001$). Compared with formula feeding and caesarean section, breastfeeding and vaginal delivery increased postnatal CMV infection respectively (OR = 3.801, 95% CI 2.474–5.840, $P < 0.001$; OR = 1.818, 95% CI 1.182–2.796, $P = 0.007$). Nevertheless, CMV-infected infants normally developed and did not show adverse clinical outcomes compared to uninfected infants.

Conclusions: Breastfeeding and vaginal delivery can increase postnatal CMV infection; however, the infection does not cause adverse events in term or late preterm infants. Detection of CMV DNA in breastmilk should not be routinely performed, and breastfeeding should be encouraged in these infants.

Background

Human cytomegalovirus (CMV) infection is ubiquitous throughout the world. The prevalence of CMV IgG in women at childbearing age is 40–70% in developed countries and > 90–100% in developing countries [1, 2]. As high as 95% CMV IgG positive puerperants can shed virus in their breastmilk due to the viral reactivation in local breast glands [3, 4], thus, breastfeeding is considered to be the most common route for postnatal transmission from CMV-IgG positive mothers. This is in agreement with the fact that primary CMV infection mostly occurs in the first year of life [5–7]. However, it is unknown to what extent breastfeeding can increase the postnatal CMV infection in infants. Additionally, CMV may shed in the secretion in obstetric canals, yet the difference in postnatal CMV infection rates in infants delivered spontaneously or by cesarean section remains elusive.

Generally, postnatal CMV infection may cause illness in very early preterm (< 32 gestation weeks) or extremely low weight (< 1500 g) infants, while the infection in full term or late preterm infants does not have overt adverse outcomes [8, 9]. However, repeated reports show that postnatal CMV infection may also result in severe diseases in full term or late preterm infants [10–14]. Consequently, detection of CMV DNA in breast milk is frequently performed in clinical practice or advised to be warranted in previous studies [8], and mothers with positive CMV DNA are commended to avoid breastfeeding their infants [15, 16], even though evidence supporting such clinical practice is not sufficient.

In our previous prospective study on the mother-to-child transmission of hepatitis B, we collected paired serum samples from mothers and their children, including cord blood samples from newborn infants, and recorded relevant maternal and children's data [17, 18]. In the present study, we conducted a secondary analysis of data from the paired mothers and children by measuring CMV IgG and IgM to investigate at what extent that breastfeeding and vaginal delivery can influence the postnatal infection of CMV, and further observe the corresponding clinical outcomes of postnatal CMV infection in the infants.

Methods

Subjects and serum specimens

In collaborative studies on the prevention of mother-to-child transmission of hepatitis B virus conducted in 5 hospitals in Jiangsu Province, China, between April 2012 and March 2015, we prospectively recruited 478 pregnant women with positive hepatitis B surface antigen [17, 18]. Serum samples from these women during pregnancy and/or at delivery were collected and kept at -30°C . The data about the pregnancy, delivery, and neonatal outcomes were prospectively collected and recorded in a computerized database by the obstetricians at each participating hospital. The umbilical blood samples from newborn infants were also collected and kept at -30°C . All the neonates received recommended combined immunoprophylaxis against hepatitis B. In the follow-up study, serum samples from those women and their 418 children (4 pairs of twins) after 7 months of age were further prospectively collected. In the present study, we aimed to conduct a secondary analysis of data from the paired mothers and children to investigate the influence of breastfeeding and vaginal delivery on postnatal infection of CMV, and further observe the infant's outcomes with postnatal CMV infection. Therefore, by further measuring CMV IgG and IgM of serum samples collected in the previous study [17, 18], we included 380 mothers who were CMV IgG positive/CMV IgM negative during pregnancy and their 384 (4 pairs of twins) children in whom umbilical blood was CMV IgM negative in the present study. Those 19 women with both negative CMV IgG and IgM were not included. Fifteen paired mothers and infants were also excluded from analysis because there was insufficient serum volume. In the present study, perinatal information, including maternal gestational age, delivery mode, birth weight and height, and neonatal complications were extracted retrospectively from the originally computerized database collected during 2012–2015 [17, 18]. Furthermore, in this study, we also retrospectively retrieved the relevant data recorded in the previous follow-up study [17, 18], including feeding mode, infant's age, height and weight, alanine aminotransferase (ALT) levels, and health condition. Formula-fed infants were fed exclusively with formula, while breastfed infants were defined as those who received exclusive breastfeeding or mixed feeding.

