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Original Research

Congenital Cytomegalovirus Infection and Maternal Primary Cytomegalovirus Infection in Universal Newborn Hearing Screening Referral Patients: A Prospective Cohort Study

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Abstract

Background: There are no detailed reports in the literature on maternal cytomegalovirus antibody screening for universal newborn hearing screening (UNHS) referral patients. We examined maternal cytomegalovirus antibody screening results and estimated the incidence of maternal primary cytomegalovirus infection among UNHS referral patients. **Methods**: During September 2013–March 2021, fresh urine samples were collected in the first week after birth from 98 neonates with UNHS referral results at 15 obstetrical institutions in Mie, Japan (the first hearing screening). We performed a real-time polymerase chain reaction analysis to detect cytomegalovirus DNA in the samples. Infants with $\geq$200 copies/mL of cytomegalovirus DNA were diagnosed with congenital cytomegalovirus (cCMV) infection. A second hearing screening was performed, and patients with positive results were sent to the otorhinolaryngologists for further examinations of congenital hearing loss. We calculated incidence rates (%) with 95% confidence intervals (CIs) for cCMV infection among patients with UNHS referral results and maternal primary cytomegalovirus infection among patients who underwent maternal cytomegalovirus antibody screening. **Results**: Among the 98 neonates with UNHS referral results (the first hearing screening), 5 were diagnosed with cCMV infection (incidence rate: 5.1%; 95% CI: 0.8–9.5). All five patients with cCMV had positive second hearing screening results and were sent to their otorhinolaryngologists. All five were diagnosed with congenital hearing loss, and four were diagnosed with congenital hearing loss secondary to cCMV infection. The remaining patient with cCMV infection was diagnosed with congenital hearing loss unrelated to cCMV infection. Of the 98 patients, 60 underwent maternal cytomegalovirus antibody screening. Among the 60 patients, six had maternal primary cytomegalovirus infection during pregnancy (incidence rate: 10.0%; 95% CI: 2.4–17.6). Of the six patients, four were positive for cytomegalovirus immunoglobulin (CMV Ig) G and IgM antibodies in maternal blood with low CMV IgG antibody avidity results during early pregnancy, while the remaining two had maternal CMV IgG antibody seroconversion during pregnancy. **Conclusions**: This is the first study to examine the maternal primary cytomegalovirus infection incidence rate in patients with UNHS referral results (the first hearing screening). We identified a 10-fold higher risk in this population (10.0%) than in the general population (0.98%).

Keywords: cytomegalovirus antibody screening; universal newborn hearing screening; congenital cytomegalovirus infection

1. Introduction

Cytomegalovirus (CMV) is the most common pathogen in transplacental infection during pregnancy and subsequent congenital anomalies in both developed and developing countries. Congenital CMV (cCMV) infection in trans-placentally-infected infants can only be diagnosed in the first few weeks of birth because newborns develop CMV antibodies due to infection with harmless CMV through breastfeeding. Thus, neonatal urine and saliva specimens are usually tested for CMV DNA. Symptoms at birth in patients with severe cCMV infection include hepatosplenomegaly, petechia, pneumonia, retinitis, small-for-gestational-age, cerebral calcification, and ventriculomegaly [1]. Complications, such as abnormal fetal heart rate patterns or fetal distress, during delivery have been reported in patients with severe symptomatic cCMV infection [2]. Moreover, the presence of persistent or late-onset neurological symptoms after birth has been

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observed in both patients with severe symptomatic and those with asymptomatic cCMV infection at birth. Among the neurological symptoms, sensorineural hearing loss (SNHL) is considered the most common. cCMV infection is the most prevalent cause of congenital SNHL at birth in 8–21% of patients [1].

Universal newborn hearing screening (UNHS) is performed for the early detection of congenital SNHL. The otoacoustic emission test is considered to have inferior sensitivity compared with the automated auditory brain-stem response (AABR) test [3]. Approximately 4–5 of 1000 neonates fail in the UNHS (using AABR test) and have to be sent to the otorhinolaryngologists for further examinations of congenital hearing loss, while the remaining pass based on the UNHS. Approximately 50% of those sent to the otorhinolaryngologists are diagnosed with congenital SNHL after further examinations [4]. In addition to the otoacoustic emission or AABR test in UNHS, auditory brain-stem response and auditory steady-state response tests, as well as temporal bone computed tomography, are used to perform detailed examinations for congenital SNHL [5]. Patients with symptomatic cCMV infection with SNHL at birth can be detected by UNHS. However, nearly 15% of children with asymptomatic cCMV infection develop SNHL later in life and, therefore, remain undetected according to the UNHS [6]. Minami *et al*. [7] reported a high risk of missing patients with cCMV infection with SNHL in UNHS (13 of 44 patients with cCMV infection passed UNHS bilaterally).

