Congenital Cytomegalovirus Infection as a Cause of Sensorineural Hearing Loss in a Highly Immune Population

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Background: The burden of congenital cytomegalovirus (CMV)-associated sensorineural hearing loss (SNHL) in populations with CMV seroprevalence approaching 100% is unknown. The purpose of this study was to assess the rate, associated factors, and predictors of SNHL in CMV-infected infants identified by newborn screening in a highly seropositive maternal population.

Methods: Newborns with positive saliva CMV-DNA that was confirmed by virus isolation in the first 2 weeks of life were enrolled in a prospective follow-up study to monitor hearing outcome.

Results: Of 12,195 infants screened, 121 (1%) were infected with CMV and 12 (10%) had symptomatic infection at birth. Hearing function could be assessed in 102/121 children who underwent at least one auditory brainstem evoked response testing at a median age of 12 months. SNHL was observed in 10/102 (9.8%; 95% confidence interval: 5.1–16.7) children. Median age at the latest hearing evaluation was 47 months (12–84 months). Profound loss (>90 dB) was found in 4/5 children with bilateral SNHL while all 5 children with unilateral loss had moderate to severe deficit. The presence of symptomatic infection at birth (odds ratio, 38.1; 95% confidence interval: 1.6–916.7) was independently associated with SNHL after adjusting for intrauterine growth restriction, gestational age, gravidity, and maternal age. Among 10 infants with SNHL, 6 (60%) were born to mothers with nonprimary CMV infection.

Conclusions: Even in populations with near universal immunity to CMV, congenital CMV infection is a significant cause of SNHL demonstrating the importance of CMV as a major cause of SNHL in children worldwide. As in other populations, SNHL is more frequently observed in symptomatic CMV infection.

Key Words: cytomegalovirus, congenital infection, hearing loss, Brazilian children

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Congenital cytomegalovirus (CMV) infection has been reported to be an important cause of hearing loss in infants born in North America and northern Europe. Population-based studies in Sweden,[1] Canada,[2] and United States[3,4] have reported that 9.3% to 17% of infants with congenital CMV infection will have sensorineural hearing loss (SNHL). The rates of SNHL reported by these studies ranged between 22% and 41% in children with clinically apparent or symptomatic infection and between 6% and 16% in those with subclinical or asymptomatic infection.

Recent studies have reported that hearing loss occurs at a similar frequency in children born to mothers who had primary CMV infection during pregnancy and offspring of women with nonprimary infection with documented preconceptional seroimmunity.[5,6] These studies showed that 7% to 10% of infected infants from mothers with nonprimary infection had SNHL, whereas 11% to 15% of infants born to mothers with primary infection had SNHL. However, the prevalence and natural history of CMV-associated SNHL in maternal populations with near universal CMV seroimmunity have not been well defined. This feature of the natural history of congenital CMV infection is particularly relevant in regions of the world with transitional economies such as South America, Africa, and southern Asia where near universal seroimmunity to CMV in maternal population has been reported. In a recent study, we have shown that the birth prevalence of congenital CMV infection (1%) and the proportion of congenitally infected infants with symptomatic infection in our population with maternal seroprevalence rate of 96.7% to be similar to that found in the populations with lower CMV seroprevalence rates.[7] Thus, more precise definition of the role of congenital CMV infection as a cause of hearing loss in offspring of women from highly seroimmune population is of considerable importance because understanding the rates of SNHL is relevant to the issue of vaccine prevention of maternal CMV infection. The objective of the current study was to assess the rate, associated factors, and predictors of CMV-induced SNHL in a highly seropositive maternal population.

PATIENTS AND METHODS

Study Population

Between March 2003 and May 2009, 121 infants with congenital CMV infection were identified from a prospective screening of 12,295 newborns in 2 public hospitals of Ribeirão Preto, State of São Paulo, Brazil. The first maternity hospital (MATER) provides care for low-risk parturients. The second hospital, Clinical Hospital of Faculty of Medicine of Ribeirão Preto, University of São Paulo, not only serves as a referral center for high-risk parturients but also provides care for low-risk parturients. Infants with congenital CMV infection were identified by the detection of CMV DNA in saliva or urine specimens collected within the first 2 weeks of life and confirmed by virus isolation in tissue culture.[8] The study was approved by the Research Ethics Committee of the University Hospital (Processes 4782/2002, and

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9145/2004), and written informed consent was obtained from all mothers.

