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Congenital Cytomegalovirus Infection and Permanent Hearing Loss in Rural North Indian Children

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Abstract

Background—Congenital cytomegalovirus infection (cCMV) is a leading non-genetic cause of permanent congenital or early-onset hearing loss (PCEHL). Although cCMV rates are high despite near-universal seroimmunity, the contribution of cCMV to PCEHL in the developing world is unclear.

Methods—Neonates at a rural north Indian hospital were screened for cCMV by saliva PCR and hearing by distortion product otoacoustic emission (DPOAE) testing. CMV positive infants and those not passing newborn hearing screening (NHS) were evaluated by auditory brainstem response to confirm PCEHL. Infants with cCMV and those with PCEHL were tested for mutations within the GJB2 gene.

Results—Of the 1720 infants screened, 40 (2.3%) did not pass NHS and 20 (1.2%) were CMV positive. ABR testing confirmed unilateral or bilateral PCEHL in 11 (0.64%) children who either did not pass NHS or CMV positive. PCEHL was 20-fold higher in neonates with cCMV (2/20, 10%) than those without (9/1700, 0.5%; $p<0.01$). None of 11 infants with PCEHL had connexin 26 mutations.

Conclusion—PCEHL incidence is high in India, with cCMV contributing significantly despite near universal seroimmunity. Our findings also demonstrate the feasibility and the utility of simultaneous newborn screening for both cCMV and hearing loss in a resource-limited setting.

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Keywords

Cytomegalovirus; congenital; hearing loss; screening

Cytomegalovirus (CMV) is a frequent cause of congenital infection worldwide.[1,2] Congenital CMV infection (cCMV) is a leading non-genetic cause of permanent hearing loss (PCEHL) in children.[3] The prevalence of cCMV is directly related to the CMV seroprevalence in the women of child-bearing age such that higher rates of cCMV are consistently observed in populations with higher seroprevalence.[4–6] The CMV seroprevalence rates are >95% in resource-limited settings such as Brazil, India, and sub-Saharan Africa[2,7–9] with the rate of cCMV greater than 1% in these countries.[7.8.10] The findings from studies in Brazil with maternal CMV seroprevalence >95% have shown that the rate of hearing loss in children with cCMV are similar to the rates seen in the US and other lower seroprevalence settings.[8,11] These studies suggests that CMV-associated hearing loss is a cause of significant burden in resource-limited settings, even in the presence of near-universal CMV seropositivity in women of childbearing age.

The number of children with disabling hearing loss is increasing worldwide.[12,13] The challenge of hearing loss is more pronounced in developing countries where >700,000 babies are born annually with or acquire permanent hearing loss early in life.[14] Of the 120 million infants born annually in developing countries, about 737,000 or 6/1,000 live births are likely to have permanent congenital or early-onset hearing loss (PCEHL) compared with 2 per 1000 live births in developed countries.[14,15] Without a systematic effort for early detection shortly after birth, PCEHL is a hidden handicap that usually becomes evident after the first year of life secondary to delayed speech and language.[16] Similarly, most infants with cCMV have no obvious clinical abnormalities at birth and therefore are not identified as being at risk for PCEHL.[1,2] Because the prevalence rates of both cCMV and hearing loss at birth are significantly higher in developing countries, early detection of cCMV and hearing loss to prevent permanent disabilities associated with PCEHL is a significant unmet medical need in these settings. However, newborn hearing screening does not occur in most of the developing world including India. The objectives of the current study are to determine the frequency of CMV-associated hearing loss in a rural north Indian population by screening newborns for CMV and hearing status.

MATERIALS and METHODS

Study setting

The Comprehensive Rural Health Services Project (CRHSP) of the Center for Community Medicine, All India Institute of Medical Sciences (AIIMS) in Ballabgarh near Delhi, India. The CRHSP provides comprehensive preventive, health promotion and intervention services for a population of approximately 90,000 in Ballabgarh and surrounding villages.

Study subjects and specimens

Infants born at CRHRP between December 2010 and May 2012 were screened for hearing within the first 2 days after birth. All live born infants were eligible to participate in the

study and enrollment into the study occurred every day of the week except Sunday. Mothers were approached postpartum to obtain written informed consent for their infant's enrollment in the study. Saliva samples were obtained from the newborns within the first 2 days after birth using cotton tipped swabs in viral transport medium and transported to the virology laboratories at AIIMS and kept at 4°C until testing [Dar, 2008]. Saliva specimens were collected at least one hour after breastfeeding.