Maternal CMV antibody screening can be performed to identify women with primary CMV infection during pregnancy with a high risk of subsequent cCMV infection. In maternal antibody screening, CMV-specific immunoglobulin (Ig) G and IgM antibodies and IgG avidities are measured in pregnant mothers. Some previous studies have reported on maternal primary CMV infection in Japan, including ours [8–11]. In these reports, low CMV IgG avidity or IgG seroconversion to positive results was found in all pregnant women with primary CMV infection. Even in mothers with CMV IgG seroconversion during pregnancy, with the highest rate of in-utero CMV transmission, the rate of cCMV infection in infants is nearly half.

To the best of our knowledge, no previous study has provided detailed results of maternal CMV antibody screening for UNHS referral patients. In this study, we examined maternal CMV antibody screening results and identified the incidence rate of maternal primary CMV infection among UNHS referral patients for the first time.

2. Materials and Methods

In Mie, Japan, UNHS (using the AABR test) is performed at about 4 days after birth (the first hearing screening) among almost all neonates, except when the mothers do not wish to accept that the test be performed. We prospectively enrolled neonates with referral results in the first hearing screening at each obstetrical institution in Mie, Japan and collected their fresh urine samples during the first week after birth. We performed a real-time polymerase chain reaction analysis to detect CMV DNA from the samples at Mie University Hospital in Mie, Japan, as previously described [9,10]. Infants with $\geq$200 copies/mL of CMV DNA in fresh urine samples were diagnosed with cCMV infection. In patients with cCMV infection, additional viral isolation and subunit analysis of CMV glycoprotein B were performed using the CMV DNA-positive urine samples at the National Hospital Organization Mie National Hospital in Mie, Japan, as previously described [12].

While the urine tests were being performed, the second hearing screening was being performed in neonates with the first hearing screening referral results on the same day or the next day after that. Patients who were diagnosed with or without cCMV infection, but had the second hearing screening referral results, were sent to the otorhinolaryngologists for further examination of congenital hearing loss, comprising auditory brain-stem response and auditory steady-state response tests, as well as temporal bone computed tomography. Congenital hearing loss was diagnosed within the first 3 months of their birth, whereby the audiological test results were abnormal. Even in patients with congenital hearing loss and cCMV infection, the temporal bone computed tomography test was performed to identify bony malformations as a cause of SNHL.

We have been conducting maternal CMV antibody screening at 24 obstetrical institutions in Mie, Japan since 2013 as part of the "Cytomegalovirus in Mother and Infant-Engaged Virus Serology (CMieV)" program. In this program, all pregnant women are screened for CMV IgG and IgM antibodies (Denka Company Limited, Tokyo, Japan) during early pregnancy. We also conducted CMV IgG antibody avidity tests in pregnant women with positive CMV IgG and IgM antibody results. IgG antibody avidity tests were performed at Aisenkai Nichinan Hospital in Miyazaki, Japan, as previously described [9,13]. Alternatively, we repeated the tests for CMV IgG and IgM antibodies during the late pregnancy in pregnant women with negative CMV IgG antibody results during the early pregnancy. We considered the results as follows: low CMV IgG avidity or IgG seroconversion, positive IgG and negative IgM, high IgG avidity, and no IgG seroconversion as maternal primary infection, a past infection, non-primary infection, and no infection (uninfected), respectively.

We calculated the incidence rates (%) with 95% confidence intervals (CIs) for cCMV among all patients with referral results in UNHS (the first hearing screening) and maternal primary CMV infection among patients who underwent maternal CMV antibody screening. SPSS software version 27 (IBM Corp., Armonk, NY, USA) was used for statistical analyses.