This study was approved by the ethics committee of each participating hospital. The study was performed in accordance with the ethical standards in the Declaration of Helsinki. All the pregnant women provided written informed consent and consented to follow up of their infants; each infant's informed consent was assigned by his/her mother in the previous study conducted in 2012–2015 [17, 18]. Therefore, relevant data and serum samples of the mothers and their children were used in the current study via an exemption approved by the institutional review board of each participating hospital.

Quantification of CMV IgG and IgM

Serum samples were quantitatively tested for CMV IgG using a commercial enzyme-linked immunosorbent assay kit (Dia.Pro Diagnostic Bioprobes, Milano, Italy) as previously reported [6]. The kit contains human plasma derived calibrators with CMV IgG at concentrations of 0, 0.5, 1, 2, 4, and 8 IU/ml. In the measurement, paired maternal and neonatal blood, diluted 1:101 with diluent (2% casein, 10 mM Tris-citrate buffer and 0.1% Tween 20), were tested in parallel, and positive and negative controls provided in the kit were also included. Calibration curve was established for each test, and the IgG level of serum samples was further quantified. Based on the manufacturer's instruction, the diluted sample with a concentration > 0.5 IU/ml was considered positive for CMV IgG, thus, the sample with a concentration \leq 0.5 IU/ml was considered negative. When the IgG concentration was beyond the upper detection limit (8 IU/ml), the sera were retested by further dilution.

CMV IgM was measured by the CMV IgM capture immunoassay (Dia.Pro Diagnostic Bioprobes). In each measurement, serum samples were diluted same as in detection of CMV IgG, and positive and negative controls were included as previously reported [6]. As recommended by the manufacturer, the cut-off value was calculated as follows: cut-off = OD450 for negative control + 0.250. The test result was interpreted as a ratio of the sample OD450 and the cut-off value (S/Co). The sample was considered positive if S/Co value was > 1.2, indeterminate if it was 1.0–1.2, and negative if it was < 1.0. The indeterminate sample was retested; the sample was considered positive if S/Co value was 1.0–1.2 or > 1.2, and negative if it was < 1.0.

Statistical analysis

Data were analyzed with the SPSS software (SPSS Standard version 11.0, SPSS Inc., Chicago, IL). Continuous variables normally distributed were expressed as mean \pm standard deviation and compared by two-sample or paired t-test. Quantitative data non-normally distributed are presented as median and range, and compared by Man-Whitney U test. Categorical variables were reported as number and percentage, and compared by χ^2 analysis or Fisher's exact test where appropriate. Logistic regression analyses were further performed to determine the independent role of the feeding and delivery mode in postnatal CMV infection of the infants; the results were expressed by the adjusted odds ratios (OR) with 95% confidence intervals (CI). A two-sided *P* value fewer than 0.05 was considered statistically significant.

Results

General characteristics of the study subjects

Totally, 380 mothers with CMV IgG positive/IgM negative and their 384 children (4 pairs of twins) were included in the study. The women were 27.5 ± 4.3 years old at delivery, and their children were at the age of 10.2 ± 2.3 months at follow-up. Of the children, 190 (49.5%) were breastfed and 194 (50.5%) others were exclusively formula-fed. The mothers who breastfed their babies and whose infants were formula-fed had similar maternal (27.9 ± 4.1 vs. 27.1 ± 4.4 , *P* = 0.068) and gestational ages at delivery (38.9 ± 1.4 vs. 38.7 ± 1.7 , *P* = 0.213). The children in the two groups also had comparable gender ratios (formula-fed 109/83 vs. breastfed 104/82) and ages (formula-fed 10.5 ± 2.9 vs. breastfed 9.8 ± 2.2 months), although the difference of age had statistical significance (*P* = 0.008).

Seroprevalence of CMV IgG in children with different feeding patterns

Previous study showed that transplacentally transferred maternal CMV IgG in infants disappears before 6–8 months age because of the natural IgG decay [6, 19]. In the present study, all infants participated were over 7 months age, therefore, we measured the CMV IgG as a marker of CMV infection in the children. Of the total 384 children at follow-up, 177 (46.1%) were CMV IgG positive and 207 others (53.9%) were negative (Table 1). As shown in Table 1, 62.6% (119/190) of the breastfed children were CMV IgG positive, while 29.9% (58/194) of formula-fed children were CMV IgG positive. The positive rate of CMV IgG was significantly higher in breastfed children than in formula-fed children ($\chi^2 = 41.403$, $P < 0.001$). Overall, the seroprevalence of CMV IgG in breastfed group at different ages was each higher than that in formula-fed children (Table 1). However, in either of breastfed or formula-fed groups, the seroprevalence of CMV IgG was relatively constant in children at the age of different months, and did not show an increasing trend with growing ages (Table 1).

