Original Article

Prevalence of Congenital *Cytomegalovirus* Infection among Hospitalized Neonates in Tehran, Iran

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Abstract

Background: *Cytomegalovirus* (CMV) can vertically transmit from infected mothers to fetuses and causes congenital infection in newborns. Unfortunately, there have been limited data available on the prevalence of congenital CMV (cCMV) infection among Iranian neonates at higher risk of infection.
Objectives: The current study aimed to assess the prevalence of cCMV infection among hospitalized neonates in Tehran, Iran, and investigate the diagnostic values of CMV polymerase chain reaction (PCR) on Guthrie cards in comparison to those reported for urine specimens.
Methods: This cross-sectional study was carried out on the hospitalized neonates with 3 weeks of age. The urine specimens and Guthrie cards were taken from each eligible newborn. Total nucleic acid was extracted from the samples and tested by PCR for the presence of CMV deoxyribonucleic acid. The cCMV infection was confirmed in the newborns, and the infected neonates underwent further evaluation.
Results: Out of 224 newborns, CMV infection was identified in 11 neonates (4.9%). There were no factors in association with cCMV infection. The sensitivity and specificity of dried blood spot (DBS) samples for the identification of newborns with cCMV infection were 90% and 99%, respectively.
Conclusion: A significant number of hospitalized neonates in the present study were infected with cCMV infection. The results of the current study revealed that Guthrie cards had adequate sensitivity for the identification of CMV infection in the hospitalized newborns. Since symptomatic newborns with cCMV infection have a higher chance for the development of early- or late-onset sequelae of infection, it is recommended to diagnose and treat this group of newborns.

Keywords: Congenital CMV infection, Newborns, Prevalence

1. Background

Cytomegalovirus (CMV), a betaherpesvirus, can vertically transmit from infected mothers to fetuses during pregnancy and causes congenital CMV (cCMV) infection in newborns with serious complications depending on the characteristics of populations, including maternal immunity (primary or non-primary) and/or racial factors (1). In total, the global incidence of cCMV infection among newborns is reported within the range of 0.2-2.2%, (2). Neonates with cCMV infection are mostly asymptomatic at birth, thereby remaining undiagnosed. Additionally, the clinical manifestations of newborns with symptomatic cCMV infection are not sufficiently specific to prompt the pediatrician to order a CMV test before 21 days of birth (3). Seizures, petechiae, and microcephaly are three common symptoms associated with cCMV infection at birth. However, both symptomatic and asymptomatic neonates can develop the late sequelae of cCMV infection, more frequently in the symptomatic cases (4).

In developed countries with moderate maternal seroprevalence (40-70%), psychomotor impairment and sensorineural hearing loss (SNHL) are reported as two common sequelae following cCMV infection (5). It is estimated that about 40% of all SNHL cases (in children up to 5 years of age) are attributed to CMV, and more than half of all cases may fail to be identified during neonatal hearing screening test due to the late onset of this complication (6). For many years, the diagnosis of cCMV infection has been performed using direct isolation of the virus from saliva and urine specimens with serological methods, such as the detection of immunoglobulin G (IgG)/immunoglobulin M antibodies and/or antigen pp65 in perilymphatic fluid and blood. However, the culture method is relatively difficult and expensive and takes about 2 or 3 days to get the results, and serological tests have less sensitivity in comparison to gold standard assays. In the last decades, these traditional cumbersome detection assays have been replaced with highly sensitive and specific assays, such as polymerase chain reaction (PCR) (7).

Based on the evidence, it was demonstrated that the early detection of cCMV infection, if associated with useful interventions in newborns, not only could prevent unnecessary diagnostic testing in the future

but also may help to improve the language outcomes in children with early- or late-onset SNHL ([8]). Nevertheless, the definitive detection of cCMV infection is only possible by testing urine, saliva, and/or blood specimens within 21 days of neonates' life ([9]). More precisely, multiple studies have suggested using dried blood spot (DBS) samples for cCMV screening in light of reliable sensitivity and specificity. According to the published data, manual-based extraction methods (e.g., heat shock assay or phenol-chloroform extraction method) with nested PCR gel-based assays have the highest sensitivity for the detection of CMV deoxyribonucleic acid (DNA) in DBS samples ([10]).