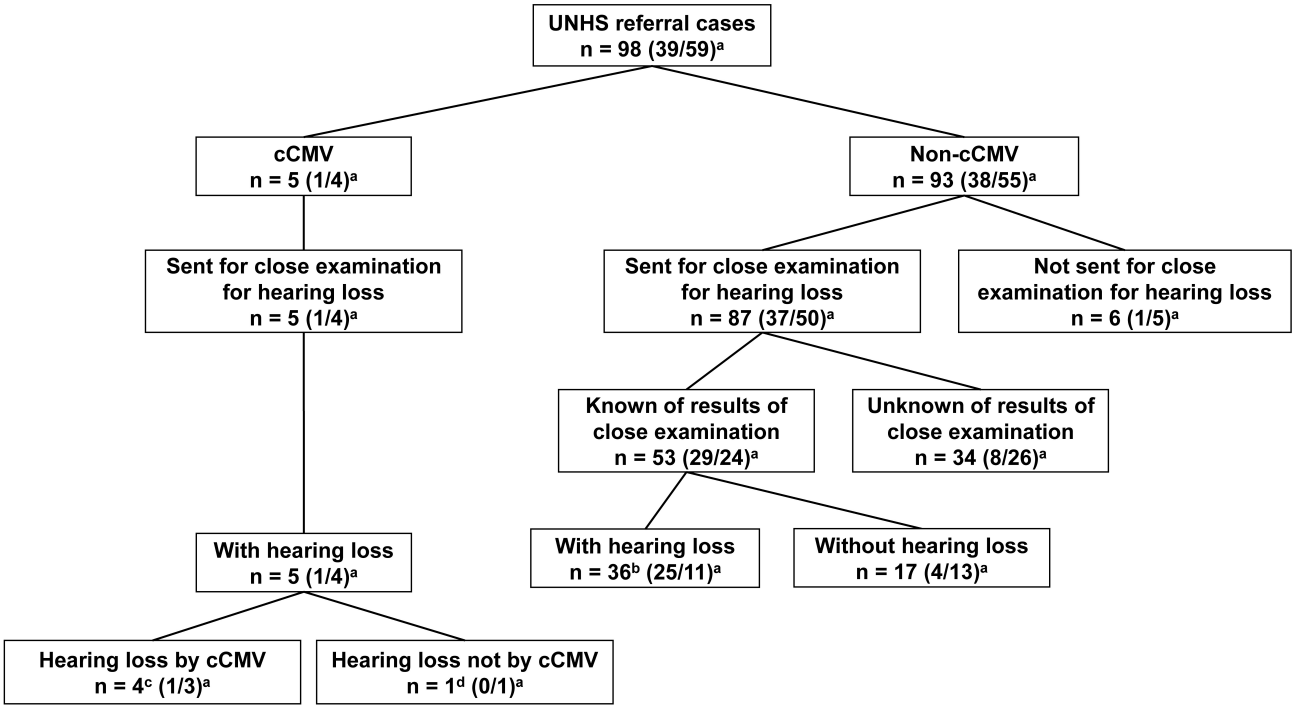

Fig. 1. Presence or absence of congenital cytomegalovirus infection and congenital hearing loss in participants. UNHS, universal newborn hearing screening; cCMV, congenital CMV; CMV, cytomegalovirus. [a]Bilateral/Unilateral referral patients, [b]Cases 6–41, [c]Cases 1–3, 5, [d]Case 4.

3. Results

During September 2013–March 2021, 98 neonates with referral results in UNHS (the first hearing screening) at 15 obstetrical institutions (1 university hospital, 4 general hospitals, 3 private hospitals, and 7 private clinics) in Mie, Japan, were enrolled and fresh urine samples were collected. The median number of gestational weeks at birth was 38 (range: 25–41) weeks, and the median birth weight was 3039 (range: 534–4453) g. The admission rate in the neonatal intensive care unit was 31.6% (31 of 98 patients). Thirty-nine and 59 patients had bilateral and unilateral referral results in the first hearing screening, respectively.

Among the 98 referred patients in the first hearing screening, five had $\geq$200 copies/mL of CMV DNA detected in fresh urine samples and were diagnosed with cCMV infection (incidence rate: 5.1%; 95% CI: 0.8–9.5). All five cCMV patients showed positive results in the second hearing screening performed on the same day as the first hearing screening or the next day, and they were sent to the otorhinolaryngologists. All five patients with cCMV infection (none of them underwent tests for deafness genes) were diagnosed with congenital hearing loss, and four (Cases 1–3, 5) were diagnosed with congenital hearing loss secondary to cCMV infection. The remaining patient with cCMV infection (Case 4) had unilateral cochlear nerve canal stenosis and ipsilateral narrow internal auditory canal and was diagnosed with congenital hearing loss unrelated to cCMV infection (Fig. 1).

Four of the five patients with cCMV infection underwent maternal CMV antibody screening. All four patients with cCMV infection had maternal primary CMV infection during pregnancy. Of the four patients, three (Cases 1–3) were positive for CMV IgG and IgM antibodies in maternal blood with low CMV IgG antibody avidity results during early pregnancy; the remaining patient (Case 4) had positive CMV IgG antibody seroconversion results during pregnancy. Detailed information on the five patients with cCMV infection (Cases 1–5) is shown in Table 1.

Of the 98 patients, 93 were not diagnosed with cCMV infection. Among the 93 patients not diagnosed with cCMV infection, 87 showed positive results in the second hearing screening performed on the same day as the first hearing screening or the subsequent day, and they were sent to the otorhinolaryngologists. We obtained the otorhinolaryngologists' detailed examination results for congenital hearing loss for 53 of the 87 patients. Of the 53 patients, 36 were diagnosed with congenital hearing loss (Fig. 1 and **Supplementary Table 1**).