To further validate the CMV IgG in children was resultant from the infection, rather than from the transplacentally acquired maternal IgG, we compared the CMV IgG levels between each CMV IgG positive child and his/her mother. The mean concentrations in children at different age subgroups were higher than that in their mothers, especially in 7–8 and 9–10 months age groups (Table 2), demonstrating that the CMV IgG in these children were acquired by postnatal CMV infection. Additionally, we tested CMV IgM in the 177 CMV IgG positive children, and 36 (20.3%) children were positive, with 25 (21.0%) from the 119 breastfed group and 11 (19.0%) from the 58 formula-fed group (Table 3).

Seroprevalence of CMV IgG in children with different delivery modes

CMV may exist in genital secretions of CMV IgG positive women [20]. To clarify whether vaginal delivery can increase the likelihood of mother-to-child transmission of CMV, we compared the positivity rate of CMV IgG in 172 vaginally delivered children with that in 212 children delivered by cesarean section (Table 4). The overall positive rate of CMV IgG in vaginally delivered children was higher than that in those delivered by caesarean section (55.2% vs. 38.7%, $\chi^2 = 10.474$, $P = 0.001$). Further stratified analysis showed that while the breastfed children had similar positive rate of CMV IgG between virginally delivered children and those delivered by cesarean section (65.3% vs. 60.0%, $P = 0.453$), formula-fed children delivered virginally had a significantly higher positive rate of CMV IgG than formula-fed children delivered by caesarean section (42.9% vs. 21.4%, $P = 0.001$). Further logistic regression analysis showed that, compared with formula feeding, breast milk could increase the seropositive rate of CMV in the infants ($P < 0.001$, OR = 3.801, 95% CI 2.474–5.840). Similarly, vaginal delivery was also independently associated with higher seroprevalence of CMV in the infants ($P = 0.007$, OR = 1.818, 95% CI 1.182–2.796).

Influence of acquired CMV infection on child's growth

To determine whether postnatally acquired CMV infection may affect the infant's growth, we compared the child's weight and height between the CMV IgG-positive and -negative children respectively. The children between the two groups had comparable birth weights and heights, gender ratios and ages. Table 5 shows that the CMV IgG-positive and -negative children had comparable weights and heights at the follow-up.

CMV IgG positive alone usually represents past CMV infection and positivity for both CMV IgG and IgM usually indicates an active infection of CMV. In the present study, a total of 36 infants were found to be both CMV IgG and IgM positive, however, these children did not have particular clinical manifestation at follow-up. Of them, 33 had measured alanine aminotransferase (ALT) level and 3 others did not measure ALT due to the hemolysis; 5 (15.1%) children had mild elevation of ALT (42.0–107.2 U/L) without clinical presentations. Of the 98 children with positive CMV IgG alone and 144 children with negative CMV IgG, 14 (14.3%) and 20 (13.9%) also had mild elevation of ALT respectively. The ALT abnormal rates among these three groups of children had no statistical difference ($\chi^2 = 0.036$, $P = 0.982$).

Discussion

In the present study, we showed that breastfeeding and vaginal delivery can each increase the mother-to-child transmission of CMV, with the OR 3.801 and 1.818 respectively; however, no obviously adverse influence on child's growth and development was observed in these infected children who were born over 32 gestational weeks. Therefore, the influence of postnatally acquired CMV infection should not be overemphasized. This study indicates that breastfeeding should not be contraindicated for infants born to CMV IgG positive parturients, despite of the presence of CMV DNA in breast milk. Similarly, although vaginal delivery can increase mother-to-child transmission of CMV, elective caesarean section should not be recommended to prevent the mother-to-child transmission, and spontaneous delivery should be encouraged.

