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Clinical and Laboratory Observations

Retrospective Diagnosis of Congenital Cytomegalovirus Infection at a School for the Deaf by Using Preserved Dried Umbilical Cord

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Key words: sensorineural hearing loss; real-time PCR.

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ABSTRACT

A retrospective diagnosis of congenital cytomegalovirus infection was made for three (12%) of 26 students with either bilateral profound or severe sensorineural hearing loss at a School for the Deaf in Japan by detecting viral DNA with real-time PCR from dried umbilical cords that had been preserved at home.
INTRODUCTION

Although congenital cytomegalovirus (CMV) infection is the leading cause of sensorineural hearing loss (SNHL) in developed countries,¹ its impact hasn’t been precisely recognized because of the difficulty in making the diagnosis beyond the neonatal period.²

Recent studies³ proved the efficacy of dried blood spots on Guthrie cards as materials to retrospectively investigate congenital infections; however, they are usually discarded within a year in Japan. The umbilical cord is kept nicely clean and dry as a symbol of mother-child bond and has been shown to be useful for retrospective diagnosis of congenital CMV infection⁴-⁹. We used preserved dried umbilical cords to determine the prevalence of congenital CMV infection at a school for the deaf in Japan.

PATIENTS AND METHODS

Study subjects

Thirty-six students with bilateral SNHL were enrolled at Nagasaki-Prefectural School for the Deaf with written consent. Further virological investigations were made for only 26 subjects (13 boys and 13 girls) whose cord samples yielded $10^3$ copies or more of tubulin gene DNA per µl by real-time PCR. The school is the only one that provides education suitable for children who have either profound (>90 dB) or severe (71-90 dB) bilateral HL without severe mental or physical handicap in Nagasaki Prefecture. The study protocol was approved by the ethical committee of Nagasaki University School of Medicine.
Sample preparation and real-time PCR

A little piece (approximately 30 mg) of each umbilical cord sample was cut off using a clean cutter knife. To avoid cross-contamination, blades were changed for each sample. DNA was extracted by using the QIAamp DNA Mini kit (QIAGEN).

Real-time PCR assays, performed as described previously,\textsuperscript{10} were sensitive enough to detect as few as 3 copies in a reaction. For the DNA specimens used, the lower detection limit was estimated to be approximately 100 copies of CMV genome per µg DNA.

Statistical Analysis

Statistical analyses were performed using StatView-J 4.02 (Abacus Concepts Inc, Berkeley, CA). A classical chi\textsuperscript{2} test was used for the 2X2 tables.

RESULTS

Among the 26 subjects tested, CMV-DNA was detected in three (12%; Table 1). None of them drew particular medical attention at birth; however, intrauterine growth retardation (IUGR) was pointed out in two (67%), KR3 and KR24, of the three, while only two (9%) out of the 23 CMV-negative subjects had IUGR ($p = 0.052$). The etiology of IUGR could not be determined in any of them, since neither maternal illnesses nor pregnancy-associated complications were identified, except that a mother of one patient (KR3) had a history of aseptic meningitis during her pregnancy ($p = 0.115$). None of the
three positive for CMV had a family history of HL, as compared to nine (39%) of the 23 negative for CMV ($p = 0.261$). Parents deferred genetic studies on hereditary hearing loss. None of the three positive for CMV had history of administration of ototoxic drug, meningitis, mumps, recurrent otitis media or head trauma. HL was asymmetric in two (KR3 and KR19) of the three, but in none of the CMV-negative subjects ($p = 0.0269$). One of them (KR3) developed SNHL with a late onset: she had been healthy until 10 years of age when she noticed unilateral hearing impairment and gradually developed bilateral severe SNHL by 14 years of age. No delayed onset of HL was observed in any of the CMV-negative subjects ($p = 0.115$). Since the progression of her HL was very insidious, its actual onset was obscure. Progressive hearing loss was observed in another subject (KR19). In contrast, progressive HL was not found in any of the CMV-negative subjects ($p = 0.0092$). None of the study subjects had other neurological deficit such as psychomotor retardation, emotional disturbance, autism or epilepsy, based on the admission policy in this school.

**DISCUSSION**

The impact of a congenital CMV infection on the pathogenesis of SNHL has been largely unknown in Japan, partly because it is very difficult to make the diagnosis beyond the neonatal period. Guthrie cards, which have been used to make retrospective diagnoses of congenital CMV infection, are stored for only a year in Japan.