The mothers of 60 of the 98 infants underwent maternal CMV antibody screening. Among the 60 maternal patients, six had primary CMV infection during pregnancy. Among the six maternal patients, four had CMV IgG and IgM antibodies detected in blood with low CMV IgG antibody avidity results during early pregnancy; the remaining two had CMV IgG antibody seroconversion during pregnancy. Three (Cases 1–3) of the four maternal patients, who

Table 1. Information of the five patients with congenital cytomegalovirus infection (cases 1–5).

No.	Sex	GWs of birth (week)	Birth weight (g)	Side of "refer" in UNHS/Actual SNHL	Degree of SNHL	Cause of SNHL	Amount of CMV DNA in neonatal urine (log10) (copy/mL)	Viral isolation of neonatal urine	Subunit of gB	Abnormality other than hearing loss	Maternal age (years)	Maternal parity (para)	Maternal CMV antibody screening	Subdivision of maternal CMV antibody screening
1	F	37	2244	Right/Right	Severe	cCMV	5	+	1	Brain MRI	30	0	Primary infection	IgG+, IgM+, Low IgG avidity
2	M	37	3090	Left/Left	Severe	cCMV	4	+	3	Brain MRI	30	0	Primary infection	IgG+, IgM+, Low IgG avidity
3	M	40	3482	Both/Both	Severe (right), moderate (left)	cCMV	4	-	NA	Nothing	24	0	Primary infection	IgG+, IgM+, Low IgG avidity
4	F	38	2458	Right/Right	Severe	Not cCMV*	3	+	1	Nothing	27	1	Primary infection	IgG seroconversion
5	F	38	3840	Left/Left	Severe	cCMV	4	+	1	Brain MRI	34	1	No screening	No screening

GW, gestational week; UNHS, universal newborn hearing screening; SNHL, sensorineural hearing loss; CMV, cytomegalovirus; gB, glycoprotein B; cCMV, congenital cytomegalovirus; MRI, magnetic resonance imaging; Ig, immunoglobulin; NA, not available; F, female; M, male.

*Stenosis of cochlear nerve canal and narrow internal auditory canal.