CMV Screening

Extraction of DNA from saliva specimens was done using a commercially available kit (QIAamp Viral RNA Minikit, Qiagen Inc, CA, USA). Amplification of CMV DNA was performed by a nested PCR for using primers targeting the gB gene as described previously.[7] The amplified products were resolved on agarose gel electrophoresis. DNA preparation from CMV AD 169 strain was used as the positive control. The samples that were positive by the nested PCR were retested by a previously described real-time PCR assay for confirmation.[17]

Newborn hearing screening (NHS)

Hearing screening was performed by a medical officer using Audx Pro otoacoustic emission (OAE) equipment (Natus/Bio-Logic, San Carlos, CA). Distortion Product Otoacoustic Emissions (DPOAE) testing occurred within a day of birth and those infants not passing on initial DPOAE testing were screened again by DPOAEs between 2 and 4 weeks of age either at the health center or during home visits.

Auditory brainstem evoked response (ABR) testing

Infants positive for CMV, as well as those who did not pass their second DPOAE hearing screen were evaluated by ABR using a broadband click stimulus to determine hearing threshold in the ENT clinics of AIIMS. The threshold obtained by ABR was the lowest intensity at which wave V could be detected and replicated. Hearing loss was defined as an ABR click-threshold greater than 40 dB nHL. To rule out conductive hearing loss, children whose hearing loss was confirmed by ABR also had tympanometry and evaluated by an otolaryngologist. Infants with permanent hearing loss were evaluated in AIIMS ENT clinic as part of standard clinical management for hearing rehabilitation.

Connexin 26 mutations

Infants with cCMV and uninfected infants with confirmed PCEHL were tested for the presence of mutations within the GJB2 gene that have been associated with hearing loss by an allele-specific PCR and nucleotide sequence analysis of exon 2 of the GJB2 gene.[18]

Data analysis

Maternal demographic data and newborn information was recorded on standardized case report forms. The categorical variables were compared with Fisher's Exact test, and continuous variables were analyzed using a 2-sided *t* test or Wilcoxon rank sum test, as appropriate.

RESULTS

Characteristics of study population and CMV Screening Results

Between December 2010 and May 2012, 1720 infants were screened for hearing and cCMV. Of the 1720 infants tested, twenty babies (1.2%; 95% CI 0.73–1.8) were positive for CMV by saliva PCR. All 20 samples were also positive by the real-time PCR assay (Figure 1). The demographic characteristics of study infants according to cCMV status are shown in Table 1. There were no differences in maternal age, gestational age and birth weight between CMV-infected and uninfected infants. More cCMV positive infants (20%) were small for gestational age compared with CMV negative infants (7.4%), although this difference did not reach statistical significance (p=0.06). One infant with cCMV had symptomatic infection with jaundice, hepatomegaly, and splenomegaly.

Newborn hearing screening results

During the initial DPOAE screening in the hospital, 362 (21%) of the 1720 infants failed the test in one or both ears. Of these 362 infants, a 2nd DPOAE screening was completed for 333 (92%) infants, 27 were lost to follow-up and two infants underwent ABR testing without a 2nd screening. Of the 333 infants who underwent a 2nd DPOAE screening, 293 infants passed and 40 infants did not pass the 2nd screening, for an overall refer rate of 2.3% (40/1720). Of those infants who did not pass NHS, 23 referred in one ear and 17 in both ears (Figure 1). Among the 20 CMV positive infants, 19 passed NHS and one infant failed the 2nd DPOAE hearing screen.

Hearing loss in the study population

Of the 59 infants who were either CMV positive (19), failed NHS (39) or both (1), ABR testing was completed for 50 infants including all CMV-infected babies. The median age of ABR testing was 4 months (range 1 to 20 months). Two infants with cCMV (10%) including one infant who did not pass NHS were confirmed to have PCEHL and both children had bilateral severe to profound hearing loss. Of the 30 children who were CMV negative, did not pass NHS, and underwent ABR testing, 9 infants were confirmed to have hearing loss on ABR testing, an overall rate of PCEHL of 5 per 1000 in this population of unselected infants. The hearing loss was bilateral in 7/9 children with thresholds ranging from 50dB to 100dB.

Connexin mutations

None of the 11 children with confirmed PCEHL had GJB2 mutations detected by the allele specific PCR and nucleotide sequencing.

