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Original Article

Identifying key clinical markers for congenital cytomegalovirus infections: a PCR-confirmed case-control study

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Abstract

Background Congenital cytomegalovirus (CCMV) infection is the leading cause of congenital infections worldwide. Clinical manifestations of CCMV are highly variable and make the clinical diagnosis difficult, especially in settings where advanced diagnostic tools are not available.

Objective To identify a cluster of clinical manifestations indicative of CCMV and analyze for correlations with PCR-confirmed diagnoses.

Methods This case-control study was conducted at a tertiary care hospital in Malang, East Java, and included 40 neonates clinically suspected to have CCMV. PCR specimens from urine or saliva were collected and analyzed to evaluate clinical manifestations of suspected CCMV. Demographic and clinical data were organized and analyzed using SPSS.

Results Of neonates with suspected CCMV, 32.5% (n=13) had PCR-confirmed CCMV. The median age for PCR testing post-suspected CCMV was 8.50 (range 3.75 to 24.25) days. Significant correlations emerged between PCR-confirmed CCMV and symptoms such as microcephaly, jaundice, purpura, thrombocytopenia, acute liver injury, hepatomegaly, feeding difficulties, and anemia. However, seizures, low birth weight, ventriculomegaly, and intrauterine growth restriction did not show significant associations, indicating their limited utility as solitary markers for CCMV. The clinical symptoms associated with CCMV were confirmed by PCR, emphasizing the significance of certain sign and symptom clusters, such as microcephaly with thrombocytopenia (OR 41.60; P<0.001), or the traditional triad of jaundice, purpura, and hepatosplenomegaly/acute liver injury (OR 41.60; P<0.001).

Conclusion Several clinical manifestations are significantly associated with PCR-confirmed CCMV infection, underscoring the diagnostic value of specific symptom combinations in identifying CCMV infection. These combinations are microcephaly and thrombocytopenia and the classic triad of jaundice, purpura, and hepatosplenomegaly/acute liver injury. **[Paediatr Indones. 2025;65:XXX; DOI: https://doi.org/10.14238/pi65.3.2025.XXX]**.

Keywords: *congenital cytomegalovirus infection; neonates; PCR; clinical manifestations*

Congenital cytomegalovirus (CCMV) is a viral infection transmitted from mother to fetus during pregnancy by transplacental crossing.[1] Cytomegalovirus (CMV) is a DNA virus belonging to the herpesvirus family, which includes herpes simplex virus, varicella-zoster virus, and Epstein-Barr virus.[2,3] Unlike other viral infections, CMV can spread across the placental barrier, leading to fetal infection. The CCMV infection is the leading cause of congenital infections worldwide, accounting for approximately 0.2-2.2% of all live births in developed countries and up to 6.0% in developing countries.[4,5]

The CCMV infection can present with a wide range of clinical manifestations, from asymptomatic disease to severe disease. Notably, approximately 10% of CCMV cases are symptomatic. Such individuals are at high risk for long-term neurodevelopmental disability, which is found in 36-90% of survivors with symptomatic CCMV.[6] However, early diagnosis of CCMV in children is often difficult due to the

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nonspecific nature of the multiple signs and symptoms. Clinical manifestations of CCMV are highly variable, including sensorineural hearing loss, microcephaly, developmental delay, as well as hepatic, pulmonary, and hematologic abnormalities.[1,7] This wide range of symptoms makes the clinical CCMV diagnosis difficult, especially in settings where advanced diagnostic tools are not available. Therefore, a well-characterized set of signs and symptoms can provide a basis for the development of sensitive and specific screening protocols for CCMV, increasing the probability of correct diagnosis in different clinical settings and ultimately improving the morbidity of the disease.[8]

The diagnosis of CCMV is usually confirmed by the detection of the virus or its components in urine, saliva, or blood specimens of newborns in the first three weeks of life, suggesting congenital rather than postnatal infection. Serological tests to detect CMV-specific antibodies (IgM and IgG) are of limited use in neonatal diagnosis due to transplacental transfer of maternal antibodies, which may confound the results. However, serology can help assess maternal CMV status during pregnancy.[9] On the other hand, PCR is considered the gold standard for the diagnosis of CCMV due to its high sensitivity and specificity. However, the limited availability of PCR testing in some settings, especially in resource-poor settings or smaller healthcare facilities, may hinder the timely diagnosis and treatment of CCMV.[1] This limitation highlights the importance of defining a set of clinical manifestations to assist clinicians in the symptom recognition of CCMV before proceeding to definitive testing for CCMV.[10]

The lack of a universally accepted screening strategy for CCMV in neonates represents a critical gap in current clinical practice. Although targeted screening of newborns with specific signs and symptoms occurs in some regions, there is no consensus on which signs and symptoms should trigger the examination.[11] Having a standard criteria of statistically established symptoms can inform policy decisions about universal and targeted screening approaches, which can lead to more uniform practices in health systems. Accordingly, we aimed to outline a cluster of signs and symptoms correlated with CCMV, as confirmed by PCR test results.