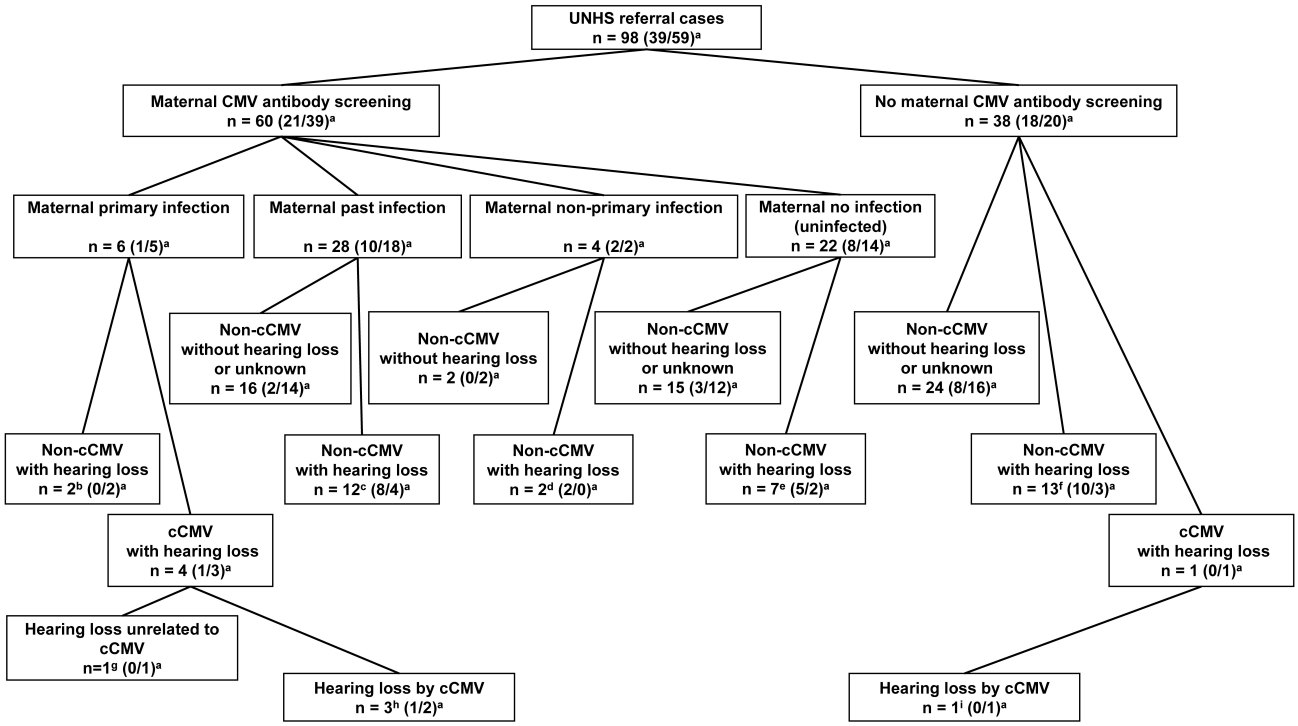

Fig. 2. Presence or absence of maternal cytomegalovirus antibody in participants and results of antibody screening and congenital hearing loss. UNHS, universal newborn hearing screening; cCMV, congenital CMV; CMV, cytomegalovirus. [a]Bilateral/Unilateral referral patients, [b]Cases 6, 7, [c]Cases 8–19, [d]Cases 20, 21, [e]Cases 22–28, [f]Cases 29–41, [g]Case 4, [h]Cases 1–3, [i]Case 5.

were positive for CMV IgG and IgM antibodies and had low CMV IgG antibody avidity results, had infants diagnosed with cCMV infection with congenital hearing loss. The infant of the remaining maternal patient (Case 6) was diagnosed with non-cCMV congenital hearing loss. The infant of one (Case 4) of the two maternal patients with CMV IgG antibody seroconversion results had cCMV infection with congenital hearing loss unrelated to cCMV infection (the patient with unilateral cochlear nerve canal stenosis and ipsilateral narrow internal auditory canal mentioned above), and the other (Case 7) had non-cCMV-related congenital hearing loss. In 54 of the 60 patients who underwent maternal CMV antibody screening, 28, 4, and 22 patients had a past infection, non-primary infection, and no infection, respectively. There were no patients with cCMV infection among them (Figs. 2,3).

4. Discussion

CMV is the most common viral cause of mother-to-fetus infections, affecting approximately 1% of all live births worldwide, with a prevalence of 0.6–0.7% in developed countries [6]. Congenital hearing loss onset in patients with cCMV infection often occurs after the neonatal period and is therefore not detected by UNHS. The incidence rates of cCMV detected in UNHS referral patients have been reported in previous studies, ranging from 0.9% to 5.0% [14–19]. Stehel *et al.* [16] reported an incidence rate of 5.0% (24 of 483 patients), which was closest to that (5.1%) of the current study. The differences in the incidence rates may be attributed to the study population and admission rate in neonatal intensive care units. The rate of neonatal intensive care unit admission affects the detection of congenital hearing loss in UNHS referral patients [6], and the incidence rate of cCMV infection in UNHS referral patients would likely vary according to the study population to some extent. Although the admission rate in this study was nearly one-third of the total, no further comparisons could be made with the previous studies. This needs to be addressed in further studies.

cCMV infection is the most prevalent cause of congenital SNHL at birth in 8–21% of patients [6,16,20–23]. Rates of congenital SNHL secondary to cCMV infection increase to 25% by 4 years of age, owing to patients with late-onset SNHL related to cCMV infection [6,24]. In the current study, the rate of cCMV infection in congenital SNHL patients at birth was 12.2% (5/41 patients), which is consistent with the previous reports mentioned above, although not all five patients had SHNL due to cCMV infection. This means that even if a UNHS referral patient is positive for cCMV infection, it does not necessarily mean that the SNHL is caused by cCMV infection. Even in patients with cCMV infection with SNHL, a detailed otorhinolaryngological examination is necessary.

