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Incidence and Prognosis of Congenital Cytomegalovirus Infection, a Retrospective Single-Center Experience, Tehran, Iran

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Abstract

Background: Congenital cytomegalovirus (cCMV) is the most common intrauterine viral infection, affecting up to 2.5% of live births worldwide; it is also the most common non-hereditary cause of sensorineural hearing loss (SNHL) in infants.
Objectives: This study aimed to evaluate the frequency of cCMV and the incidence of sensorineural hearing loss at a large referral hospital in Tehran.
Methods: In our cross-sectional study, all infants born between March 2019 and April 2020 (one year) at Mahdiyeh Obstetrics and Gynecology Hospital were enrolled in the present study, and their urine samples were collected for CMV PCR in the first 2 days of life. PCR test results divided these infants into two groups, with and without congenital cytomegalovirus infection. For both groups, the otoacoustic emission screening test (OAE) was performed at birth and one month of age; the auditory brain response test (ABR) was then performed for infants with hearing impairment.
Results: Urine samples of 859 were collected for cytomegalovirus PCR testing; 70.3% of specimens were from male infants. Neonatal urine samples were tested for the presence of cytomegalovirus by PCR; 847 of the specimens (98.6%) were negative, and 12 (1.4%) were positive for cytomegalovirus, CI: (95%). The prevalence of congenital cytomegalovirus infection was 1.18% in girls and 1.49% in boys, revealing no significant difference between the two groups. All infants with congenital cytomegalovirus infection were full-term, between 38 and 42 weeks of gestational age. The first OAE test was impaired in 4 cases (33%) with congenital cytomegalovirus infection.
Conclusions: In our study, congenital CMV infection prevalence was 1.4%. We recommend hearing screening tests (OAE and AABR) be performed for all neonates. If impaired, the infant should also be evaluated for cCMV infection in addition to auditory follow-up. It is recommended that this study be continued in a multicenter manner with a larger number of samples and a longer period to fully evaluate the prevalence of complications in cCMV.

Keywords: Congenital Cytomegalovirus Infection, Newborn Screening, Sensorineural Hearing Loss

1. Background

Congenital cytomegalovirus (cCMV) infection is the most common of the intrauterine infections grouped as TORCH (toxoplasmosis, rubella, cytomegalovirus, herpes simplex, and other organisms including syphilis, parvovirus, and varicella zoster) syndrome. In a prospective 22-year study by Foulon et al., the prevalence of this congenital infection is reported from 0.5 to 2.5% (1). The prevalence of this infection in Iran has been reported from 0.3 to 4.9%, according to different studies (2-5). Congenital CMV infection is the most common cause of acquired sensorineural hearing loss (SNHL) in infants. Although congenital cytomegalovirus infections are asymptomatic in 85 - 90% of cases, 0.5 to 20% of these asymptomatic patients develop sensorineural hearing loss at birth or a few years later. (6) According to a prospective multicenter registry recorded by Foulon et al. (1) in 2007 - 2018, the rate of hearing loss in symptomatic patients was 63%, while it was 8% in the asymptomatic children with cCMV at initial testing. In their study, a

child is classified as symptomatic when $\geq$ 1 significant abnormality is found on physical examination, central nervous system imaging, hearing tests, fundoscopy, and blood tests. Patients with a late diagnosis of cCMV due to delayed-onset hearing loss are considered asymptomatic. An important outcome of cCMV infection was the delayed onset of hearing impairment in 10.6 % of symptomatic patients and 7.8 % of asymptomatic children with cCMV. Another important finding of the study was that more than 29% of patients with symptomatic infection were using some kind of hearing aid; this figure was 1.6% in asymptomatic children (7). Hearing screening at birth and during childhood greatly helps to identify patients with sensorineural hearing loss due to congenital CMV infection. Nevertheless, this screening is not mandatory in many countries, including Iran.

2. Objectives

The aim of this study was to evaluate the frequency of congenital cytomegalovirus infection and the incidence of sensorineural hearing loss in infected neonates born between March 2019 and April 2020 at Mahdiyeh Obstetrics and Gynecology Hospital (a large referral hospital with about 1000 live births per year) in Tehran.

