

Received: 2016.03.18
Accepted: 2016.06.30
Published: 2017.03.11

Detection of Cytomegalovirus (CMV) Infection in Wheezing Infants by Urine DNA and Serum IgG Testing

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Data Interpretation D
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Source of support: Departmental sources

Background: The aim of this study was to investigate the involvement of CMV infection in wheezing infants and the association between CMV-DNA and immunoglobulins (Igs).




Material/Methods: A total of 243 wheezing infants and 3,000 parturients were enrolled in this study. The infants were randomly grouped to receive blood HCMV-DNA tests (n=46) or urine HCMV-DNA tests (n=197). Furthermore, all participants had serum CMV-specific IgM and IgG testing. Afterwards, 10 HCMV-IgG positive infants were randomly selected for simultaneous blood and urine HCMV-DNA tests, and 25 HCMV-IgG positive puerperants were randomly selected for urine HCMV-DNA tests.

Results: The detection rate of urine HCMV-DNA was significantly higher than that of blood HCMV-DNA (67.5% vs. 13.0%, $p < 0.001$). Fifteen (6.2%) and 190 (80.0%) infants showed positive CMV-specific IgM and IgG results ($p < 0.001$), respectively. Among the 10 HCMV-IgG positive infants tested further, only two infants had positive HCMV-DNA blood tests, while all of the 10 infants had positive HCMV-DNA urine tests. However, HCMV-DNA was not detected in the urine of the 25 randomly selected parturients positive for HCMV-IgG.

Conclusions: CMV infection may be one of the causes of wheezing in infants; CMV infection can be detected by urine-HCMV-DNA and serum HCMV-IgG testing. Infants were more susceptible to CMV infection than parturients.

MeSH Keywords: **Immunoglobulin G • Infant • Sequence Analysis, DNA**

Full-text PDF: <http://www.medscimonit.com/abstract/index/idArt/898589>

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Background

Cytomegalovirus (CMV) infection, a human infectious disease caused by the human CMV (HCMV) [1], is a serious threat to human health [2,3]. CMV infection is widely prevalent in China, and most often occurs in infancy [4–6]. Immediate early antigen (IEA) is generated in CMV replication in the early stage (1–3 hours), whereas early antigen (EA) is generated after 3 hours, and late antigen (LA), such as structural protein pp65, is generated at 6–24 hours. CMV resides long-term or life-long once it has invading the human body [7,8]. CMV is asymptomatic in most immunocompetent individuals, but immunosuppressed individuals, including fetuses and young infants, may have obvious symptoms for CMV infection [9]. Many organs including lung, retina, liver, and gastrointestinal tract are damaged by CMV [10,11]. However, the target organ of CMV is related to the age of patient. For example, damage to the central nervous system and various congenital malformations are only found in a fetus that has a congenital intrauterine CMV infection. Infant hepatitis and pneumonia are common in CMV infection infants, while older children are asymptomatic [12].

Infants with wheezing disease can have similar symptoms but different etiology. Bronchial asthma is the most common wheezing disease in children, and the incidence rate of asthma has been increasing over the past decades. Bronchiolitis is the most common wheezing disease for infants and babies. In bronchiolitis, the infiltrated inflammatory cells caused by respiratory syncytial virus (RSV) are mainly neutrophils and lymphocytes [13], rather than eosinophils as is found in asthma. In previous reports, wheezing in infants was found to be predominantly caused by adenovirus, rhinovirus, and respiratory syncytial virus [14].

There are several studies that have reported the association of CMV infection and wheezing in infants. In Denmark, CMV infection was detected in 26.2% of patients with respiratory tract infections using an indirect immunofluorescence test in the culture of nasopharyngeal aspirates, throat swabs, or urine; which demonstrated the close relationship between CMV infection and respiratory diseases [15]. Morisawa et al. also reported primary CMV infection as an important cause of wheezing attacks in infants in Japan [16] and found that 18% of wheezing infants had CMV-specific IgM and IgG positive tests from nasopharyngeal aspirates cultures. However, the frequency of CMV-associated wheezing in infants in China has not been reported. Hence, we enrolled 243 wheezing infants in this study to investigate the involvement of CMV infection in wheezing infants and test for CMV-specific IgM and IgG, as well as CMV-DNA in blood and urine. Furthermore, we investigated the association between CMV-IgG and CMV-DNA in wheezing infants and selected parturients. These study results could provide an important resource to aid in the clinical diagnosis of CMV infection in infants with wheezing.

Material and Methods

Samples collection

This study was approved by the Ethical Committee of Wuxi No. 8 People's Hospital, and informed consents were obtained from the parents of the wheezing infants, and from the parturients included in the studied. A total of 243 wheezing infants (aged 6 months to 1.5 years old) and 3,000 parturients who were hospitalized in our hospital from September 2011 to March 2012 were recruited into this study. The infants were randomly enrolled to receive HCMV-DNA testing (Figure 1A). Among these infants, 46 had DNA blood tests, and 197 had DNA urine tests. Furthermore, blood samples were collected from all participants for serum HCMV-IgM and HCMV-IgG assays (Figure 1B, 1C). Ten HCMV-IgG positive infants were randomly selected for both blood and urine HCMV-DNA tests (Figure 1B), and 25 HCMV-IgG positive parturients were randomly selected for urine HCMV-DNA tests (Figure 1C).