DISCUSSION

In this study, we screened newborns in a rural setting in northern India for both cCMV and PCEHL and show high prevalence of congenital CMV infection and provide reliable estimates of permanent congenital or early onset hearing loss and cCMV in this population. This is the first study in rural Indian population demonstrating the feasibility of implementing newborn hearing screening (NHS) in conjunction with CMV screening. The

high prevalence of cCMV (1.2%) observed in this population with near-universal CMV seroprevalence was previously reported in a smaller study[7]. The prevalence of hearing loss in this population, ~5 per 1000 is higher that the reported hearing loss prevalence in developed country settings but similar to the rates of PCEHL from developing country populations. We also show that cCMV is an important cause of hearing loss in this population. Our study did not include data on long-term audiological follow-up of children with cCMV with normal hearing beyond infancy and thus, the occurrence of delayed-onset hearing loss could not be ascertained. Therefore, the actual number of children with CMV-associated hearing loss may have been underestimated.

The importance of cCMV as a cause of PCEHL has not been well documented in resource-limited setting with high CMV seroprevalence. A study from Brazil showed that 11% of children with cCMV had hearing loss.[11] Findings of our study are similar to those findings and we observed PCEHL in 10% of infants with cCMV. This is in contrast to 0.5% prevalence of PCEHL in uninfected infants. One of the 20 (5%) CMV-infected infant also did not pass NHS which is more than twice the rate of uninfected infants (2.3%) who did not pass NHS. A similar finding of higher referral rate on NHS in infants with cCMV was reported from a previous study in the US.[19]

Given the fact that about 25 million infants are born in India each year[20], it is estimated that between 250,000 and 300,000 infants with cCMV are born annually in India. The findings from our study suggest that 25,000 to 30,000 infants born each year will suffer from CMV-associated hearing loss. Therefore, cCMV is likely a significant cause of disease burden in infants and young children in India and likely other resource-limited settings with high CMV seroprevalence.

In addition to the data on the prevalence of cCMV and the burden of CMV-associated hearing loss, the findings of this study also provide reliable information on the prevalence of PCEHL in rural north Indian population. Further, we demonstrate the feasibility of implementing newborn hearing screening program in this population. Screening of infants for both hearing and CMV will also allow the identification of CMV-related hearing loss thereby permitting early intervention measures including antiviral therapy and hearing rehabilitation to decrease permanent disabilities. Most NHS screening programs in the US include at least two screening tests and infants who are referred on the 2nd screening test will undergo a diagnostic ABR testing. The refer rate after the 2nd OAE screening (2.3%) is comparable to most NHS screening programs in the U.S. and Europe. Although, it is preferable to conduct hearing screening after the first 24 hours of life to avoid interference by amniotic fluid in the ear canal, it was not possible in our study because most babies were discharged from the health center. Therefore, the 2nd DPOAE screening was conducted after discharge either during a home visit or at the CRHSP. A limitation of this study is the loss to follow-up between the 1st and 2nd OAE screening and between the 2nd screening and ABR testing. However, most infants (92%) who failed the 1st OAE screening had the 2nd screening test and ABR testing could be completed in 85% of those who did not pass the 2nd OAE screening. Nonetheless, this is the first study to demonstrate the feasibility of NHS in rural Indian population and provide reliable estimates of the prevalence of PCEHL at birth and early infancy. The finding that 5 per 1,000 infants had PCEHL indicates the high

prevalence of hearing impairment early in life in rural Indian population and highlights the need for more studies in different population groups to define the burden and to develop effective strategies for identification of hearing loss early in life to prevent or minimize long-term disabilities. As the vast majority of the population in India lives in rural areas and almost half of all deliveries occur at home, our study provides a framework for implementing NHS at rural health centers and the feasibility of conducting screening at home.

We have examined for the presence of mutations in the GJB2 that code for connexin 26 protein in the 11 children with confirmed PCEHL. Using allele-specific PCR and nucleotide sequencing, none of previously reported mutations in GJB2 in Indian children with non-syndromic hearing loss were found.[18] However, the number of children examined for GJB2 mutations in this study is small.

As only newborn saliva samples were tested for CMV and breastfeeding is common in this population, it is possible that the positive PCR in some of the children was due to the presence of breast milk CMV in saliva. The samples were collected at least an hour after breastfeeding to minimize this possibility. In addition, it has been shown that CMV shedding in breast milk usually occurs after the 1st week postpartum.[21] As the screening saliva PCR results were not confirmed by testing saliva or urine specimens obtained within the first 3 weeks of life, the possibility of contamination of saliva specimens with CMV present in the breast milk was not completely eliminated in our study. However, we did not observe false-positive CMV PCR of saliva specimens in our previous study and therefore, it is unlikely that the positive PCR results in our study were due to contamination of saliva from CMV in breast milk.[7] In addition to the loss to follow-up during screening and ABR testing, another limitation of our study is that ABR testing was completed at varying ages (between 3 and 9 months) in children who were either CMV positive or did not pass NHS. Therefore, accurate information on the age of onset of PCEHL could not be determined. However, our findings do provide reliable data on the overall prevalence of PCEHL in early infancy.