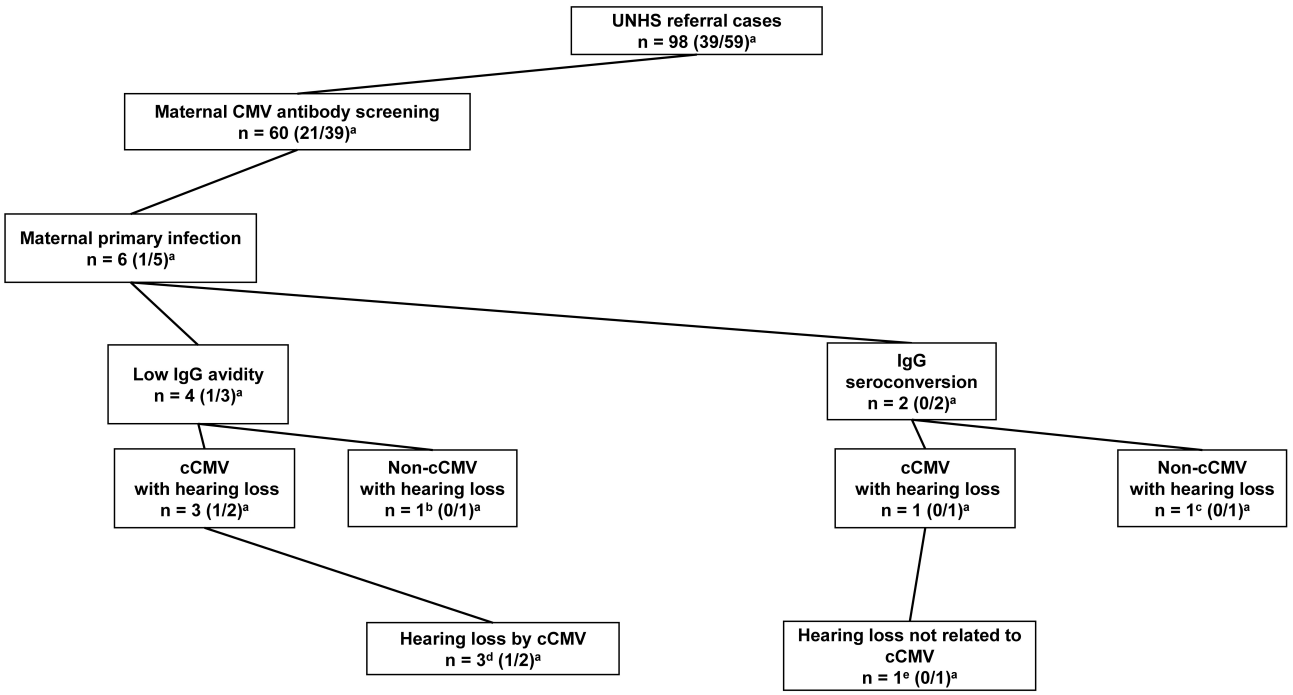

Fig. 3. Primary cytomegalovirus infection in maternal cytomegalovirus antibody screening. UNHS, universal newborn hearing screening; cCMV, congenital CMV; CMV, cytomegalovirus. [a]Bilateral/Unilateral referral patients, [b]Case 6, [c]Case 7, [d]Cases 1–3, [e]Case 4.

In a maternal CMV antibody screening, low IgG avidity is an indicator of primary infection, whereas IgG seroconversion confirms primary infection. Thus, the incidence rate of cCMV infection in mothers with IgG seroconversion (almost half) is higher than that in those with low IgG avidity (<10%) [10]. However, in this study, the cCMV infection rate was higher in mothers with low IgG avidity relative to those showing IgG seroconversion. The UNHS referral population likely includes more patients with symptomatic cCMV infection. However, this is the first study to examine the incidence rate of maternal primary CMV infection in UNHS referral patients in Japan. The incidence rate of maternal primary CMV infection in the general population is reportedly 1–2% in Western Europe and the United States [1]. Indeed, we reported an incidence rate for primary CMV infection among all serologically screened pregnant women in Mie, Japan as 0.98% (95% CI: 0.85–1.13%) [10,25]. The current incidence rate of maternal primary CMV infection in UNHS referral patients (10.0%) was 10-fold higher than that in the general population. The number of patients with true congenital hearing loss among the UNHS referral patients is larger than that of the general population. As cCMV infection is a major contributor to congenital hearing loss, many mothers with maternal primary CMV infection (the most important factor in the occurrence of cCMV infection) may have been included in a population comprising UNHS referral patients.

Both maternal primary and non-primary infections (including past infections) can cause cCMV infection in infants. In countries with a low or intermediate CMV seroprevalence, the occurrence of cCMV infection after maternal primary and non-primary infections is reportedly approximately equal. For example, the maternal CMV seroprevalence as well as the seroprevalence for cases of cCMV infection occurring after maternal primary and non-primary infection (including past infection) is 60%, 52%, and 48%, in France, respectively; whereas in Finland, these were 72%, 47%, and 53%, respectively [1,26,27]. There are currently insufficient data on cCMV infection following maternal non-primary infection in Mie, Japan. However, because of the similar rate of CMV seroprevalence in Mie, Japan (66%), a similar ratio of cCMV infection following maternal primary and non-primary infections may exist [8]. Herein, all four UNHS referred neonates with cCMV infection whose mothers underwent CMV antibody screening were positive for maternal primary CMV infection, with no cCMV diagnosis following maternal non-primary infection (including past infection). The reason for this is unclear since the occurrence of congenital hearing loss in patients with cCMV infection is considered the same in maternal primary and non-primary infections [1]. Congenital hearing loss in patients with cCMV infection after non-primary maternal CMV infection may be difficult to detect in UNHS. Further studies are warranted to evaluate this clinically important possibility.