Detection of HCMV-DNA by quantitative real-time PCR

Mononuclear cells were isolated from the blood samples using Tficoll separation solution (Shanghai Hua Jing Biotech Company). The urine samples were centrifuged for 10 minutes at 1,500 rpm and then the sediment was collected [17]. The DNA from mononuclear cells and from the sediment of urine samples were extracted using a DNA extraction kit according to the manufacturer's instructions. Quantitative real-time reverse transcription-PCR (qRT-PCR) was performed to determine CMV using TaqMan probe and Roche Light Cycler fluorescence PCR detector. The results were considered CMV positive in samples with a CT value ≤ 37.0 .

Detection of serum HCMV-IgM and HCMV-IgG

The blood samples were centrifuged at 1000 g for 10 minutes to separate serum. Then, the serum samples were used to detect the HCMV-IgM and HCMV-IgG levels by colloidal gold labeled immuno-chromatographic test kit (DaAn Gene Co., Ltd., SUN Yat-Sen University) according to the manufacturer's guidelines.

Statistical analysis

Statistical analysis of data was performed using SPSS 19.0 software (Statistical Package for the Social Sciences). Comparison of Ig positive results of the wheezing infants was conducted using McNemar test. Chi-square test was used to compare the percentage values between two groups; $p < 0.05$ was considered statistically significant.

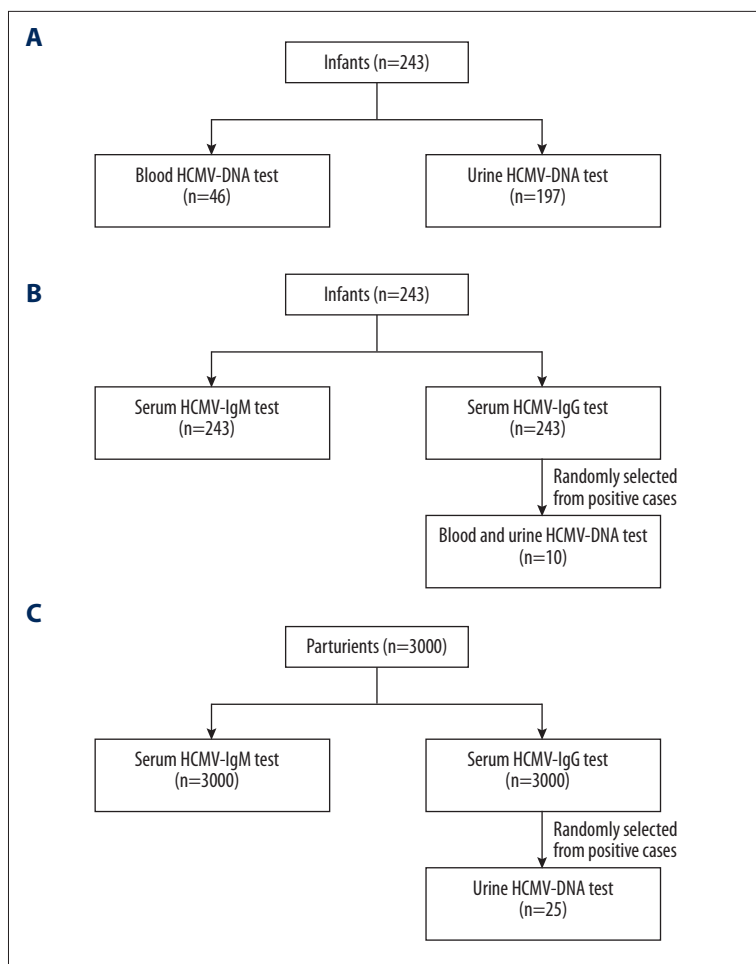


Figure 1. Study flow. (A) The enrolled 243 infants were randomly grouped for blood (n=46) and urine (n=197) HCMV-DNA tests. (B) All enrolled 243 infants received serum HCMV-IgM and HCMV-IgG tests. Afterwards, 10 HCMV-IgG positive infants were randomly selected for blood and urine HCMV-DNA tests. (C) A total of 3,000 parturients were tested for serum HCMV-IgM and HCMV-IgG. Afterwards, 25 HCMV-IgG positive parturients were randomly selected for urine HCMV-DNA test.

Results

Association between HCMV-IgG and urine-HCMV-DNA in wheezing infants

As shown in Table 1, 13.0% (6/46) of blood samples were HCMV-DNA positive. However, urine samples showed a higher positive rate of 67.5% (133/197) ($p < 0.001$). Combined, the ratio of CMV infection in the wheezing infants was 57.2% (139/243).

Serum HCMV-specific IgM and IgG levels in the wheezing infants were determined by colloidal gold labeled immuno-chromatographic test kit. The results showed that 6.2% (15/243) and 80.0% (190/243) of samples were positive for HCMV IgM and IgG antibodies, respectively (Table 2). The result of McNemar tests showed that the HCMV IgG positive rate was higher than the IgM positive rate in wheezing infants, and wheezing infants with IgM positive were not simultaneously IgG positive ($p < 0.001$).