To summarize, the finding of the current study provide reliable data on the prevalence of congenital CMV infection, CMV-associated hearing loss, and the overall prevalence of PCEHL present at birth or during infancy. Our findings also demonstrate the feasibility of NHS in rural Indian population, the importance of implementing NHS on a wider scale in India because of the high prevalence of PCEHL at birth or during early infancy in this population. Finally, the findings also document the importance of cCMV and the significant disease burden from this intrauterine infection in this highly seropositive population.

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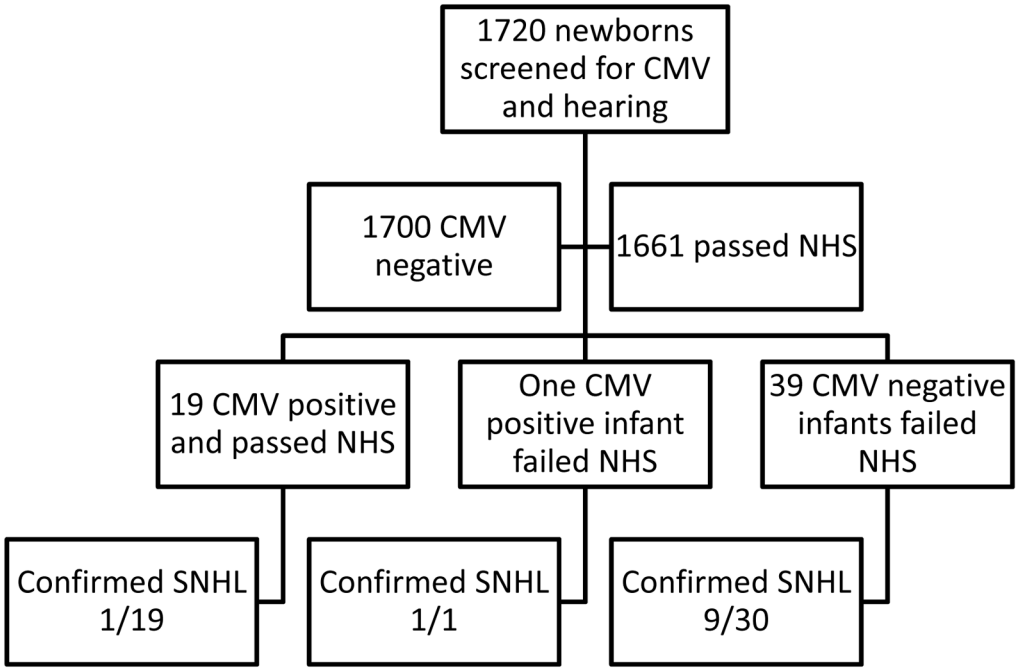

Figure 1.

Prospective screening of newborns for CMV and hearing at The Comprehensive Rural Health Services Project (CRHSP) of the Center for Community Medicine, All India Institute of Medical Sciences (AIIMS) in Ballabgarh near Delhi, India.

Table 1

Demographic characteristics and newborn hearing screening results for study infants according to CMV status

	Infants with cCMV (N = 20)	Uninfected infants (N = 1700)	p-value
	N (%)		
Female gender	7 (35)	781 (45.4)	NS
Maternal age (mean ± SD)	22.7 ± 3.7	23.8 ± 3.3	NS
Gestational Age	37.8 ±1.2	37.6 ± 1.5	NS
Birth Weight (kg, mean ± SD)	2.67 ± .53	2.80 ± .43	NS
Small for gestational age	4/20 (20)	124/1693 (7.4)	0.06
Referred on newborn hearing screening	1 (5)	39 (2.4)	0.38

Table 2

Characteristics of sensorineural hearing loss in infants with congenital CMV infection and uninfected infants

	Infants with cCMV (N=20)	Uninfected infants (N=1700)
	N (%)	
PCEHL	2 (10)	9 (0.5)
Bilateral PCEHL	2 (100)	7 (77.8)
Severity of PCEHL		
Mild (21–45 dB)	0	0
Moderate (46–70 dB)	0	5
Severe (71–90 dB)	0	3
Profound (>90 dB)	2	1