Herein, we performed a prospective enrollment study of neonatal patients who had referral results in UNHS. Therefore, since there was no enrollment at the time of

undergoing UNHS, the exact number and clinical characteristics of neonates who underwent UNHS could not be reported. However, in another study of pregnant women (cytomegalovirus antibodies), along with those included in this study, the number of pregnant women enrolled during the same study period was 44628. Therefore, we assume that approximately the same number of neonates received UNHS. Assuming that approximately 4–5 of 1000 neonates failed at the UNHS [4], about twice as many neonates as the 98 patients in this study would have been considered to have had referral results. The reason for this small number of patients with referral results would be that, UNHS was not performed when mothers did not wish to receive the test, because some patients were sent to the otorhinolaryngologists even after obtaining UNHS referral results without enrollment in this study and their urine samples were not collected. Moreover, in the assessment of the incidence rate of cCMV infection in UNHS referral patients, selection bias to an extent might be present in this study population. We performed neonatal CMV DNA tests from urine samples in all UNHS referral patients; however, we were not able to obtain all of the corresponding CMV antibody screening results from the mothers. Thus, although we identified the incidence rate of cCMV infection among all UNHS referral patients, we showed the incidence rate of maternal primary CMV infection in a limited number of patients (60 of 98 mothers). This could also contribute to a selection bias in this study.

5. Conclusions

To the best of our knowledge, this is the first study to assess the incidence rate of maternal primary CMV infection using maternal CMV antibody screening in patients with referral results in UNHS. We assessed the incidence rate of cCMV infection in UNHS referral patients at 5.1% and a 10-fold higher risk of the incidence rate of maternal primary CMV infection in the referral patient group (10.0%) as compared with the general population (0.98%).

Abbreviations

AABR, automated auditory brain-stem response; cCMV, congenital cytomegalovirus; CI, confidence interval; CMV, cytomegalovirus; Ig, immunoglobulin; SNHL, sensorineural hearing loss; UNHS, universal newborn hearing screening.

Author Contributions

AK, KTor, and TI were involved in writing the paper. MH-A was involved in data collection. MI performed CMV DNA tests. TM performed CMV IgG avidity tests. SS performed viral isolation tests. Close examination of congenital hearing loss in infants were performed by MKit, KTak, SU, and SM at the otorhinolaryngology departments. EK, MKih, and FM assisted in operating the maternal CMV antibody screening program. All authors read and approved the final manuscript.

Ethics Approval and Consent to Participate

This prospective cohort study was conducted in accordance with the Declaration of Helsinki. We obtained ethical approval (No. 2610) from the Clinical Research Ethics Review Committee of the Mie University Hospital and obtained informed consent from all participants' mothers.

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Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/j.ceog4912259.

References

[1] Leruez-Ville M, Foulon I, Pass R, Ville Y. Cytomegalovirus infection during pregnancy: state of the science. American Journal of Obstetrics and Gynecology. 2020; 223: 330–349.

[2] Kaneko M, Sameshima H, Ikeda T, Ikenoue T, Minematsu T. Intrapartum fetal heart rate monitoring in cases of cytomegalovirus infection. American Journal of Obstetrics and Gynecology. 2004; 191: 1257–1262.

[3] Kanji A, Khoza-Shangase K, Moroe N. Newborn hearing screening protocols and their outcomes: a systematic review. International Journal of Pediatric Otorhinolaryngology. 2018; 115: 104–109.