3. Methods

All infants born between March 2019 and April 2020 at Mahdiyeh Obstetrics and Gynecology Hospital were eligible for enrollment in the study. Urine samples were collected during the first day of birth with a urine bag, and specimens were kept in 2-milliliter cryo tubes at 4°C and then stored at - 80°C until the time of real-time polymerase chain reaction (RT-PCR). Viral DNA extraction was prepared by FAVORGEN (Cat. No. FATGK001, 50). Amplification was employed using the following primers: Reverse ACGACCCGTGGTCATCTTTA and forward GCGGTGGTTGCCCAACAGGA. PCR from the UL55 fragment region was done on a final volume of 20 μl, containing 10μL of cyber green master mix, 0.5 μL of each primer (10 picomol), and 5 μL of DNase and RNase free water and 4 μL of viral DNA sample. It was performed in a thermocycler (Qiagen/Corbett Rotor-Gene 6000) under the following conditions: Hold on 95°C for 3 minutes, 40 cycles by 95°C for 15 seconds, and 60°C for 40 seconds and melt temperature was 60°C up to 95°C (7). UL55 is related to glycoprotein B gene and is one of the most sensitive primers that could be used, with detection limits of 8 viral particles per ml (VP/m) (8). Urine specimens were mixed in groups of 10 samples (10°C from each specimen). In case of a positive result in each group, extraction was performed for each sample alone. This method could detect a single positive CMV sample (9, 10).

Based on PCR test results, these infants were divided into infected and non-infected groups. For both groups, the OAE (otoacoustic emission screening) hearing test was performed at birth, and for infants with impaired hearing tests, second OAE hearing tests were performed again at 1 month, and ABR at 1 year of age.

Infants with congenital cytomegalovirus infection were examined by a neonatologist and referred for ophthalmological consultation. Brain ultrasound and initial tests, including CBC, BUN, Cr, and liver function tests, were performed on all affected neonates. ABR follow-up tests on 10 infants with a positive CMV PCR from the urine sample were performed successfully at one year old.

Qualitative variables were reported as numbers and percentages. For quantitative variables assuming a normal distribution, mean and standard deviation, and for quantitative variables with an abnormal distribution, mean, minimum, and maximum were reported. The Wilson Score Interval formula was used to calculate the 95% confidence interval; all methods were carried out in accordance with relevant guidelines and regulations.

4. Results

Of the 1,200 infants born at Mahdiye Hospital during this period, approximately 1,000 infants were eligible for the study. However, due to the difficulty of collecting urine samples from infants, only 904 neonates were included. Among the infants included in the study, 859 infants had acceptable urine samples; 255 (29.7%) were girls, and 604 (70.3%) were boys. The mean birth weight of neonates was 3194 ± 548 grams, and the gestational age of 72.63% of neonates was 38 to 42 weeks, 23.98% was 35 to 37 weeks, and 3.49% was less than 34 weeks.

Out of 859 samples, 847 specimens (98.6%) tested negative, and 12 (1.4%) were positive for CMV PCR (95% CI: 0.2% - 3%). The mean age of the neonates at the time of collecting the urine sample was 1 day (6 hours-3 days). The initial OAE test was abnormal in 33% (n = 4) of neonates with congenital CMV infection and 58.6% (n = 497) without congenital CMV infection. In the follow-up of patients with an abnormal primary OAE test, in 99.2% (n = 402) of cases, the result of the second OAE test was normal. Only three infants in the second OAE test had hearing loss, none of whom had a congenital CMV infection. The ABR test of these three infants at one year of age was normal (Table 1).

In this study, patients with congenital CMV infection showed no specific clinical symptoms. Four patients

Table 1. Clinical and Demographic Data of Infants with Congenital Cytomegalovirus Infection and Disease [a]