To determine the correlation between HCMV-IgG and urine-HCMV-DNA, 10 wheezing infants who were positive for HCMV-IgG

Table 1. Wheezing infants showed a higher rate of urine CMV-DNA positive.

	Blood CMV-DNA testing (n=46)	Urine CMV-DNA testing (n=197)
Positive number, n	6	133
Negative number, n	40	64
Detection rate, %	13.0%	67.5%

were randomly selected to detect HCMV-DNA in both blood and urine. The results showed that among the 10 IgG-positive samples, only 20% (2/10) of the blood samples positively detected the DNA of CMV. However, CMV DNA was detected in urine for all the IgG positive samples (Table 3).

Table 2. Inconformity results of HCMV-specific IgM and IgG tests were obtained in wheezing infants.

	HCMV-specific IgM (n=243)	HCMV-specific IgG (n=243)
Positive number, n	15	190
Negative number, n	228	53
Detection rate, %	6.2%	80.0%

HCMV IgG positive rate was higher than IgM positive rate in wheezing infants. Wheezing infants IgM positive were not simultaneously IgG positive.

Table 3. Urine CMV-DNA was detected in wheezing infants with positive HCMV-IgG.

	Blood CMV-DNA testing (n=10)	Urine CMV-DNA testing (n=10)
Positive number, n	2	10
Negative number, n	8	0
Detection rate, %	20%	100%

Difference results between parturients and wheezing infants

A total of 3,000 blood samples from parturients were collected to detect HCMV-IgM and HCMV-IgG levels. As shown in Table 4, 80% (2,400/3,000) of samples were positive for HCMV-IgG antibody. However, none of the samples were positive for HCMV-IgM antibody. Then 25 HCMV-IgG positive parturients were randomly selected for detection of urine-HCMV-DNA to determine the correlation of serum HCMV-IgG with urine HCMV-DNA in parturients. The results showed that HCMV DNA could not be detected in the urine samples of the 25 HCMV-IgG-positive parturients.

Discussion

CMV is detected in saliva, semen, urine, emulsion and cervical secretions of infected patients and asymptomatic patients with latent infections [18]. Most CMV infected patients had latent infections, and congenital CMV infection could lead to neurological disability in children [19,20]. Thus, it is particularly important for early diagnosis as well as monitoring and treatment of CMV infection. In this study, we included 243 wheezing

Table 4. Puerperants showed a higher rate of urine CMV-DNA positive.

	Blood CMV-DNA testing (n=3000)	Urine CMV-DNA testing (n=3000)
Positive number, n	2400	0
Negative number, n	600	3000
Detection rate, %	80%	0%

infants and found that the positive rate of serum HCMV-IgM was significantly lower than that of serum HCMV-IgG. Then, 10 samples which were positive for HCMV-IgG were randomly selected for the analysis of HCMV-DNA in blood and urine to determine the correlation between HCMV-IgG and HCMV-DNA. The results indicated that there was a correlation between HCMV-IgG and urine-HCMV-DNA of wheezing infants. Furthermore, HCMV might be a cause of wheezing for infants with a detection rate of 80% by serum HCMV-IgG testing and 67.5% by urine-HCMV-DNA testing. However, HCMV-DNA could not be detected in the urine of the 25 HCMV-IgG-positive parturients. The difference in results between parturients and wheezing infants suggested that infants were more susceptible to CMV than parturients. Because kidney is a major target organ for CMV, once an infection occurs, a permanent latent infection will formed and exist for life. Therefore, urine-positive HCMV-DNA is not used as the measure for active CMV infection. Positive tests for HCMV-DNA in serum and plasma were considered as sign of active CMV infection as reported in Chinese Journal of Pediatrics in 2012 [21].

The immunological basis of asthma is an immune reaction mediated by Th2 cells; the airway chronic allergic inflammation mainly involves mast cells and eosinophils [22,23]. Th2 cells are also the predominant immunological cells for viral infection of infant wheezing, such as CMV infection. With RSV infection, the level of IL-10 secreted by dendritic cells declines, which leads to a Th2 cell dominant immune response. With influenza virus (IFZ) infection, dendritic cells secrete sufficient IL-10 to inhibit the Th2 response, and then the Th1 immune response is dominant [24]. IL-12 secreted by dendritic cells can promote a Th1 response, but inhibit Th2 response. In addition, clinical studies have shown that the level of secreted IL-12 is inversely proportional to the degree of breathing [25,26] Immune response caused by viral infection is influenced by a variety of factors. Whether HCMV can inhibit or promote Th2 response similar to RSV infection remains unknown, and should be verified in future experiments.

Conclusions

Urine-HCMV-DNA test results were associated with serum HCMV-IgG test results in wheezing infants but not parturients. Infection with HCMV might contribute to wheezing etiology in infants. The difference in the results for parturients compared

to infants illustrates that infants but not parturients might be susceptible to HCMV infection.

Conflicts of interest

None.

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