In this study, we did not define CMV infection by detecting CMV DNA, but defined the infection based on the presence of CMV IgG. Since maternal CMV IgG can transplacentally transfer to fetus, one may assume that the CMV IgG in infants is derived from the mothers. However, our previous study and others demonstrated that the maternally acquired CMV IgG in infants disappears before the age of 6–8 months [6, 19]. In the present study, all children were negative for CMV IgM at birth and were tested for CMV IgG over the age of 7 months. Moreover, the CMV IgG levels in children were higher than that in their mothers, indicating that the presence of CMV IgG in these children was not derived from their mothers, but the consequence of postnatal CMV infection. Therefore, the presence of relatively high levels of CMV IgG in these children can reliably define the postnatal CMV infection. Of the all 177 CMV IgG positive children, 36 (20.3%) showed CMV IgM positive (Table 3). The positive rate of CMV IgM appears to be lower, because the mean age of these CMV IgG positive children was only 10.1 ± 2.9 months and the CMV infection in these children should be primary. However, our previous study with detection of CMV IgG and IgM in longitudinal serum samples at ages of 1, 3.5, 8, and 24 months in a same cohort of children showed that CMV IgM was mostly positive (83.3%) at ages of 1 and 3.5 months [6], indicating the postnatal CMV infection mostly occurs before 3.5 months age. Considering that CMV IgM usually disappears within 1–3 months, ~ 20% of CMV IgM positive children in the present study were logically reasonable.

The critical findings of our present study are that, compared with children who did not experience CMV infection after birth, the postnatally CMV infected children had similar body weights and heights (Table 5) and did not have overt diseases, and that the children with active primary CMV infection (positive for both CMV IgG and IgM) had a similar rate of mild elevation of ALT, compared to the children with latent CMV infection (CMV IgG positive alone) and the children without CMV infection (negative for both CMV IgG and IgM). These results demonstrated that postnatal infection of CMV in infants with gestational age ≥ 32 weeks does not cause obvious adverse influence on the health of infants. The minimal influence on the health of infants may be associated with the presence of maternal CMV IgG in infants, which can neutralize the virulence of CMV and provide substantial protection against symptomatic diseases or sequelae [21].

The results of our study have several practical implications. First, for newborn infants with gestational age ≥ 32 weeks, the presence of CMV DNA in breast milk should not be the contraindication for breastfeeding [22]. Actually, detection of CMV DNA in breast milk of CMV IgG-positive puerperants who delivered her neonates after 32 weeks gestation is excessive and is not necessary. On the other hand, undetectable CMV DNA in breast milk in a single test cannot exclude the presence of virus, because CMV can actively replicate in epithelial cells of breast glands in $>95\%$ of CMV IgG positive puerperants in the longitudinal breast milk samples after labor [3]. Second, it should be cautious to use saliva as detection materials in defining in utero infection of CMV. The optimal approach to diagnosing in utero congenital infection of CMV is to detect CMV DNA in urine samples of newborns within 3 weeks after birth [23, 24]. However, due to the difficulty in collecting urines from newborns, saliva samples are usually used to detect CMV DNA [25–28]. In the present study, we found that the infants who were delivered virginally and/or breastfed had higher CMV infection rate than those who were delivered by caesarean section and/or formula-fed, indicating that saliva samples from the neonates can be contaminated with maternal CMV. Thus, positive result in the detection of CMV DNA in saliva samples from newborns who were delivered virginally and/or breastfed may not be infected congenitally, but be false positive caused by contamination of maternal CMV [28–30]. Indeed, the congenital infection rate estimated by detection of CMV DNA in saliva samples appears to be higher than that based on detecting CMV DNA in urine samples [24, 31].

The main limitation in this retrospective study is that we excluded congenital CMV infection just by detecting CMV IgM in cord blood, but not by detecting CMV DNA in urine samples of the newborns within three weeks after birth [32]. However, because the number of the infants was similar in the breastfed and formula-fed groups in the study, the potential bias should be minimal, even if congenital infection really existed. Another limitation is that we were not able to retrieve the data to investigate the clinical features, such as liver function and platelet counts, in the infants during acute infection phase. Therefore, we could not confirm whether acute CMV infection in those infants has caused relevant symptomatic diseases. However, at the follow-up, the health conditions in CMV-infected infants had no difference, compared to the uninfected infants. Moreover, the proportion of abnormal liver function in those 33 infants with active CMV infection was not significantly higher than that in latent or non-infected infants, indicating that the postnatal CMV infection has minimal influence on the health and development of infants, although acquired infection associated with postnatally breastfeeding has been confirmed [33].

Conclusion

Breastfeeding and vaginal delivery can increase the risk of postnatal CMV infection; however, the infection does not cause obviously adverse events in the infants born over 32 gestational weeks. Therefore, detection of CMV DNA in breastmilk should not be routinely performed, and breast-feeding and vaginal birth should be recommended.

