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Research Article

# Amelioration of Congenital Cytomegalovirus Disease in Premature Neonates by Pasteurization of Natural Mother's Breast Milk

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## Abstract

**Objective:** To investigate if Pasteurization of Mother's Breast Milk (PMBM) prior to giving it to her baby alters the clinical course of CMV in premature neonates born with CMV in their urine.

**Study design:** When a delivery occurred before 34 weeks of GA, maternal blood and baby's urine were tested for the presence of CMV-IgG antibodies and CMV respectively. If the mother was seropositive for CMV, her Expressed Breast Milk (EBM) was pasteurized prior to giving it to her baby. Here we describe the study protocol and clinical course of a cohort of four babies who were positive for CMV in their urine at birth and received biological mother's PMBM.

**Results:** While 42/44 (96%) mothers were seropositive, 4/52 (7.7%) babies were positive for the presence of CMV in their urine. None of these babies had signs or symptoms of CMV disease at birth or during the entire infancy.

**Conclusion:** In this preliminary study, PMBM prior to its use seems to ameliorate clinical course of CMV in premature babies. More such studies are warranted.

**Keywords: Congenital CMV; Maternal CMV-IgG antibody; Premature Baby; Maternal Breast Milk; Pasteurization**

## Abbreviations

AU: Arbitrary Units; BM: Breast Milk; BW: Birth Weight; CMV: Cytomegalovirus; cCMV: Congenital Cytomegalovirus; DBM: Donor Breast Milk; EBM: Expressed Breast Milk; DMH: Deenanath Mangeshkar Hospital; GA: Gestational Age; IgG: Immunoglobin G; IgM: Immunoglobin M; NICU: Neonatal Intensive Care Unit; PCR: Polymerase Chain Reaction; PMBM: Pasteurized Mother's Breast Milk; VLBW: Very Low Birth Weight; VPT: Very Preterm

## Introduction

Cytomegalovirus (CMV), acquired trans-placentally or postnatally from Mother's Breast Milk (MBM), is an important health problem worldwide especially among preterm babies <34 weeks of gestation [1-9]. Recently, we described an extremely preterm baby who developed septic shock secondary to maternal to infant transmission of CMV *via* MBM [10]. In a prospective study, we reported the prevalence of seropositive rate of maternal CMV in USA and India who delivered a baby at <34 weeks of GA and congenital CMV in these neonates [10]. In response to the index case, we developed a protocol to prevent maternal to infant transmission of CMV *via* MBM. We performed holder pasteurization of natural mother's EBM prior to feeding her baby. It has been well established that holder pasteurization kills CMV [1,3,5,9]. Here we describe the protocol to prevent maternal to infant transmission of CMV *via* MBM and the outcome of a cohort of four babies who had CMV in their urine at birth and received their mother's PBM [10].

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## Materials and Methods

All four babies who had CMV in their urine at birth were admitted in the Neonatal Intensive Care Unit (NICU) at Deenanath Mangeshkar Hospital (DMH) in Pune, India [10]. Pertinent maternal and neonatal data was collected from the electronic medical records [10]. Clinical protocol to draw maternal blood for the measurement of serum CMV-IgG antibodies, check urine for the presence of CMV at birth and to administer pasteurized MBM was approved by the Institutional Review Board at DMH (IHR-2021-Feb-UD-398) [10].

### Quantitation of maternal serum CMV IgG antibodies

Soon after birth, all mothers who delivered a baby at <34 weeks GA were approached for the measurement of serum CMV-IgG antibodies [10]. The rationale for testing their blood was explained and verbal consent to draw ~2 ml of blood was obtained [10]. Maternal serum CMV-IgG antibody testing was performed using an automated quantitative enzyme linked fluorescent assay [10]. Results are reported as Arbitrary Units (AU) /ml: Negative: <4, Equivocal: 4-6, Positive: >6 [10].

Physical examination and routine laboratory tests were performed to determine if these neonates had signs of CMV disease [3-5]. These included presence of skin petechiae or a rash, hepatosplenomegaly, chorioretinitis, complete blood count including manual differential, platelet count, total and direct serum bilirubin and cranial ultrasound imaging. In one baby with persistent CMV in the urine, liver function

tests were also performed around two months of age.

### Urine for CMV

Bagged urine specimens were obtained from all infants upon admission, prior to discharge and after discharge. All samples were processed for the detection of CMV-DNA by RT-PCR [10].

