doi:10.2223/JPED.1436

0021-7557/06/82-01/46
**Jornal de Pediatria**
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# The prevalence of congenital cytomegalovirus infection in newborn infants at an intensive care unit in a public hospital

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**Abstract**

**Objective:** To determine the prevalence of congenital cytomegalovirus infection in newborn infants admitted to an intensive care unit in a public hospital in Porto Alegre.

**Methods:** A cross-sectional study of 261 newborn infants born at a public hospital in the city of Porto Alegre in 2003 and admitted to the intensive care ward. Urine samples were collected within 7 days of birth and a polymerase chain reaction-PCR performed to test for cytomegalovirus DNA.

**Results:** The prevalence of congenital cytomegalovirus infection among the study population was 0.8% (95% CI: 0.097-2.86). It was not possible to assess risk factors because this prevalence was so low.

**Conclusions:** The prevalence of congenital cytomegalovirus infection in an intensive care unit at a public hospital in Porto Alegre was not considered elevated and was comparable with prevalence rates found by other studies.

*J Pediatr (Rio J). 2006;82(1):46-50:* Cytomegalovirus, congenital cytomegalovirus, polymerase chain reaction, newborn, neonate.

## Introduction

Cytomegalovirus (CMV) belongs to the *herpesviridae* family and is a common cause of infection among humans. The virus undergoes periods of activity and periods of latency and, once a woman has been infected, the virus remains in the host's body indefinitely and can reactivate at any point.[1]

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Financial funding: FIPE-HCPA.

Manuscript received May 19 2005, accepted for publication Aug 24 2005.

**Suggested citation:** Miura CS, Miura E, Mombach AB, Chesky M. The prevalence of congenital cytomegalovirus infection in newborn infants at an intensive care unit in a public hospital. J Pediatr (Rio J). 2006;82:46-50.

The virus is transmitted to fetuses via the transplacental route. The earlier the virus is transmitted to the fetus, the worse prognosis and the greater the chance of severe malformations (MF). The maternal infection can either be primary (in women who had never been infected before) or recurrent (by viral reactivation or reinfection by other viral strains).[2] When the infection is primary, the chances of transmission to the fetus are higher and the frequency of severe sequelae is greater.

Ten percent of infected newborn infants (NB) will be symptomatic infected. Mortality among this subset can reach 30%, and 90% may suffer serious sequelae.[3] The principle characteristics of symptomatic infections are petechiae, hepatosplenomegaly, jaundice, microcephalia, retarded intrauterine growth, prematurity, periventricular cerebral calcifications , miscarriage, inguinal hernia and chorioretinitis.[4] Ten to fifteen percent of the asymptomatic NB may exhibit delayed clinical manifestations such as

deafness, mental retardation and chorioretinitis, which can all appear during the first two years of life.[5,6]

Diagnosis is established by isolating the virus in culture. The preferred source of test samples is urine because CMV titers are elevated in urine. The viral tissue culture technique is considered the gold standard and it takes 2 to 6 weeks for the virus to replicate and be identified. The PCR (polymerase chain reaction) method for identifying viral DNA also offers high sensitivity and specificity. The PCR method offers certain advantages, namely faster results (24 to 48 hours), reduced sample volume requirements and the fact that samples can be frozen and stored.[7]

Congenital CMV infections are currently the most common intrauterine infections worldwide, with a prevalence of 0.2 to 2.2%. Furthermore, they are the principal infectious cause of malformations of the central nervous system (CNS) and the principal cause of deafness and learning difficulties in childhood and have a major social impact.[8,9] In selected populations, such as newborn infants in neonatal intensive care units (NICU), this prevalence can climb even higher, primarily because critically ill NB are likely to have the same risk factors. Several different studies associate adolescent mothers, black race, sexual activity with multiple partners, single marital status, multiparity, low socioeconomic status and contact with sources of CMV (at daycare, for example) with increased risk of congenital infection.[2,10]

The prevalence of this infection in our region is not yet known. A number of studies performed in Brazil have observed prevalence rates that vary, depending on study population, from 0.39 to 6.8%.[11-14]

Early diagnosis of this infection is important both for therapeutic interventions minimizing morbidity and mortality among symptomatic NB and also for determination of the risk of future sequelae in asymptomatic NB.[15]

The objective of this study was to determine the prevalence of congenital CMV infection among NB at the ICU of a public hospital in Porto Alegre.

## Patients and methods

A cross-sectional prevalence study was carried out, involving all of the NB who were admitted to the neonatal ICU (NICU) of a public hospital in Porto Alegre from May to December 2003. The sample size was based on a study in which the prevalence of CMV infection at a neonatal ICU was 6.8%.[13] The sample size calculation was performed using Epi-Info 6.0, and returned a figure of 241 patients to achieve a confidence interval of 95% and a margin or error of 3%. The study took place from 05/05/2003 to 04/12/2003. All children born at the hospital and admitted to the NICU was eligible for the study. Patients transferred in from other institutions were excluded to avoid sampling bias, since the hospital in question is a referral center for congenital infections.