At present, screening for cCMV infection in newborns is only performed in one country (i.e., USA), and in other countries CMV testing is limited to those who have suspicious symptoms or signs referable to cCMV infection. In a previous study, it has been shown that the frequency of cCMV infection among asymptomatic neonates born in the six cities of Tehran, Iran, is about 0.34% in total ([11]). However, there have been limited data available on the prevalence of cCMV infection among Iranian neonates.

2. Objectives

The present study aimed to assess the prevalence of cCMV infection among the hospitalized neonates with less than 3 weeks of age in Tehran and evaluate the diagnostic values of CMV PCR of DBS samples in comparison to those reported for the standard urine specimens of newborns.

3. Methods

3.1. Population Study
This cross-sectional study was carried out on the hospitalized newborns referred to university-affiliated hospitals in the western regions of Tehran within April 1st to September 2017. The pediatrics units of these public hospitals serve as the main referral centers for newborns' diseases in Tehran province and admit patients from all socioeconomic statuses. Before sampling, all the neonates' parents were approached about the participation of their neonates in the study. The newborns with > 21 days of age and/or with no DBS cards were excluded from the present study.

The main symptoms and characteristics of the neonates were documented according to the hospital records. The demographic data and related maternal factors of the newborns were also documented using a questionnaire. Informed consent was obtained from the neonates' parents for participation in the study. The present study was approved by the Ethics Committee of Iran University of Medical Sciences, Tehran, Iran.

3.2. Sample Collection
The urine specimen was taken from each neonate using sterile bags, decanted into the collection tubes, and transported to the laboratory at the Virology Department of Tehran University of Medical Sciences, Tehran, Iran. Newborns' Guthrie cards (Whatman 903) were retrieved after the completion of metabolic screening tests in the central reference laboratory. Briefly, two of five circles from each Guthrie card were cut out by scissors and stored into a sealed plastic bag containing desiccants and then transported to the research laboratory at the Institute of Immunology and Infectious Diseases (in Hazrate-Rasool University Hospital, Tehran, Iran) within 24 h and stored at 4°C until further processing ([9]).

3.3. Laboratory Tests
Total DNA was extracted from urine samples using a high pure PCR template purification kit (Roche Diagnostics, GmbH. Germany) according to the manufacture's instruction and subsequently evaluated for the presence of *CMV UL83* gene with a CE-IVD quantification PCR kit (R-GENE, Biomerieux, France), as described by the manufacturer. The detection limit of this kit was 2.6 $\log_{10}$ copies/ml, and all the experiments were conducted with the PCR platform of ABI 7500 (Applied Biosciences).

Genomic DNA was extracted from the DBS samples in triplicate using a highly sensitive thermal shock assay according to the modified protocol ([12]). In brief, a punch of each DBS sample (with a diameter of 3 mm) was soaked in 30 µl of Minimum Essential Medium (MEM, Sigma Aldrich, USA), incubated overnight (4°C), and then heated in a thermocycler with a temperature of 55°C for 60 min, 100°C for 7 min, and 0°C for 2 min (Eppendorf, Germany). Finally, after a centrifugation step (3320×g for 15 min), the supernatant (20 µl) was frozen at −80°C overnight and tested for the presence of CMV DNA with an in-house nested PCR assay as previously described (11).

3.4. Clinical Evaluation of Neonates
All the parents of newborns with CMV positive PCR tests were informed of the screening results of their neonates and approached for cardiac examination and echocardiography (in case of indication). The infected neonates were included in a 2-year follow-up study for other evaluations, such as central nervous system examination; computerized tomography scan, and audiometric and ophthalmic examinations, by relevant specialists. The CMV treatment was initiated based on the severity of the symptoms and physician's decision.