[4] Sato T, Nakazawa M, Takahashi S, Mizuno T, Ishikawa K, Yamada T. Outcomes of regional-based newborn hearing screening for 35,461 newborns for 5 years in Akita, Japan. International Journal of Pediatric Otorhinolaryngology. 2020; 131: 109870.
[5] Masuda S, Usui S. Comparison of the prevalence and features of inner ear malformations in congenital unilateral and bilateral hearing loss. International Journal of Pediatric Otorhinolaryngology. 2019; 125: 92–97.
[6] Lombardi G, Garofoli F, Stronati M. Congenital cytomegalovirus infection: treatment, sequelae and follow-up. The Journal of Maternal-Fetal & Neonatal Medicine. 2010; 23: 45–48.
[7] Minami SB, Yamanobe Y, Nakano A, Sakamoto H, Masuda S, Takiguchi T, *et al*. A high risk of missing congenital cytomegalovirus-associated hearing loss through newborn hearing screening in Japan. Journal of Clinical Medicine. 2021;10: 5056.
[8] Kaneko M, Sameshima H, Minematsu T, Kusumoto K, Yamauchi A, Ikenoue T. Maternal IgG avidity, IgM and ultrasound abnormalities: combined method to detect congenital cytomegalovirus infection with sequelae. Journal of Perinatology. 2013; 33: 831–835.
[9] Toriyabe K, Morikawa F, Minematsu T, Ikejiri M, Suga S, Ikeda T. Anti-cytomegalovirus immunoglobulin M titer for congenital infection in first-trimester pregnancy with primary infection: a multicenter prospective cohort study. Journal of Perinatology. 2017; 37: 1272–1277.
[10] Shimada K, Toriyabe K, Kitamura A, Morikawa F, Minematsu T, Ikejiri M, *et al*. Primary cytomegalovirus infection during pregnancy and congenital infection: a population-based, mother–child, prospective cohort study. Journal of Perinatology. 2021; 41: 2474–2481.
[11] Tanimura K, Tairaku S, Morioka I, Ozaki K, Nagamata S, Morizane M, *et al*. Universal Screening with Use of Immunoglobulin G Avidity for Congenital Cytomegalovirus Infection. Clinical Infectious Diseases. 2017; 65: 1652–1658.
[12] Shimada K, Toriyabe K, Kitamura A, Morikawa F, Ikejiri M, Minematsu T, *et al*. Characteristics and serology of pregnant women with cytomegalovirus immunoglobulin G seroconversion during pregnancy in Japan. Taiwanese Journal of Obstetrics and Gynecology. 2021; 60: 621–627.
[13] Ebina Y, Minematsu T, Sonoyama A, Morioka I, Inoue N, Tairaku S, *et al*. The IgG avidity value for the prediction of congenital cytomegalovirus infection in a prospective cohort study. Journal of Perinatal Medicine. 2014; 42: 755–759.
[14] Melamed R, Shemer-Avni Y, Shany E, Kurtzman L, Gorali R, Landau D. Targeted and universal screen in term and preterm infants for congenital CMV infection. Infectious Diseases. 2020; 52: 730–735.
[15] Fowler KB, McCollister FP, Sabo DL, Shoup AG, Owen KE, Woodruff JL, *et al*. A Targeted Approach for Congenital Cytomegalovirus Screening within Newborn Hearing Screening. Pediatrics. 2017; 139: e20162128.
[16] Stehel EK, Shoup AG, Owen KE, Jackson GL, Sendelbach DM, Boney LF, *et al*. Newborn Hearing Screening and Detection of Congenital Cytomegalovirus Infection. Pediatrics. 2008; 121: 970–975.
[17] Beswick R, David M, Higashi H, Thomas D, Nourse C, Koh G, *et al*. Integration of congenital cytomegalovirus screening within a newborn hearing screening programme. Journal of Paediatrics and Child Health. 2019; 55: 1381–1388.
[18] Vancor E, Shapiro ED, Loyal J. Results of a Targeted Screening Program for Congenital Cytomegalovirus Infection in Infants who Fail Newborn Hearing Screening. Journal of the Pediatric Infectious Diseases Society. 2019; 8: 55–59.
[19] Ari-Even Roth D, Lubin D, Kuint J, Teperberg-Oikawa M, Mendelson E, Strauss T, *et al*. Contribution of targeted saliva screening for congenital CMV-related hearing loss in newborns who fail hearing screening. Archives of Disease in Childhood - Fetal and Neonatal Edition. 2017; 102: F519–F524.
[20] Korver AMH, de Vries JJC, Konings S, de Jong JW, Dekker FW, Vossen ACTM, *et al*. DECIBEL study: Congenital cytomegalovirus infection in young children with permanent bilateral hearing impairment in the Netherlands. Journal of Clinical Virology. 2009; 46: S27–S31.
[21] Goderis J, De Leenheer E, Smets K, Van Hoecke H, Keymeulen A, Dhooge I. Hearing Loss and Congenital CMV Infection: a Systematic Review. Pediatrics. 2014; 134: 972–982.
[22] Avettand-Fenoël V, Marlin S, Vauloup-Fellous C, Loundon N, François M, Couloigner V, *et al*. Congenital Cytomegalovirus is the second most Frequent Cause of Bilateral Hearing Loss in Young French Children. The Journal of Pediatrics. 2013; 162: 593–599.
[23] Barbi M, Binda S, Caroppo S, Ambrosetti U, Corbetta C, Sergi P. A wider role for congenital cytomegalovirus infection in sensorineural hearing loss. The Pediatric Infectious Disease Journal. 2003; 22: 39–42.
[24] Nance WE, Lim BG, Dodson KM. Importance of congenital cytomegalovirus infections as a cause for pre-lingual hearing loss. Journal of Clinical Virology. 2006; 35: 221–225.
[25] Hyde TB, Schmid DS, Cannon MJ. Cytomegalovirus seroconversion rates and risk factors: implications for congenital CMV. Reviews in Medical Virology. 2010; 20: 311–326.
[26] Leruez-Ville M, Magny J, Couderc S, Pichon C, Parodi M, Bussières L, *et al*. Risk Factors for Congenital Cytomegalovirus Infection Following Primary and Nonprimary Maternal Infection: a prospective neonatal screening study using polymerase chain reaction in saliva. Clinical Infectious Diseases. 2017; 65: 398–404.
[27] Puhakka L, Lappalainen M, Lönnqvist T, Niemensivu R, Lindahl P, Nieminen T, *et al*. The Burden of Congenital Cytomegalovirus Infection: a Prospective Cohort Study of 20 000 Infants in Finland. Journal of the Pediatric Infectious Diseases Society. 2019; 8: 205–212.