Newborn Evaluation and Definition of Congenital CMV Disease

All infants identified as congenitally infected underwent a clinical evaluation, ophthalmological examination, and computed tomography (CT) scan of the brain. Congenitally infected neonates were classified as small for gestational age (<fifth percentile) or appropriate for gestational age (≥fifth percentile) according to a standard reference curve.[9] Microcephaly was defined as described previously.[7] Infants were classified as symptomatic if they presented with at least one of the following findings suggestive of congenital infection including petechiae, cholestatic jaundice (conjugated bilirubin level >2 mg/dL), hepatosplenomegaly, purpura, microcephaly, seizures, chorioretinitis, or abnormal cranial CT.[7,10] Infants who were small for gestational age were not classified as having symptomatic infection if they did not exhibit any of the typical CMV-related findings.[10]

Audiologic Evaluation

The audiologic protocols consisted of an auditory brainstem evoked response (ABR) testing for all congenitally CMV-infected infants within the first year of age and children younger than 3 years of age. During follow-up visits, pure tone conditioned play audiometry measurement was performed in children older than 3 years of age. The ABR register was performed using a standard protocol after infant's sedation with chloral hydrate when necessary.[11] All infants underwent otoscopic examination to detect middle-ear disorders before testing. The ABR threshold was defined as the lowest level at which the wave V could be detected and replicated. SNHL was suspected when the first ABR test showed air conduction thresholds above 30 dB in an infant with normal middle ear function. Confirmation of SNHL was made after at least 2 ABR evaluations performed on different occasions. A child was considered to have normal hearing when the first ABR threshold was ≤30 db and confirmed by subsequent ABR and/or pure tone audiometry measurements.

In March 2006, a newborn hearing screening program consisting of transient otoacoustic emission (OAE) testing (AccuScreen, Madsen, Denmark) of all infants born at the study hospital was instituted. The results of the OAE screening test was reported as pass or fail.

Definition of Maternal CMV Infection

Maternal CMV infection was considered primary when a CMV-specific immunoglobulin G (IgG) seroconversion occurred during pregnancy or when the first prenatal serum specimen contained CMV IgG antibodies of low avidity with a subsequent increase in the avidity index in the sample obtained at delivery. Women with CMV IgG antibodies before pregnancy and those with high avidity CMV IgG antibodies without CMV IgM within the first 25 weeks of gestation were classified as having nonprimary CMV infection.[12]

Statistical Analysis

Statistical analysis was performed by logistic regression models using the LOGISTIC procedure of SAS 9.0 statistical package, SAS Institute. Associations between newborn findings, maternal characteristics, and hearing loss were analyzed initially by crude odds ratios (ORs) with 95% confidence intervals for each OR. To avoid confounding effects, multiple logistic regression models were performed simultaneously for each variable and adjusted ORs were calculated to determine the predictors independently associated with hearing loss.

RESULTS

Among 121 infants with congenital CMV infection, 12 (10%) had clinical findings at birth that were consistent with symptomatic congenital infection, 5 had multisystem disease (1 died within the first week of life), and 4 showed at least one clinical finding. The remaining 3 infants had only cranial computerized tomography findings including abnormalities of neuronal migration, leading to polymicrogyria (1 infant), white matter gliosis and supratentorial ventriculomegaly (1 infant), and myelination delay in association with lissencephaly (1 infant). Of 12 symptomatic infants, 5 received 6 weeks of intravenous ganciclovir therapy (6 mg/kg/per day in 2 doses) and 4 of them had multisystem disease. None of the infected infants had abnormal findings on ophthalmologic examination. Intrauterine growth restriction was observed in 35 of 121 (28.9%) infants. Among 12 symptomatic infants, 8 (66.7%) were small for gestational age.

Hearing Outcome in Children With Congenital CMV Infection

The overview of the study population and the results of hearing evaluations are shown in Figure 1. Of the 121 infants, 102 (84%) with congenital CMV infection underwent at least one ABR assessment, and the median age of initial ABR testing was 12 months (range: 15 days–51 months; 18 of 102 [17.6%] <6 months, and 32 of 102 [30%] <12 months of age). Three infants had conductive hearing loss at the time of this analysis, and 14 infants (1 symptomatic) were lost to follow-up after undergoing only one ABR test. All of these 14 infants had normal hearing. Of the 102 children who underwent at least one ABR testing, 10 (9.8%; 95% confidence interval [CI]: 5.1–16.7) were demonstrated to have SNHL. Their median age at the latest hearing evaluation was 47 months (range: 12–84 months). Using more stringent criteria that included at least 2 ABR assessments and follow-up for at least 12 months, SNHL was confirmed in 10 of 85 (11.8%; 95% CI: 6.1–19.9) children; 6 of 11 with symptomatic (54.5%; 95% CI: 25.9%–81.3%), and 4 of 75 (5.3%; 95% CI: 1.7–12.4) with asymptomatic congenital CMV infection. Five children had bilateral hearing loss and among these, 4 (3 with multisystem disease and 1 asymptomatic in the neonatal period who had no abnormalities in the CT scan) had profound loss (>90 dB) and the remain-