3.5. Quality Control
For the prevention of cross-contamination between patients' specimens, the scissors were cleaned by 0.1 M of hydrochloric acid before making cuts, and a blank Whatman card was punched before

and after taking each patient's sample ([13]). The CMV positive and negative control samples in different dilutions were prepared from the blood samples of three infected and two healthy newborns (using the similar procedure of the study samples), respectively, and used as standard controls in each run of PCR. The DBS samples with positive results for CMV DNA were tested again with reverse transcription PCR for the quantification of CMV load in each sample.

3.6. Statistical Analysis

Univariate analyses were performed by Fisher's exact test and Chi-squared test to compare the values of risk factors between the two groups of CMV infected and non-infected newborns. Statistical analyses were carried out using MedCalc Statistical Software (version 15.8; MedCalc Software bvba, Ostend, Belgium; https://www.medcalc.org; 2015). A p-value of less than 0.05 was considered statistically significant ([14]).

4. Results

In this study, 238 hospitalized newborns with 21 days of age were recruited from the university medical centers of Tehran with the exclusion of 14 cases due to unwillingness to continue the study and/or absence of Guthrie cards. Out of 224 neonates, 11 cases were positive for CMV DNA, yielding an infection rate of 4.9% (95% CI: 2.4-8.6) in total. There were no risk factors statistically associated with cCMV infection in newborns. Table 1 tabulates the main characteristics of the neonates and their mothers in association with cCMV infection.

The clinical reasons mainly leading to neonates' hospitalization were intrauterine growth restriction, jaundice, and low birth weight. There were no differences in the prevalence of clinical symptoms in newborns with and without cCMV infection. Table 2 shows clinical symptoms and signs observed in

Table 1. Analysis of demographic and medical characteristics of newborns with congenital *Cytomegalovirus* infection

Characteristics	CMV-Positive n (%)	CMV-Negative n (%)	P-value
Gender			
Male	7 (6.0)	115 (94.0)	0.5
Female	4 (4.0)	98 (96.0)	
Gestational age			
≤37 weeks	4 (5.9)	67 (94.1)	0.7
>37 weeks	7 (4.7)	146 (95.3)	
Type of delivery			
Vaginal	8 (6.6)	120 (93.4)	0.2
Cesarean	3 (3.2)	93 (96.8)	
Maternal age			
≤27 years	6 (4.5)	132 (95.5)	0.6
>27 years	5 (6.1)	81 (93.9)	
Birthweight (g) (mean±SD)	2500.0±250	2750.0±500	0.1
Body length (cm) (mean±SD)	46.5±0.5	47.0±1.5	0.2
Head circumference (cm) (mean±SD)	33.5±0.5	34±1.0	0.1

CMV: *Cytomegalovirus*; SD: Standard deviation

Table 2. Comparison of main clinical symptoms between neonates with and without congenital *Cytomegalovirus* infection

Variable	Congenital *Cytomegalovirus* infection		P-value
	Positive n (%)	Negative n (%)	
Intrauterine growth restriction			
Yes	6 (4.4)	135 (95.6)	0.5
No	5 (6.4)	78 (93.6)	
Jaundice			
Yes	8 (6.6)	121 (93.4)	0.2
No	3 (3.2)	92 (96.8)	
Low birthweight			
Yes	5 (4.3)	114 (95.7)	0.5
No	6 (6.0)	99 (94.0)	
Petechiae			
Yes	4 (8.5)	47 (91.5)	0.2
No	7 (4.2)	166 (95.8)	
Seizures			
Yes	1 (4.5)	22 (95.5)	0.8
No	10 (5.2)	191 (94.8)	
Hearing screening results			
Abnormal	3 (13.0)	23 (87.0)	0.07
Normal	8 (4.1)	193 (95.9)	

Table 3. Comparison of dried blood spot to urine samples in screening for congenital *Cytomegalovirus* infection

Urine	Dried blood spot		Total
	Positive	**Negative**	
Positive	10	1	11
Negative	2	211	213
Sensitivity (95% CI)	90.9% (58.7-99.7%)		
Specificity (95% CI)	99.0% (96.6-99.8%)		
Positive predictive value (95% CI)	83.3% (51.5-97.9%)		
Negative predictive value (95% CI)	99.5% (97.4-99.9%)		

neonates with and without cCMV infection.