	CMV Positive (n = 12, 1.40%)	CMV Negative (n = 847, 98.6%)	Total (N = 859)
Sex			
Male	9 (1.49)	595 (98.51)	604 (100)
Female	3 (1.18)	252 (98.82)	255 (100)
Mean gestational age (w)	38.4 ± 0.5	37.9 ± 1.95	37.9 ± 1.93
Mean birth weight (gr)	3131.6 ± 437.6	3195.6 ± 549.8	3194.7 ± 548.2
Result of the first OAE test			
Normal	8 (2.23)	350 (97.77)	358 (41.68)
Abnormal	4 (0.8)	497 (99.20)	501 (58.32)
Result of 2nd OAE test			
Normal	10 (2.49) [b]	392 (97.23)	402 (99.25)
Abnormal	0	3 (100)	3 (0.74)
Result of ABR			
Normal	10 (100) [b]	3 (100)	3 (100)
Abnormal	0	0	0

[a] Values are presented as No. (%) or mean ± SD.
[b] Two CMV-positive patients did not come for follow-up hearing tests.

had laboratory disturbances in the form of increased AST, and one patient had a transient increase in ALT. In the follow-up 6 months later, the serum levels of transaminases were normalized in all 4 patients. None of the infants with congenital cytomegalovirus infection had abnormalities on the ophthalmological examination and brain ultrasound.

According to the congenital CMV infection protocol, infants without clinical symptoms do not need to be treated with ganciclovir; therefore, none of the CMV-positive infants received ganciclovir (11).

5. Discussion

The reported prevalence of congenital CMV varies from 0.2% - 2% (average 0.65%). There is a higher overall rate in regions with higher maternal seroprevalence. (12) In developing countries, the prevalence of congenital CMV infection varies between countries and may be as high as 6 - 14% (13, 14). Karimian et al., from Isfahan, reported a positivity rate of congenital CMV at 0.5% on testing neonatal urine samples in 2016 (5). Fahimzad et al. estimated the CMV prevalence based on testing neonatal saliva samples as 0.3% (4). In our study, the prevalence of congenital CMV was 1.4%. The difference in the statistics obtained in these three studies could be due to the area of residence of the participants in the study or the method of virus detection.

The gestational age of neonates in our study was between 38 - 42 weeks old, and preterm infants were not included in our study. In the study done by Lorenzoni et al. in 2013, the prevalence of congenital CMV infection in preterm neonates was 3.03% (15).

In our study, 70.3% of specimens were from males and 29.7% from females. The reason was difficulty in collecting urine with bags in girls. Accordingly, 70% of congenital CMV cases were male. No significant relationship was found between gender and the prevalence of congenital CMV infection in our patients.

Approximately 90% of neonates with congenital CMV infection are asymptomatic, and 10% have clinical manifestations, including hepatosplenomegaly, jaundice, microcephaly, hearing loss, and intrauterine growth retardation (16). In our study, out of 859 neonates, 12 had a congenital CMV infection. All of these infants underwent a complete clinical examination in infancy and a complete ophthalmologic examination by an ophthalmologist. No clinical signs of CMV infection were observed in any of the cases. Brain ultrasounds performed to assess brain damage were normal in all infants with cCMV in our study. Also, paraclinical tests were performed for anemia, hyperbilirubinemia, and increased transaminases. Three of the infants had transient transaminase increases.

Infants with congenital CMV infection were also examined by ABR; 10 infants passed the test successfully, and unfortunately, the test could not be successfully completed in two infants. Hearing loss due to congenital

CMV infection is often bilateral, progressive, and moderate to severe in severity; in cases asymptomatic at birth, hearing loss is often unilateral. In a study by Goderis et al., in 2016, it was stated that 4.5% of children with normal hearing on the initial evaluation had delayed hearing loss (17). Considering that the duration of our study was one year, it was not possible to follow up with children after one year of age. It is recommended that infants with congenital CMV infection be periodically evaluated for audiometry every 6 months until the age of 4 - 6 years (11).

We had major limitations in our study. Due to the coincidence of our study with the start of the COVID-19 pandemic, it was extremely difficult to recruit and follow up with patients. Patients did not go to the hospital due to the fear of contracting COVID-19. Therefore, the follow-up of the patients was difficult.

5.1. Conclusions

We recommend a hearing screening test (OAE or ABR) be performed for all neonates, and if impaired, in addition to auditory follow-up, the infant should also be evaluated for cCMV infection. According to the neonatology's references, the patient needs antiviral treatment if the infant has a symptomatic cCMV infection, particularly in patients with CNS or hepatic involvement.

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Footnotes

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