The umbilical cord is detached from newborn babies within two weeks after birth and contains blood coagula and endothelial cells; therefore, the presence of CMV
DNA in those samples should indicate congenital infections. A series of preserved umbilical cords from healthy infants (data not shown) or infants with symptomatic postnatal CMV infection were demonstrated to be negative for CMV DNA by real-time PCR. In a series of studies, retrospective diagnosis of congenital CMV infection has been successfully made by using preserved umbilical cords from Japanese children, thus implying that this unique material may provide opportunities for clinicoepidemiological studies of congenital infections.

Recently, Ogawa et al. conducted a large-scale retrospective study on the etiological role of congenital CMV infection in SNHL using preserved umbilical cords and identified congenital CMV infection in 10 (15%) patients with SNHL among 67 tested. The rate of congenital CMV infection-related cases in the current study (12%) was comparable to it even though the study subjects were considerably different between the two studies. While SNHL was the only clinical manifestation in the current study subjects, those in their study included patients with mental retardation, cerebral palsy or autism. Therefore, the prevalence of congenital CMV infection in patients with SNHL alone may be similar to that in patients with SNHL complicated with other neurological sequelae in Japan.

Patients with CMV-associated SNHL in this study had no family history of HL but were frequently associated with IUGR. Such clinical manifestations as delayed onset, and asymmetric and progressive nature of HL have been reported in congenitally CMV-infected children, thus re-emphasizing the necessity of making an accurate diagnosis of and conducting a long-term follow-up for infants with an asymptomatic...
congenital CMV infection.

In conclusion, a retrospective diagnosis using preserved umbilical cord implicated the impact of a congenital CMV infection in Japanese children; however, further studies performed in a prospective manner will provide greater insight regarding the epidemiology of congenital CMV infection and its relationship to SNHL in Japan.
ACKNOWLEDGMENTS

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REFERENCES


Table 1. Characteristics of 3 cases of CMV-associated SNHL

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<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td>Age tested (years)</td>
<td>16</td>
<td>11</td>
<td>4</td>
</tr>
<tr>
<td>CMV genome (copies/10^6 cells)</td>
<td>3.6x10^3</td>
<td>1.8x10^2</td>
<td>5.6x10^3</td>
</tr>
<tr>
<td>Gestational age (wks)</td>
<td>38</td>
<td>40</td>
<td>39</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>1800</td>
<td>3050</td>
<td>2478</td>
</tr>
<tr>
<td>IUGR</td>
<td>yes</td>
<td>no</td>
<td>yes</td>
</tr>
<tr>
<td>Special event(s) during pregnancy</td>
<td>yes*</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>Family history of HL</td>
<td>no</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>Degree of HL (dB; left/right)</td>
<td>65/122**</td>
<td>98/124**</td>
<td>118/134</td>
</tr>
<tr>
<td>Age of onset (years)</td>
<td>&lt;10***</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Progressive HL****</td>
<td>yes</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>Psychomotor retardation</td>
<td>no</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>Emotional disturbance</td>
<td>no</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>Convulsion</td>
<td>no</td>
<td>no</td>
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</tbody>
</table>

*Aseptic meningitis during pregnancy.
** Asymmetric HL (defined as >20-dB difference at thresholds between worse and better ears).
***Delayed-onset (applied here to a patient(s) who had no suspicion of HL at 18-month and 42-month regular health check-ups including auditory assessment with a questionnaire and appeared to have developed HL thereafter).
****Defined when sensorineural decrease in hearing of >10 dB was documented on 2 separate evaluations.
Table 2.  CMV-associated SNHL versus non-CMV SNHL

<table>
<thead>
<tr>
<th></th>
<th>CMV+</th>
<th>CMV-</th>
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<tbody>
<tr>
<td></td>
<td>n = 3</td>
<td>%</td>
<td>n = 23</td>
</tr>
<tr>
<td>Family history of HL</td>
<td>0</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>IUGR</td>
<td>2</td>
<td>67</td>
<td>2</td>
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<tr>
<td>Maternal viral illness*</td>
<td>1</td>
<td>33</td>
<td>0</td>
</tr>
<tr>
<td>Asymmetric HL</td>
<td>2</td>
<td>67</td>
<td>1</td>
</tr>
<tr>
<td>Delayed onset HL</td>
<td>1</td>
<td>33</td>
<td>0</td>
</tr>
<tr>
<td>Progressive HL</td>
<td>2</td>
<td>67</td>
<td>0</td>
</tr>
</tbody>
</table>

*Aseptic meningitis during pregnancy