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Universal neonatal cytomegalovirus screening using saliva – Report of clinical experience

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ABSTRACT

Objectives: To analyze the results of a neonatal universal screen for congenital cytomegalovirus (CMV) using saliva real-time polymerase chain reaction (rt-PCR).
Study design: During one year (15/5/2011–15/5/2012), saliva was collected from 9845 infants (97% of 10,137 newborns). Viral DNA was extracted by Magna-Pure LC (Roche) and was tested for the presence of CMV IE and gB genes. Urine culture was collected from positive infants for confirmation. For all infants with congenital CMV maternal data were collected and head ultrasound, blood count, liver enzymes, retinal examination and auditory brainstem response testing were performed. Parents were notified in advance and had the option to avoid screening. The ethical committee approved retrospective analysis of the data.
Results: Fifty six infants (0.57%) had a positive saliva assay. Of these, 47 were confirmed by urine rt-PCR and culture, in another one maternal sero-conversion was documented during pregnancy (48 infants). Twenty-eight mothers (28/47, 60%) had primary infection during pregnancy, 14 (30%) had non-primary infection, and no serological data were obtained from five (10%). Four infants (8.5%), two with prenatal diagnosis of CMV and normal fetal brain imaging and two born to mothers sero-positive before pregnancy, exhibited symptoms related to CMV and were offered antivirals. Hearing impairment was diagnosed in two infants (late onset HI in one case).
Conclusions: Saliva rt-PCR assay is a feasible and effective means of universal neonatal CMV screening that can detect affected infants who might benefit from treatment and follow-up. The long-term clinical significance of screening and its cost effectiveness are yet to be determined.
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1. Background

Congenital cytomegalovirus (CMV) infection is the most common intra-uterine infection in developed countries with major neuro-developmental implications, including hearing impairment (HI) [1]. The real burden of its consequences is not fully appreciated due to many undiagnosed asymptomatic infants born to mothers who were previously exposed to CMV and are not aware of the possibility of transmitting it to the fetus during pregnancy. Lately, with the implementation of universal newborn CMV screening in several medical centers around the world, the true burden of congenital CMV infection has become more obvious. Dollard et al. [2] summarized data from 15 projects of newborn screening for congenital CMV that included 120,000 infants and estimated that about 13.5% of asymptomatic and 40–58% of symptomatic infants will suffer from sequelae. The most promising and practical approach to CMV newborn screening, using saliva real-time polymerase chain reaction (rt-PCR) described by Boppana et al.,

Abbreviations: ABR, auditory brainstem response; CMV, cytomegalovirus; HI, hearing impairment; IUGR, intrauterine growth restriction; LSV, lenticulostriate vasculopathy; rT-PCR, real-time polymerase chain reaction; SNHL, sensory neural hearing loss; TEAOE, transient evoked otoacoustic emissions; US, ultrasound.

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Yamamoto et al. and others [3–5], enables us to identify asymptomatic infants who previously went undiagnosed. These infants are now closely monitored and evaluated in terms of development and hearing status at birth and later on. Townsend et al. [6] reported their experience with 176 infants with congenital CMV infection among 50,000 infants screened. Sequelae were found in 14% of the asymptomatic infants, non-primary maternal infections contributed substantially to the burden of disease. Though universal newborn hearing screening currently successfully detects infants with congenital hearing impairment, many children with congenital asymptomatic CMV who will develop late-onset hearing loss are likely to be missed. Fowler et al. [7] monitored 388 infected newborns diagnosed with CMV at birth for six years. While 5.2% were detected with sensory-neural hearing loss (SNHL) at birth, by the age of 72 months 15.4% of the children were found to have HI (thresholds >20 dB). The ability to offer antiviral treatment to symptomatic infants and the opportunity to identify and habilitate hearing impaired infants as early as possible [8] make the case for CMV screening.

In Israel, rates of congenital CMV, detected by urine PCR were found to be 0.4% and 1% in two different hospitals serving socio-economically different populations [9]. The Chaim Sheba Medical Center is a large tertiary hospital which serves mostly Jewish population, with around 10,000 labors per year. In a previous report we summarized the results of our neonatal screening in which we used a single primer rt-PCR to detect CMV DNA from umbilical blood. We have detected CMV DNA in 0.27% of screened infants [10]. This low rate could be explained by the low sensitivity of single primer PCR in blood samples as described by Boppana et al. [11], and by the fact that most infected infants are not viremic at birth. The gold standard assay for diagnosis of congenital CMV is urine culture. Since urine collection is technically difficult in infants, and since saliva has been shown to be sensitive in detection of congenital CMV infection [3–5], we decided to screen all infants born in our Medical Center using rt-PCR to detect both IE and gB genes in saliva (presuming better sensitivity using 2 genes).