Methods

This case-control, preliminary study was performed in neonates at Saiful Anwar General Hospital (SAGH), Malang from February to July 2023. Inclusion criteria for the case group were neonates aged ≤25 days who were treated or followed up in the Perinatology Unit or Pediatric Outpatient Clinic at SAGH and had signs and symptoms of suspected CCMV infection such as thrombocytopenia, jaundice, ventriculomegaly, microcephaly, and/or IUGR. Control group subjects were infants with signs and symptoms of CMV, but with negative PCR tests. Infants diagnosed with CMV infection older than 25 days of age and lacking confirmation from PCR testing for CMV were excluded. Informed consent and ethical clearance were exempted due to the use of medical records as the source of data.

The eligible subjects were chosen by purposive sampling by extracting the CMV PCR record from the Central Laboratory of Clinical Pathology (SAGH, Malang) testing registry. CMV PCR specimens were either urine or saliva, tested by qualitative PCR, and then expressed as either "detected" or "not detected" per laboratory protocol. The demographic baseline characteristics of subjects and the clinical signs and symptoms that led to CMV PCR testing were recorded.

The study employed a comprehensive data analysis approach to evaluate the clinical manifestations of suspected CCMV to its PCR-confirmed diagnostic outcomes. Demographic and clinical data were systematically organized using Microsoft Office Excel spreadsheets. Subsequently, statistical analysis was performed using *Statistical Package for the Social Sciences (SPSS), IBM 25.0,* where Chi-square tests were applied to assess categorical variables.[12] The distribution of clinical manifestations between subjects was further mapped to qualitatively ascertain patterns indicative of CCMV. The level of significance was set at P value < 0.05 and odds ratios (ORs) were also recorded with their respective 95% confidence intervals (CI) to provide an understanding of the associations observed.

Results

Forty neonatal subjects with clinically suspected CCMV were included (**Table** 1). PCR-confirmed CCMV was detected in 13 (32.5%) subjects. The median age at diagnosis, i.e., the age at which PCR testing was done, was 8.50 [interquartile range (IQR) 3.75 to 24.25)] days, indicating a moderate spread in the ages at diagnosis within the cohort. The distribution of sex and gestational age at birth was relatively balanced within the cohort.

The CCMV clinical manifestations between groups are summarized in **Table 2**. PCR-confirmed CCMV and the following clinical parameters (by themselves) were significantly associated: microcephaly, jaundice, purpura, thrombocytopenia, acute liver injury, hepatomegaly, feeding problems, and anemia ($P < 0.05$ for all). These signs had higher probability of occurring in the CCMV-positive group. For example, microcephaly and jaundice were more common in PCR-positive subjects, with ORs of 12.80 and 11.67, respectively. In contrast, conditions such as ventriculomegaly and intrauterine growth restriction (IUGR) were not significantly different between the groups, suggesting that not all medical conditions traditionally associated with CCMV were likely to result in a positive diagnosis when PCR confirmation is taken into account. These findings emphasize the complex nature of CCMV clinical manifestations. **Figure 1** depicts the comparison of clinical manifestations between PCR-confirmed CMV cases and controls.

Microcephaly was significantly higher in the CCMV-positive group (OR 12.80; 95%CI 2.48 to 65.98; P=0.001). Jaundice, purpura, acute liver injury, and hepatomegaly were also significantly more common in infected neonates. As it is impractical to denote a single sign or symptom to establish a particular diagnosis, the need for a composite set of clinical manifestations is imperative.

There were no significant associations between CCMV and seizure occurrence, low birth weight (LBW), ventriculomegaly, or intrauterine growth retardation (IUGR), which suggested that while these conditions may co-occur with CCMV, they were not definitive markers for the infection in isolation.

A systematic approach, considering a constellation of clinical findings, increases the diagnostic accuracy and is crucial for clinicians to identify those infants who should undergo PCR testing for CCMV. The particular combinations of symptoms observed in our study suggests that these sets of symptoms, when present together, offers a stronger predictive value for CCMV infection than any single symptom alone.