Feeding protocol: Pending results of the maternal serum CMV, all babies were fed either PDBM obtained from the BM bank at DMH or mother's colostrum (up to 20 ml) collected within 5 days of delivery. If the mother was seronegative, her baby received standard NICU feeding regimen. If the mother was seropositive, Dr. Kalane met with the mother to explain implications of her CMV status, to assess social and educational background, mother's willingness to provide EBM for longer duration and to assess if there were contraindications to using EBM [10]. If the mother was seropositive and considered a suitable candidate, MBM was pasteurized prior to feeding her baby. A written informed consent was obtained from each mother regarding the use of PEBM. If the natural MEBM was not available or insufficient, the baby received PDBM or the formula designed for premature babies until discharge from the hospital. While the mother was allowed to hold her baby close to her breast for non-nutritive sucking and skin to skin contact (Kangaroo care), she was not allowed to do direct nipple feeding.

### Holder pasteurization

Donor or natural MEBM was pasteurized in the BM bank of DMH using the HSC pasteurizer PAS 10000 (HSC-Inox, Lyon, France). Manufacturer's instructions as well as the Human Breast Milk Banking Association of North America guidelines regarding collection, handling, storage, transportation and the use of MEBM were strictly followed [11].

Bacterial culture after pasteurization was performed only for the DBM. PEBM was stored at 4°C if used within 48 hours. Otherwise, it was stored frozen at -18°C.

### Hearing screening

It was performed at the time of discharge according to the NICU protocol and again during infancy.

### Developmental evaluation

After obtaining parental consent, neonates were examined at the Small Steps Morris Child Development Centre located at DMH. Neurodevelopmental assessment was performed using the standard Bayley scales of infant and toddler (BSID-III) [12,13]. Anthropometric measurements were also performed. Parents were educated when indicated regarding home intervention program. All data was recorded in a structured format.

## Results

Forty-four (36 inborn+8 out born) women were recruited in the study [10]. Maternal blood was collected for the measurement of CMV-IgG antibodies before 5 days after delivery. Pertinent maternal and neonatal data for four babies who were positive for CMV in their urine upon admission are shown (Table 1). The number of CMV copies/ml at birth were 47, 130, 185 and 5024 (Table 1). In three babies, urine for CMV was negative upon repeat examination at 15, 23, 34 days of age (Table 1). Only one infant continued to have CMV in his urine for almost a year (Table 1). The peak urine CMV colony count was observed at ~2 months of age (Table 1). Mothers of all four babies with CMV in the urine were seropositive for CMV-IgG antibodies (Table 1). All four babies did not have any signs, symptoms or laboratory data suggestive of active CMV disease at birth or during infancy. Even the baby with persistent CMV in the urine did not have signs or symptoms of active CMV disease. His liver function tests at two months of age were within the normal range: SGPT 21, SGOT-65, Alkaline phosphatase 469, Total protein 5.7, Albumin 3.8 and Globulin 1.9. All four babies had normal cranial ultra sound performed prior to discharge: None had intraventricular hemorrhage, periventricular leukomalacia or calcifications in the CNS. None of the babies had abnormal hearing screening at discharge or on repeat examination (Table 1). None developed signs of ROP.

### Neurodevelopmental outcomes

With the exception one baby who had transient abnormal muscle tone at ten months of age needing a short course of physical therapy, three other babies had normal developmental assessment, appropriate for the corrected GA. Baby with abnormal tone did not have abnormal cranial ultrasound. At 17 months of age his developmental assessment was totally normal.

## Discussion

In this study we followed clinical course of a cohort of four premature babies who had acquired CMV before birth. If the mother is seropositive, CMV gets reactivated during pregnancy and lactation in the mammary gland in 96% of women while CMV is excreted in the BM in 80% [10]. Ongoing viral load from MBM was eliminated by PMBM prior to feeding her baby.

Among three babies, urine for CMV was negative at 15, 23, 34 days of life and they had normal growth and neurological development and no hearing loss during infancy. We hypothesize that the viral load from MBM was eliminated by pasteurization. Therefore, the natural course of CMV disease was ameliorated.

In one patient urine was still positive at 1 year of age (Table 1). This baby did not have hearing loss but had abnormal neurodevelopmental assessment at ten months of age needing short course of physical therapy. We could not determine with certainty if the abnormal neurodevelopmental findings were due to CMV or severe prematurity. It is unlikely abnormal findings were due to CMV since he did not have signs or symptoms of CMV disease at any time. These included normal physical examination, laboratory tests and cranial ultrasound. His neurodevelopment was totally normal at 17 months of age. Transient abnormal neurodevelopmental findings are common among extremely premature neonates. Therefore, we speculate that elimination of CMV load from maternal BM by pasteurization lead to an amelioration of CMV disease. Opposing views are welcome.

High concentration of maternal serum CMV-IgG antibodies means she has current, past or reactivated CMV infection [10]. For reasons described earlier, we chose to quantitate CMV IgG antibodies only [10]. Reasons for wide variations in the maternal serum IgG level remain unknown.