A urine sample was collected during the first week of life from each participating NB, under aseptic conditions using a collector bag, and sent to the Clinical Pathology Service (Microbiology Unit), where they were stored in a freezer until processing. These urine samples were tested in duplicate for the presence of viral DNA by PCR. Infection was defined as when both test results were positive. The PCR methodology employed was developed in-house and is used for diagnosis by the Clinical Pathology Service at the hospital and is validated by means of comparison with viral cultures from urine (gold standard).

Detection of CMV is based on amplifying a specific DNA sequence from the glycoprotein B gene of the virus, using the nested PCR technique. Nested PCR uses two amplification reactions with two pairs of different primers (external and internal) for the same genome, resulting a method that is both more specific and more sensitive.

The PCR reactions used 16 mM $(NH4)_2SO_4$, 67 mM Tris-HCL (pH 8.8 at 25 ºC), 1.5 mM $MgCl_2$, 0.01% (w/v) of Tween-20 (PCR buffer, Advanced Biotechnologies), 0.25 mM of each deoxynucleotide triphosphate (Advanced Biotechnologies Ltd.), 0.1 µM of each specific primer (R&D Systems Ltd.) and 0.625 units of Taq polymerase (Advanced Biotechnologies Ltd.). Genetic material was purified from a 140 µl urine sample using a Qiagen QIAamp kit according to the manufacturer's instructions. For the PCR reaction, 10 µl of the extracted DNA was used for the first amplification, making a final total reaction volume of 50 µl. Amplification with internal primers was performed in a mixture identical to that described above with the exception of the total volume, which was 25 µl, and the fact that 2 µl from the first reaction was added as the reaction sample. The two amplification stages were performed in a Techne thermocycler (Flexigene®). All samples were tested in duplicate. The amplification for the first PCR reaction was executed under the following conditions: initial denaturing for 1 minute and 40 seconds at 94 °C, followed by 33 cycles of 20 seconds at 94 °C, for denaturing, 20 seconds at 50 °C for annealing, 20 seconds at 72 °C for extension. amplification for the second PCR reaction was executed under the following conditions: an initial denaturing for 45 seconds at 94 °C followed by 33 20-second cycles at 94 °C for denaturing, 20 seconds at 50 °C for annealing and 30 seconds at 72 °C for extension. Amplification products were detected by electrophoresis, using 10 µl from the second PCR reaction in agarose gel at 2% containing 0.5 µg/ml of ethidium bromide. The DNA bands were viewed in the gel using an ultraviolet transilluminator. The tests took an average of 6 hours to perform.[15]

The database was compiled in Excel and data analysis was performed with SPSS version 12.0.

The project was approved by the Ethics Committee at our institution and in all cases informed consent was obtained from parents or guardians.

## Results

Two hundred and sixty-one neonates born at the hospital were admitted to the neonatal ICU during the study period. Two patients were excluded because they had received transfusions of blood products before urine collection (0.76%), two because consent was not obtained (0.76%), one because the urine sample was lost (0.38%), two died before collection (0.76%) and four were lost because they were discharged early, before urine collection (1.5%).

The study population comprised 145 male NB (58%) and 105 female NB (42%). The principal causes of admission to the ICU were prematurity (111 cases, 44.4%), respiratory dysfunction (64 cases, 25.6%), sepsis (31 cases, 12.4%) and hypoglycemia (21 cases, 8.4%). The mean weight of the newborn population studied was 2,412±900 g and mean gestational age was 35.7±3.7 weeks. The majority of the NB had adequate intrauterine growth (67.9%), while 26.9% were small for gestational age (SGA) and 5.2% were large for gestational age. Maternal age varied from 14 to 48 years (mean 25.8±7.3 years). More than 80% of the population of recently-delivered mothers were married or had a stable relationship. Two hundred and thirty-seven (94.8%) of the 250 mothers had received some prenatal, with 175 attending four or more consultations and 62 from one to three.

Two of the 250 NB enrolled on the study presented positive PCR for CMV in urine, which equates to a prevalence of 0.8% (95% CI: 0.097-2.86). There were no cases in which the duplicate tests did not agree.

The mother of one of the patients infected by CMV was a primaparous black adolescent (16 years) student with a fixed partner. This was a planned pregnancy with an event-free prenatal that went to full term when a male weighing 3,105 g was delivered vaginally with normal examination, SNAPE-PE (Score for Neonatal Acute Physiology-Perinatal Extension) of 0 and respiratory dysfunction that progressed well. Contact with this mother was lost.

The mother of the other patient was a white 30 year-old housewife. This was her second pregnancy, it was planned and there had been an ovarian infection during prenatal and she had been carrying twins, but one fetus had died. Delivery was vaginal, birth weight was 1,260 g, gestational age was 30 weeks, SNAPE-PE was 0 and sex was male. The child presented petechiae at birth and persistent tachypnea for several days. This patient continued to be followed by our service. At 1 year and 6 months he was still excreting CMV in urine, had normal neurological development for his corrected age and his sight and hearing examination findings were normal.