To gain insight about the pattern of various clinical manifestations in cases of CCMV, a heat map was constructed to visualize the signs and symptoms distribution between cohorts (**Figure 2**). The heat map was plotted with individual cases on the vertical axis and clinical features on the horizontal axis, with green shading indicating the presence of a specific clinical feature in a particular case. Heat map analysis revealed specific patterns highlighting clusters of clinical manifestations associated with PCR-confirmed CCMV. These patterns form the basis for suggested combinations of clinical manifestations that may raise suspicion for congenital CMV infection (**Table 3**).

Table 3 outlines the proposed selection of clinical manifestation combinations that were highly indicative of CCMV, emphasizing their potential diagnostic value. A concurrent presentation of microcephaly and jaundice was found exclusively in CCMV-positive cases (7/13), with no occurrence in controls, demonstrating a OR of 5.5 (95%CI 2.67 to 11.34) indicating that this combination of symptoms may be pathognomonic for CCMV when detected in a clinical setting. The pairing of microcephaly with acute liver injury or hepatomegaly was more common in CCMV-positive cases (5/13) compared to controls (3/27) (OR 5.00; 95%CI 0.97 to 25.77). Most notably, the classic triad of jaundice, purpura, and

Table 1. Demographic and clinical characteristics of subjects with suspected CCMV

Characteristics	(N=40)
CMV confirmation by PCR, n (%)	
Detected	13 (32.5)
Not detected	27(67.5)
Median age at diagnosis (IQR), days	8.50 (3.75-24.25)
Gestational age criteria, n (%)	
Preterm	18 (45%)
Full term	22 (55%)
Sex, n (%)	
Male	22 (55%)
Female	18 (45%)

Table 2. Analysis of CCMV clinical manifestations between PCR-positive and PCR-negative (control) groups

Parameters	PCR-positive (n=13)	PCR-negative (n=27)	OR (95% CI)	P value
Seizures, n (%)				
Yes	4	11	0.65 (0.16 to 2.64)	0.542
No	9	16		
LBW/ VLBW, n (%)				
Yes	10	14	3.10 (0.69 to 13.80)	0.130
No	3	13		
Microcephaly, n (%)				
Yes	8	3	12.80 (2.48 to 65.98)	0.001**
No	5	24		
Jaundice, n (%)				
Yes	10	6	11.67 (2.41 to 56.49)	0.001**
No	3	21		
Purpura, n (%)				
Yes	9	3	18.00 (3.35 to 96.73)	< 0.001**
No	4	24		
Thrombocytopenia, n (%)				
Yes	12	4	69.00 (6.92 to 688.1)	< 0.001**
No	1	23		
Ventriculomegaly, n (%)				
Yes	2	1	4.73 (0.39 to 57.7)	0.189
No	11	26		
Acute liver injury, n (%)				
Yes	7	1	30.33 (3.12 to 295.2)	< 0.001**
No	6	26		
Hepatomegaly, n (%)				
Yes	11	1	143.0 (11.72 to 1745.3)	< 0.001**
No	2	26		
IUGR, n (%)				
Yes	2	1	4.73 (0.39 to 57.70)	0.189
No	11	26		
Feeding difficulty, n (%)				
Yes	11	3	44.00 (6.41 to 302.0)	< 0.001**
No	2	24		
Anemia, n (%)				
Yes	8	1	41.60 (4.22 to 410.2)	< 0.001**
No	5	26		

Statistical analysis with Chi-square test. P<0.05 is considered significant, *P<0.05, **P<0.01; LBW=low birthweight; VLBW=very low birthweight

hepatosplenomegaly or acute liver injury was strongly predictive of CCMV, with an occurrence of 8/13 in the PCR-positive group and 1/27 in the controls (OR 41.60; 95%CI 4.29 to 410.2). Microcephaly and thrombocytopenia are two other symptom combinations that have a significance predictive value for CCMV, with an occurrence in the PCR-positive group and in the controls (OR 41.60; CI 4.29 to 410.2). These two combinations of symptoms are the most common sign of congenital cytomegalovirus.

Discussion

Cytomegalovirus is the most common cause of congenital infections, impacting between 0.2% and 2.0% of newborns in North America and Europe. This rate escalates to as much as 6.1% in the developing countries.13 Cytomegalovirus is responsible for up to 25% of cases of non-hereditary sensorineural hearing loss in children. In cases of CCMV with no symptoms, the likelihood of having neurological problems was