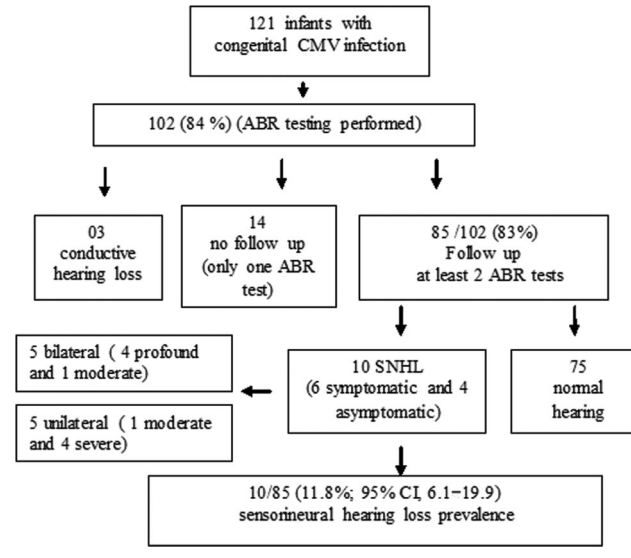

FIGURE 1. Overview of the study population.

TABLE 1. Characteristics of 85 Infants With Congenital CMV Infection According to Sensorineural Hearing Status

	Hearing Loss (n = 10)	Normal Hearing (n = 75)	Crude OR (95% CI)	Adjusted OR (95% CI)
Type of congenital CMV infection				
Asymptomatic (n = 75)	4 (5.3%)	71 (94.7%)	1.0	1.0
Symptomatic (n = 10)	6 (60.0%)	4 (40.0%)	26.6 (5.3–134.1)	38.1 (1.6–916.7)
Intrauterine growth restriction				
Adequate for gestational age (n = 63)	4 (6.4%)	59 (93.6%)	1.0	1.0
Small for gestational age (n = 22)	6 (27.3%)	16 (72.7%)	5.5 (1.4–22.0)	7.3 (0.7–72.9)
Gestational age (wk)				
Term ≥37 (n = 60)	8 (13.3%)	52 (86.7%)	1.0	1.0
Preterm <37 (n = 25)	2 (8.0%)	23 (92.0%)	1.7 (0.3–9.0)	7.2 (0.5–106.2)
Gender				
Male (n = 49)	3 (6.1%)	46 (93.9%)	1.0	1.0
Female (n = 36)	7 (19.4%)	29 (80.1%)	3.7 (0.9–15.5)	12.4 (0.9–163.9)
Maternal age (y)				
≥20 (n = 55)	3 (5.5%)	52 (94.5%)	1.0	1.0
<20 (n = 30)	7 (23.3%)	23 (76.7%)	5.3 (1.3–22.2)	1.1 (0.1–22.8)
Gravidity				
Multiparous (n = 37)	2 (4.3%)	45 (95.7%)	1.0	1.0
Primiparous (n = 44)	8 (21.1%)	30 (78.9%)	6.0 (1.2–30.2)	9.5 (0.5–182.7)

CMV indicates congenital cytomegalovirus; CI, confidence interval; OR, odds ratio.

ing child with multisystem disease during neonatal period had moderate SNHL (60 dB). All 5 children with unilateral involvement (2 symptomatic and 3 asymptomatic) had moderate to severe SNHL (60–90 dB).

Of the 85 children, 65 (76%) whose hearing status could be ascertained completed follow-up for at least 36 months of age and underwent multiple hearing evaluations (median of 4 tests, range: 2–6). Their median age at the latest hearing evaluation was 56 months (range: 36–84 months). None of the 65 children had progression in the hearing deficit.

The demographic characteristics and newborn findings were compared among study children with at least 2 ABR evaluations according to their hearing status (Table 1). No association was observed between hearing loss and intrauterine growth restriction, gestational age, gender, maternal age, or gravidity. Although univariate analyses showed that children with abnormal findings at birth and those born to mothers younger than 20 years of age were more likely to develop hearing loss, only symptomatic congenital CMV infection remained independently associated with hearing loss. Additional risk factors for SNHL were observed in 1 infant who was born prematurely (32 weeks) and received intravenous aminoglycosides for more than 5 weeks after birth.

Newborn Hearing Screening Findings

Of 10 infants, 7 with confirmed hearing loss had been tested by OAE within the first month of life and 6 of these infants failed OAE. In all 6 children, ABR confirmed loss in the same ear in which OAE testing resulted in failure, suggesting that hearing loss was present at birth. In 1 child who had normal hearing at 2 months of age, ABR testing at 10 months of age revealed unilateral profound SNHL (109 dB). Among 75 infants with normal hearing, 30 had been screened by OAE in the neonatal period. Two of them had failed OAE in 1 ear, however, subsequent testing by OAE and at least 2 ABR assessments revealed normal hearing. Delayed-onset SNHL could not be excluded in 3 children in whom hearing loss was detected at the time of their first ABR evaluations at older ages (21, 28, and 40 months) and did not undergo OAE testing during the neonatal period.