The median viral load in the DBS samples of the infected newborns was 4.5 $\log_{10}$ copies/ml (range: 2.70-6.25 $\log_{10}$ copies/ml). In comparison to the results of urine PCR, the sensitivity and specificity of DBS were 90.9% (95% CI: 58.7-99.7) and 99.0% (95% CI: 96.6-99.8), respectively (Table 3).

5. Discussion

The CMV infection is the most common viral congenital infection around the world [15]. The prevalence of cCMV infection among hospitalized newborns in Tehran, the capital of Iran, during 6 months of the study period was 4.9%. To the best of our knowledge, the current study has provided the documented data for the first time in Iran on the rate of cCMV infection among neonates admitted to hospitals at birth by testing newborns' DBS cards and urine specimens. The overall prevalence rates of cCMV infection among asymptomatic and symptomatic neonates in Iran were reported within the range of 0.3-58% in total [16-18]. In addition, similar findings were reported by a systematic review in which cCMV infection among the newborns of populations with high maternal CMV seroprevalence was reported to be about 6.1% [19]. The findings of the current study are in line with the aforementioned data from Tehran and other developing countries.

Based on previous studies, most of the Iranian pregnant women (within the range of 85-95%) have a positive titer for anti-CMV IgG in their serum due to CMV infection before pregnancy [20, 21]. This finding means that over 98% of newborns with cCMV infection in Iran are asymptomatic at birth out of which 10% will develop the late-onset sequelae of infection in the form of SNHL and psychomotor delay [22]. Although multiple studies indicated that the rate of cCMV infection with severe symptoms at birth is lower in populations with high maternal seroprevalence, the results of the current study revealed that 27.2% of CMV-infected cases failed in the primary hearing screening test [23].

Several studies have demonstrated that a DBS sample is not associated with adequate sensitivity for using as the sample choice in CMV detection assays [24, 25]. On other hand, some studies have reported the beneficial aspects of using DBS samples in the diagnosis of infected neonates who are more prone to develop the late-onset sequelae of infection [26, 27]. Based on the results of the present study, the sensitivity and specificity of the DBS samples for the identification of newborns with cCMV infection were 90.9% and 99%, respectively. Although in this study the most sensitive assays (i.e., heat shock protocol and nested-PCR assay) were used which were noted in the literature for the diagnosis of CMV DNA, other studies have also reported the high sensitivity and specificity of DBS samples for the detection of CMV DNA in neonates at birth [28].

The median viral load in the DBS samples of Iranian infected neonates was 4.5 $\log_{10}$ copies/ml, similar to the median DBS viral load reported for symptomatic French newborns (4.26 $\log_{10}$ copies/ml) using an identical PCR diagnostic assay [29]. Finally, there were some limitations in the present study which should be considered in interpreting the obtained findings. Firstly, the prevalence of cCMV infection was assessed in the newborns referred to the hospitals of Tehran. In addition, the sample size was relatively small. Therefore, the results of the current study may not be generalizable to the whole population of neonates in Iran.

6. Conclusion

In the present study, the incidence of cCMV infections among the hospitalized newborns in Tehran was calculated as 4.9% in total. More importantly, the sensitivity and specificity of the DBS samples in comparison to the standard urine specimens for the detection of cCMV infection were 90.9% and 99.0%, respectively, which was relatively high. Since newborns with cCMV infections have a chance for developing the early- or late-onset sequelae of infection, it is recommended to diagnose and treat infected neonates and propose precautionary measures to pregnant women as the best approaches for controlling the disease. Finally, it is suggested to carry out further studies with larger sample sizes and focus on neonates with specific symptoms in different parts of Iran.

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Footnotes

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