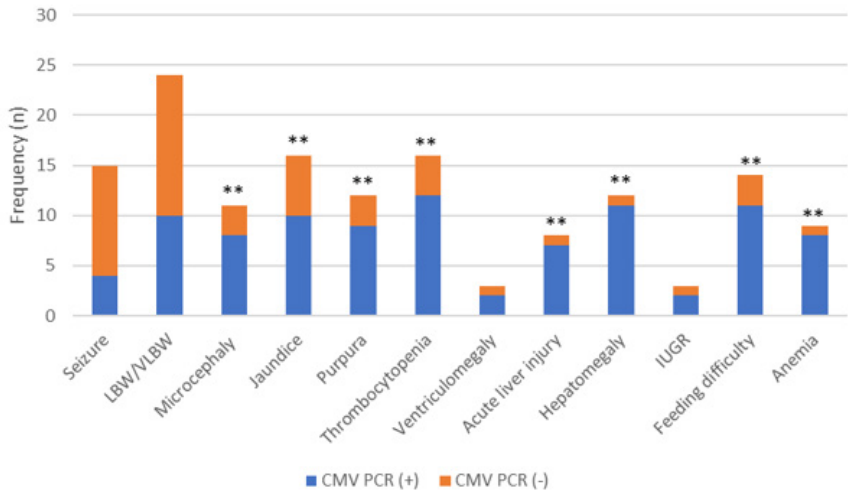

Figure 1. Comparison of clinical manifestations between PCR-confirmed CCMV cases and controls [statistical analysis with Chi-square test. P<0.05 is considered significant, *P<0.05, **P<0.01]

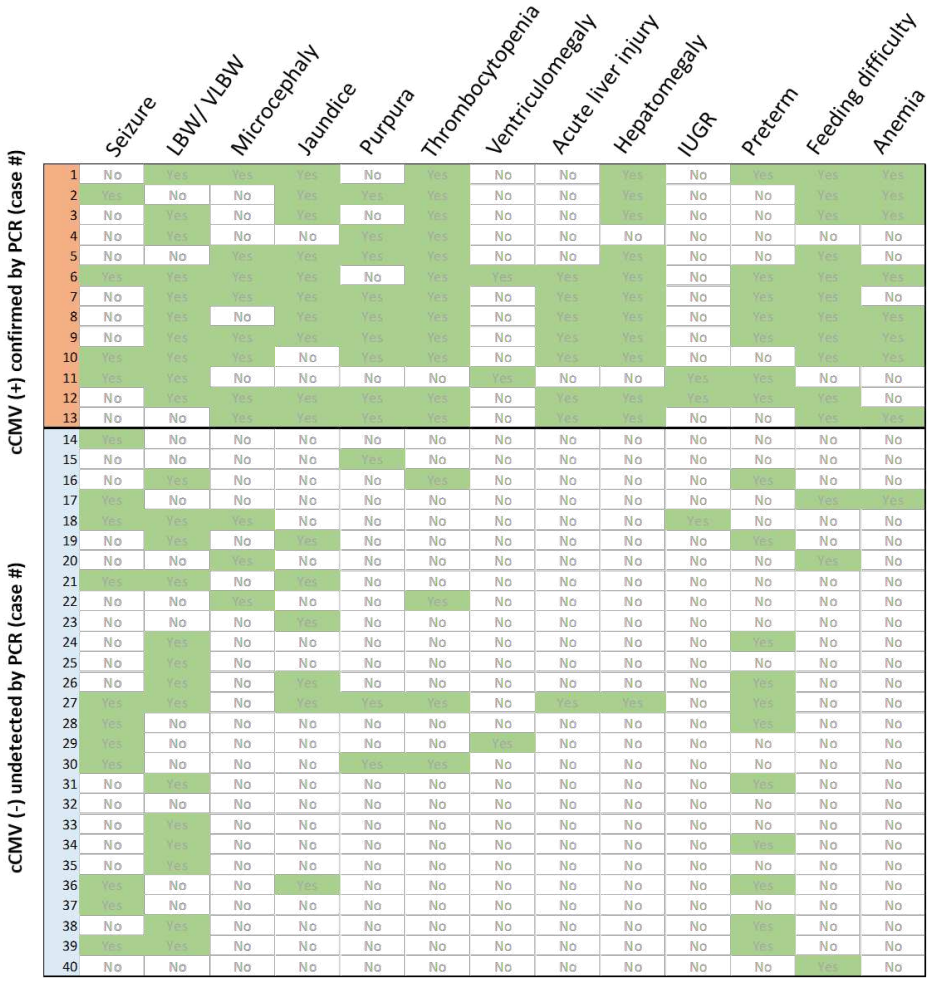

Figure 2. Heat map of the occurrence of clinical manifestations across between PCR-confirmed CMV and controls

Table 3. Analysis of proposed combinations of clinical manifestations in neonates with suspected CCMV