Abbreviations

ALT: alanine aminotransferase; CI: confidence intervals; CMV: cytomegalovirus; OR: odds ratios

Declarations

Ethics approval and consent to participate

The study was approved by the institutional ethics review committee of Nanjing Drum Tower Hospital, Wuxi Children's Hospital, the First People's Hospital of Nantong, Zhenjiang Fourth People's Hospital, Taixing People's Hospital, respectively, and was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. All the pregnant women provided written informed consent and consented to follow up of their infants, including collecting relevant data and serum samples; each infant's informed consent was assigned by his/her mother in the previous study. Therefore, serum samples of the mothers and their children were used in the current study via an exemption approved by the institutional review board of each participating hospital.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

JC, YZ and Y-HZ conceived and designed the study, analyzed the data and prepared the manuscript. LC, CX, LL, BX, YD and YH assisted the development of the research idea, the analysis, interpretation and preparation of the manuscript. All authors have been involved in revising the manuscript critically for important intellectual content; and they approved the final manuscript.

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Tables

Table 1 Seroprevalence of CMV IgG in breast- and formula-fed children

Age (months)	Overall		Breastfed ^a		Formula-fed ^a		P value
	n	IgG+ (%)	n	IgG+ (%)	n	IgG+ (%)	
>7-8	92	46 (50.0)	47	31 (66.0)	45	15 (33.3)	0.002
9-10	172	80 (46.5)	91	59 (64.8)	81	21 (25.9)	< 0.001
11-12	85	35 (41.2)	39	22 (56.4)	46	13 (28.3)	0.009
13-26	35	16 (45.7)	13	7 (53.8)	22	9 (40.9)	0.458
Total	384	177 (46.1)	190	119 (62.6)	194	58 (29.9)	< 0.001

^a the prevalence of CMV IgG among children at different age had no statistical difference either in breastfed children ($\chi^2 = 1.62$, $P = 0.6545$) or in formula-fed children ($\chi^2 = 2.19$, $P = 0.5330$).

Table 2 Comparison of CMV IgG levels (IU/mL) between the infants and mothers

Age (months)	Infant		Mother		P value
	n	Concentration	n	Concentration	
>7-8	46	559.8 ± 239.0	45	400.0 ± 256.3	0.003
9-10	80	549.2 ± 262.1	77	426.7 ± 249.7	0.003
11-12	35	492.6 ± 255.2	35	427.2 ± 207.0	0.243
13-26	16	508.2 ± 204.6	16	386.6 ± 242.4	0.136
Total	177	537.1 ± 249.5	173 ^a	416.2 ± 241.2	< 0.001

^a four mothers delivered twin infants.

Table 3 Seroprevalence of CMV IgM in breast- and formula-fed children with positivity of CMV IgG

Age (months)	Overall		Breastfed		Formula-fed		<i>P</i> value ^a
	n	IgM+ (%)	n	IgM+ (%)	n	IgM+ (%)	
>7-8	46	12 (26.1)	31	10 (32.3)	15	2 (13.3)	0.312
9-10	80	15 (18.8)	59	11 (18.6)	21	4 (19.0)	> 0.999
11-12	35	6 (17.1)	22	3 (13.6)	13	3 (23.1)	0.801
13-26	16	3 (18.8)	7	1 (14.3)	9	2 (22.2)	> 0.999
Total	177	36 (20.3)	119	25 (21.0)	58	11 (19.0)	0.751

^a comparison between breastfed and formula-fed infants.

Table 4 Seroprevalence of CMV IgG in infants who were born by vaginal delivery or caesarean section

	Vaginal delivery		Caesarean section		<i>P</i> value
	n	CMV IgG+ (%)	n	CMV IgG+ (%)	
Breastfed	95	62 (65.3)	95	57 (60.0)	0.453
Formula-fed	77	33 (42.9)	117	25 (21.4)	0.001
Total	172	95 (55.2)	212	82 (38.7)	0.001

Table 5 Infants' parameters with respect to the status of CMV IgG

	CMV IgG+	CMV IgG $\bar{}$	<i>P</i> value
	(n = 177)	(n = 207)	
Age (months)	10.1 \pm 2.9	10.2 \pm 2.3	0.707
Male (%)	102 (57.6)	111 (53.6)	0.431
Height (cm)	75.67 \pm 4.97	74.94 \pm 5.16	0.161
Weight (kg)	10.86 \pm 2.23	10.54 \pm 2.12	0.151