## Discussion

Congenital CMV infection is the most prevalent congenital infection worldwide, varying from 0.2 to 2.2%. A study conducted by Santos et al. in Minas Gerais found a prevalence of congenital CMV infection at a ICU neonatal of 6.8%,[13] in contrast to what we observed where prevalence was 0.8% (two cases out of 250 patients). A study in Finland detected a prevalence of congenital CMV infection of 4.8% among premature NB, below 34 weeks, at a neonatal ICU, with full term NB not enrolled on the study.[16] In Milan, Italy, the observed prevalence among the general population of a neonatal ICU was 1%.[17] In a neonatal ICU in Japan a prevalence of 10% of congenital CMV infection was observed. Babies who tested positive were SGA or premature. This study failed to report important population data, such as maternal and prenatal parameters.[18] Another study, carried out in Hungary, found a prevalence of 16.7% among premature NB and of 14.7% among full term NB at a neonatal ICU.[19] These studies conducted at neonatal ICUs show an extremely variable prevalence, without giving the details of each population. Similarly, prevalence studies of all live births have also returned variable results. Panutti et al. studied, middle and low socioeconomic class populations in São Paulo and found prevalence rates of 0.39 and 0.98%, respectively. Two diagnostic methods were used (serology and viral urine culture) and an increased prevalence was demonstrated among less privileged socioeconomic classes, while the superiority of culture over assaying IgM in blood was also proven.[11] In Ribeirão Preto (São Paulo state), Yamamoto et al. demonstrated a 2.6% prevalence of congenital infection (diagnosed by isolating the virus from urine).[14] The same team from Ribeirão Preto found a similar prevalence rate among the offspring of HIV-positive mothers (2.7%) and HIV-negative mothers (2.9%, $p = 1.00$).[12] The same team have also demonstrated that there is no difference in the prevalence of this infection between full term NB (1.8%) and preterm NB (1%).[20] Recent research from Japan, Mexico and Israel obtained prevalence rates of 0.31, 0.89 and 0.7%, respectively.[22-24] Part of the difference in the results from prevalence studies seems to be more related to characteristics of the populations than of the NB. The work carried out in Ribeirão Preto[12,20] has shown us that there was no greater prevalence of congenital CMV among HIV positive mothers or among premature NB. The characteristics of the population that we studied explain the reduced prevalence of congenital CMV infection, even in a study performed within a neonatal ICU. Despite the hospital being a public

one, the population that uses it seems to be very distinct. Just 11% of the mothers studied were less than 18 years old and around 80% were married or had stable relationships, thus eliminating two important risk factors for CMV, which are adolescent expectant mothers and sexual promiscuity. Furthermore, the great majority of expectant mothers, 94.8% of them, had adequate prenatal care, receiving guidance on precautions to take during pregnancy. We could, therefore, attribute the reduced prevalence to this prenatal care. It is known that low maternal age, single marital status, underprivileged socioeconomic classes, sexual activity with multiple partners, multiparity and high population density increase the risk of risk of congenital CMV infection.[2,10] Lansky et al. reviewed the literature on factors that could reduce perinatal mortality and concluded that one such factor was good prenatal care.[25] Another contributing factor was the exclusion of transferred-in patients because our service is a referral canter and it is common for us to receive NB with suspected congenital infections. If these patients had been included the prevalence would have been higher giving a distorted picture of our service and creating a selection bias. While Santos et al. did not mention whether they had excluded referred patients, we observe that they had a study population with 70% of birth weights below 2,500 g and a majority of small for gestational age, compared with 57.2% premature NBs in our sample and just 26.9% SGA. It is probable that these studies citing higher prevalence rates for CMV at Neonatal ICUs have included patients originating from other regions, distant from the services themselves.[16,18,19]

The PCR urine diagnostic technique for identifying viral DNA has high sensitivity and specificity offers easy sample collection and rapid results. In order to avoid contamination of the samples and false positive results, barrier tips with filters were used, there were four distinct environments for the different stages of the test and negative controls were used at each amplification. Positive controls were also used to avoid false negatives. All samples were tested in duplicate. This test currently appears to be the most reliable means for diagnosing the infection in NB.[7,23] The method's cost-benefit is improved by the massive social damage caused by the neurological and auditory sequelae caused by the disease and which can be ameliorated with early diagnosis. In contrast with the available serological test methods, which produce both positive and negative false results, as the work by Neto et al. demonstrated in 2004. They used Guthrie test results from many regions in Brazil produced using ELISA-IgM serology for CMV from 15,873 NB. Thirty-nine of them had IgM tests that were positive for CMV (0.2%). These NB and their mothers were retested and just 16 (0.1% of the total) had the result confirmed.[26]

In conclusion we state that the early diagnosis of this infection would make possible treatment in serious cases, family counseling, multidisciplinary follow-up of symptomatic babies and screening and early diagnosis of complications among asymptomatic babies, thus offering greater hope for the futures of these children.

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