Recent modifications, BSID-III and BSID-IV of the Bayley Scales of Infant Development (BSID) assess cognition, motor, language, socio-emotional, and adaptive behavior [12,13] BSID-III has been used to evaluate neurodevelopment of preterm and term infants and effects of early intervention programs. Therefore, we used BSID-III in our study.

It is unlikely that the mother will have a high CMV viral load in her BM milk within the first three days post-partum [14-16]. On the other

**Table 1:** Pertinent maternal/neonatal data.

| Patient Number | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| Maternal Age | 29 | 28 | 22 | 26 |
| Mode of delivery | C/S | C/S | C/S | C/S |
| GA at birth (weeks) | 26 | 29 3/7 | 33 5/7 | 31 1/7 |
| Gender, in/out born | M/out | M/out | M/in | F/out |
| Maternal serum IgG | 26 | 83 | 48 | 51 |
| Birth Weight (gm) | 635 | 1160 | 1640 | 1435 |
| Discharge Weight (gm) | 1480 | 1942 | 1690 | 1782 |
| First urine+for CMV (day/colony count) | 3 d-5024 | 6 d-130 | 2 d-185 | 4 d-47 |
| Repeat Urine CMV (day/colony count) | 46 d -228638<br>63 d-3753750<br>110 d-748430<br>365 d-971 | 34 d-neg | 15 d-neg | 23 d-neg |
| HC cm (birth/discharge) | 24.5 / 31 | 27 / 28 | 29 / 29 | 27 / 32 |
| ROP Screening | Negative | Negative | Negative | Negative |
| BERA (age-months) | 8 m-negative | 5 m-negative | 4m-negative | 2 m-negative |
| T/D bilirubin (age-d) | 5/0.4, d 1 | 5.7/0.4, d 3 | 9/0.5, d 2 | 6.5/0.6 d 3 |
| WBC/PMN/Lym/Platelet | 5.0/42/37/135 | 16.1/28/58/110 | 14.2/72/18/225 | 8.3/54/39/235 |
| LOS (d) | 78 | 48 | 12 | 21 |
| Days received PEBM | 53 | 33 | 7 | 16 |

hand, pasteurization of colostrum may not be ideal, especially when the baby is premature, since it may lead to some loss of important proteins like the secretory immunoglobins particularly IgA [17-19]. Therefore, we chose not to pasteurize initial 20 ml of colostrum before giving it to the baby. Decision regarding giving 20 ml was arbitrary. Opposing views are respected.

Neonates with CMV, congenital or postnatally acquired, are born to women who are CMV-IgG seropositive [2,20]. If the mother is seronegative, her baby will not have cCMV or develop it postnatally from her BM [2,20]. In our study, all four babies with CMV in their urine upon admission were born to mothers who were seropositive.

While freeze-thawing of BM at -20°C for 18 hours to 10 days reduces viral load, it does not fully destroy viral infectivity [1,3,19,20]. Short term pasteurization (62°C for 5 seconds) eliminates CMV and conserves most of the nutritional and immunologic components of BM: CMV specific antibodies, enzyme activity, hormones and growth factors [3,4]. However, specific machine capable of creating a thin milk layer, heated by a stream of hot air, following a specific ramp of temperature and duration are not available commercially. Long term holder pasteurization (30 minutes at 62.5°C) is effective in completely inactivating CMV in the BM. Such pasteurizers have been used for many decades and easily available commercially. While holder pasteurization significantly reduces the amount of some beneficial biological compounds in BM, it has not been determined that these losses are clinically significant. Therefore, we chose to perform holder pasteurization.

If the infant has moderate to severe symptoms due to CMV infection, treatment with intravenous ganciclovir or oral valganciclovir is indicated [19,21]. However, because of noteworthy toxicities of these drugs, consideration of their use must balance known risks such as neutropenia and other possible risks like gonadal dysgenesis and carcinogenicity with potential benefits [22]. In a consensus statement, it was concluded that neonates with asymptomatic CMV should not be treated with antiviral therapies (level 3 evidence) [21]. Despite persistence of CMV in the urine of our extremely small infant, he remained CMV disease symptom free. Therefore, we did not initiate antiviral pharmacotherapy. Opposing views are welcome.

In summary, we propose that natural MEBM should be pasteurized prior to giving it to her baby even if the baby has acquired CMV prior to birth. Pasteurization will decrease ongoing viral load from MBM leading to an amelioration of the CMV disease. More studies are necessary to confirm our preliminary observations.

## Acknowledgement

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## Author Contributions

Dr. Devaskar and Dr. Kalane designed the study protocol. Dr. Kalane obtained IRB approval at DMH and was the PI. Miss Raste and Mrs. Haridas were the research coordinators and pasteurized all BM samples. Serum IgG measurements were performed under the supervision of Mrs. Patwardhan. Dr. Devaskar reviewed all the data and wrote the manuscript with input from Dr. Kalane.

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