2. Objective

The aim of this study was to retrospectively analyze the first year of this universal screening project and to describe clinical characteristics of infants who were positive for CMV and their audiological follow-up at 3–18 months.

3. Study design

Upon approval of the Medical Center General Manager, a project of newborn screening for CMV was launched. Beginning 15/5/2011, saliva was collected immediately after birth from all infants born at the Chaim Sheba Medical Center. On admittance to the labor and delivery room, parents were notified of the screening and were given the option to object (informed dissent). Approval of the local ethics committee was obtained for retrospective analysis of data collected from infants born during the period 15/5/2011–15/5/2012. Funding was provided through an internal hospital grant reserved for clinical projects.

3.1. Virological studies

Saliva specimens were collected in Virocult tubes and transported to the Central Virology Laboratory (CVL) within 24–48 h. Viral DNA was extracted by Magna-Pure LC (Roche) and was tested by rt-PCR assay, developed at the CVL, for the presence of CMV IE and gB genes. Urine rt-PCR and virus rapid culture (Shell Vial) were used to confirm positive cases.

Infants with confirmed congenital CMV infection underwent the following workup: pregnancy history, thorough physical examination, complete blood count (CBC), serum liver enzymes, head ultrasound (US), retinal examination and hearing evaluation. When positive results were obtained after infant discharge, the parents were contacted by an infectious diseases specialist and referred to perform workup as soon as possible. All infants were evaluated at the pediatric infectious diseases clinic before reaching 4 weeks of age. Infants were defined as "symptomatic" when one or more positive findings were detected upon investigation: intra-uterine growth restriction (IUGR), petechiae, hepato-splenomegaly, microcephaly, elevated liver enzymes or direct bilirubin, thrombocytopenia, abnormal brain imaging, chorioretinitis and SNHL, as defined hereafter.

3.2. Hearing evaluation

All infants were tested using transient evoked otoacoustic emissions (TEOAE) as part of a universal hearing screening test performed to all infants prior to hospital discharge. Furthermore, all infants underwent auditory brainstem response (ABR) within 10–14 days after discharge. ABR thresholds to clicks and 1 kHz tone bursts stimuli, i.e., ≤20 dB normal hearing level – nHL in both ears were considered within the normal range. Periodic audiological follow-ups were also carried out in accordance with the recommendations of the Joint Committee on Infant Hearing 2007 Position Statement [12]. All children were scheduled for audiological follow-up at regular intervals. Specifically, when initial ABR thresholds were within the normal range, children underwent behavioral audiometry and TEOAE testing at three-month intervals until the age of one year and behavioral audiometry at six-month intervals until the age of three years. In all cases of uncertainty, repeated ABR testing was immediately performed. When infants were offered antiviral treatment, ABR testing was frequently repeated. HI was defined as unilateral or bilateral SNHL greater than 25 dBHL in the 500–4000 Hz frequency region. HI severity was determined based on the Clarks classification [13].

Antiviral treatment with ganciclovir or valganciclovir was offered to selected cases of "symptomatic" congenital CMV infection, with significant central nervous system findings and/or SNHL. Periodic visits at the pediatric infectious diseases clinic took place at the ages of 3, 6 and 12 months for asymptomatic infants. "Symptomatic" cases were examined more frequently.

4. Results

During the period 15/5/2011–15/5/2012, 10,137 live infants were born at the Chaim Sheba Medical Center. Saliva was collected from 9845 infants (97%) (Fig. 1).

Fifty six (0.57%) saliva samples were positive for CMV by rt-PCR in at least one of the genes. Seven samples were weakly positive by rt-PCR (either high PCR cycle or positivity in only 1 gene). In all cases repeated saliva rt-PCR showed similar results and urine rt-PCR and cultures were negative. We assumed that these results may be related to cross-contamination from small amounts of CMV DNA in birth canal or breast milk. These children were not considered as CMV infected (false positives) and no further work-up was performed. In 47 infants (84%), congenital infection was confirmed by a positive urine rt-PCR and culture. Of note, in 7/47 saliva rt-PCR was positive only in the gB gene. In the 40 others, PCR was positive for both genes. In two additional positive cases, urine was not collected; however, one of these cases was included due to documented maternal sero-conversion during pregnancy. Thus, 48 infants with congenital CMV (0.49% of screened infants) were included, the product of 47 deliveries (2 twin pregnancies: in both

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