TABLE 2. CMV-related Hearing Loss According to Type of Maternal Infection

Hearing Status	Maternal CMV Infection (n = 85)		
	Primary (n = 3)	Nonprimary (n = 40)	Indeterminate or Samples Not Available (n = 42)
Moderate to severe unilateral HL	0	4	1
Moderate to profound bilateral HL	1	2	2
Normal	2	34	39

CMV indicates congenital cytomegalovirus; HL, hearing Loss.

Maternal Immune Status and CMV-associated Hearing Loss

The data on association between the type of maternal CMV infection and hearing loss are shown in Table 2. Serum samples were collected from 43 (50%) of the mothers of 85 children, adequately evaluated by at least 2 ABR tests, during their first prenatal visit and at delivery. Seven infants of these 43 mothers had hearing loss and 6 of them were born to mothers with nonprimary maternal CMV infection as determined by the presence of CMV-IgG antibodies before pregnancy in 1 mother and by the presence of CMV-IgG antibodies of high avidity in serum samples obtained between 6 and 25 weeks of gestation in 5. The mother of the remaining infant with SNHL had primary infection as indicated by low-avidity CMV IgG antibodies in the serum sample obtained at 9 weeks of gestation.

DISCUSSION

This prospective follow-up study of children with congenital CMV infection identified by newborn screening of Brazilian infants demonstrated that congenital CMV infection is an important cause of SNHL even in this population with near universal maternal CMV seroimmunity. The frequency of hearing loss detected in our study (9.8%) is similar to that reported for popu-

lations with lower CMV seroprevalence in which the majority of congenital CMV infections was presumed to be a consequence of primary maternal CMV infections[2,6] and for populations with higher CMV seroprevalence in the developed world.[3,13,14] Thus, the findings of our study confirm that although the prevalence of congenital CMV infection may vary with underlying CMV seroprevalence rates and demographic factors, it constitutes an important cause of SNHL worldwide.

Although the type of maternal CMV infection could only be determined in 7/10 infants with CMV-related hearing loss, 6 of these children were born to mothers with nonprimary maternal infection and only one was born to a mother with primary infection. The findings of our study confirm previous evidence from populations in the United States and Northern Europe that the frequency of hearing loss is similar in congenitally infected infants irrespective of the maternal CMV serologic status prior to pregnancy.[5,6] Further, our findings demonstrate the occurrence of bilateral and severe to profound SNHL in congenitally infected children born to women with nonprimary maternal CMV infection.

Although the exact prevalence of SNHL at birth in the study population has not been delineated, available data suggest that the prevalence of hearing loss at birth in Brazilian infants (0.96 per 1000 living newborns)[15] is similar to that observed in the United States. (1.6 per 1000)[16] and Europe (0.78 per 1000).[17] Considering the 1% birth prevalence rate of congenital CMV infection in our population, the hearing impairment because of congenital CMV infection would affect at least 6 per 10,000 live births or 1800 of the 3 million Brazilian infants born annually. Even though most (4/5, 80%) of the infants with bilateral severe hearing loss had been identified due to the presence of multisystem CMV-related signs at birth, approximately half of the children (4/10) with SNHL had no detectable clinical abnormalities at birth and therefore, would not have been identified during the neonatal period if they were not screened for CMV.

A limitation of our study is that the hearing evaluation of CMV-infected children were not performed at similar ages and at least 2 ABR assessments were only performed in 85 of 121 study children with congenital CMV infection. Of the 7 study children with confirmed CMV-associated SNHL who also underwent OAE screening during the neonatal period, only one child with normal hearing during initial testing developed SNHL at a follow-up evaluation at 12 months. However, since newborn hearing screening was not in place during the first 3 years of the study, it is not possible to determine the exact time of onset of hearing impairment or document late-onset and/or progressive SNHL in some of the CMV-infected children with SNHL. In addition, 76% of the study children were followed up for at least 36 months. Therefore, we believe that our data provide reliable estimates of CMV-associated SNHL in a highly seropositive population and suggest that current strategies to prevent morbidity associated with congenital CMV infection including the development of prophylactic vaccines to prevent primary maternal infections during pregnancy may have limited efficacy in these populations.

Similar to the findings from studies conducted in populations with different CMV seroprevalence rates in the United States and Europe, the results of our study indicated that symptomatic infants were significantly more likely to develop SNHL than those with asymptomatic infection.[1–3] Thus, the presence of CMV-related symptoms at birth is a strong predictor of hearing loss, even in populations with high maternal CMV seroprevalence rate. Our findings demonstrate that congenital CMV infection is an important cause of hearing loss, including bilateral and severe to profound deficit, even in countries with transitional economies in which maternal seroimmunity is nearly universal.

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