Parameters	PCR-positive (n=13)	PCR-negative (n=27)	OR (95% CI)	P value
Seizure & microcephaly, n (%)				
Yes	6	1	22.29 (2.29 to 216.9)	0.001**
No	7	26		
Microcephaly & jaundice, n (%)				
Yes	6	0	5.5 (2.67 to 11.34)***	< 0.001**
No	7	27		
Microcephaly & acute liver injury or hepatomegaly, n (%)	5	3	5.00 (0.97 to 25.77)	0.043*
Yes	8	24		
No				
Microcephaly & thrombocytopenia, n (%)	8	1	41.60 (4.29 to 410.2)	< 0.001**
Yes	5	26		
No				
Classic triad of jaundice, purpura, & hepatosplenomegaly or acute liver injury, n (%)	8	1	41.60 (4.29 to 410.2)	< 0.001**
Yes	5	26		
No				

Statistical analysis with Chi-square test. P<0.05 is considered significant, *P<0.05, **P<0.01

estimated to be between 5% and 15%, but this increased to 36 - 90% among those who survived symptomatic CCMV.6 Although symptomatic CCMV occurs in approximately 10% of all newborn cases,1 the challenge lies in its early detection for prompt diagnosis, which is crucial as timely intervention would significantly improve patients' outcomes.

The PCR is considered the gold standard for CCMV diagnosis, due to its high sensitivity and specificity; CMV commonly sheds in saliva or urine.14 However, the limited availability of PCR testing in some settings, especially in resource-poor settings or smaller healthcare facilities, may hinder the timely diagnosis and treatment of CCMV.

The optimal testing period for congenital or neonatal CMV infections is within 21 days. This is consistent with the American Academy of Pediatrics' recommendation for early screening of suspected cases to facilitate timely intervention. A positive CMV DNA PCR collected after 21 days of age may reflect postnatal acquisition of infection, leading to a completely different natural history and sequelae.15

According to our study, the frequency distribution of suspected congenital CMV by gender indicates that male patients are more likely than female patients (55% of males and 45% of females). Microcephaly had a significantly higher prevalence in the CCMV-positive group, a finding that echoed a previous report establishing microcephaly as a critical indicator of congenital CMV infection.16 Jaundice, purpura, and acute liver injury or hepatomegaly were also significantly more common in infected infants. These findings are congruent with the literature delineating hepatocellular dysfunction and vascular anomalies as complications of CCMV.17 As there is no single sign or symptom to establish a CCMV diagnosis, the need for a composite set of clinical manifestations is imperative.

Our findings underscore the complex clinical presentation of CCMV and the need for a multifaceted diagnostic approach. While some symptoms may be more strongly associated with CCMV, the absence of such should not preclude testing, especially in neonates with an equivocal clinical picture.9 Our study reinforces the complex nature of CCMV infection, demonstrating that while certain clinical manifestations are significantly associated with infection, it is impractical to establish a diagnosis based solely on one sign or symptom. This complexity is evidenced by the varying degrees of association between different clinical signs and PCR-confirmed CCMV. The use of clinical combinations to suggest CCMV should be considered in the context of the patient's overall clinical presentation and in conjunction with diagnostic testing such as PCR for

CMV. A previous study showed that jaundice (62%), petechiae (58%), and hepatosplenomegaly (50%) were the most frequently noted classical triad in symptomatic infants.18

The challenge remains in establishing clear guidelines for screening and diagnosis. Our study contributes to this effort by providing evidence for specific symptom combinations that are highly suggestive of CCMV. This can inform clinical decision-making, prompting consideration of CCMV in infants presenting with these symptom clusters, thereby facilitating timely and appropriate diagnostic testing.

There were some limitations of our study, including a relatively small sample size and its retrospective design, which might limit the generalizability of the findings. Additionally, the reliance on PCR testing alone may not capture all cases of CCMV, potentially leading to underdiagnosis. Since we focused on CCMV symptoms, asymptomatic CCMV infections would not have been represented. However, generating a symptomatology pattern of CCMV infection confirmed by its gold standard testing would render timely diagnosis and treatment, thus preventing the long-term consequences of CCMV infection.

In conclusion, our findings elucidated the clinical manifestations associated with PCR-confirmed CCMV infection, underscoring the diagnostic value of specific symptom combinations in detecting the disease, i.e., microcephaly and thrombocytopenia and the classic triad of jaundice, purpura, and hepatosplenomegaly or acute liver injury. Future research could benefit from incorporating larger prospective cohorts and utilizing a broader range of diagnostic tests to enhance the understanding of CCMV's clinical manifestations.

Conflict of interest

None declared.

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