Pediatr Gastroenterol Hepatol Nutr. 2024 Sep;27(5):298-312
https://doi.org/10.5223/pghn.2024.27.5.298
pISSN 2234-8646·eISSN 2234-8840

Pediatric Gastroenterology, Hepatology & Nutrition
pghn

Original Article

Efficacy and Safety of Valganciclovir in Congenital Cytomegalovirus Infection with Isolated Intrahepatic Cholestasis: A Randomized Controlled Trial

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Received: Jan 26, 2023
1st Revised: Sep 1, 2023
2nd Revised: Nov 3, 2023
Accepted: Jun 6, 2024
Published online: Sep 9, 2024

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ABSTRACT

Purpose: Cytomegalovirus (CMV) infection affects the hepatic, neurologic, hematopoietic, respiratory, gastrointestinal, and other organs, resulting in a high mortality rate and long-term sequelae. It may cause acute or chronic hepatitis, or even lead to hepatic cirrhosis. Valganciclovir (VGCV) is an effective, safe, and well-tolerated treatment for congenital CMV infection, without any serious adverse effects. This study was conducted to evaluate the clinical, biochemical, and virological profiles of infants with CMV with intrahepatic cholestasis and to determine the outcomes with or without treatment with VGCV.
Methods: Twenty infants aged <6 months diagnosed with congenital CMV infection with evidence of intrahepatic cholestasis were included in this study. Randomization was used to divide the study participants into 2 groups. The control group (n=10) was treated with only supportive management, and the intervention group (n=10) was treated with oral VGCV at 16 mg/kg/dose 12 hours a day for 6 weeks plus supportive treatments. Physical examinations and biochemical, serological, and virological tests were performed at the time of diagnosis and at the end of 6 weeks and 6 months.
Results: The control and intervention groups were compared in terms of clinical and laboratory parameters such as jaundice, dark urine, pale stool, hepatomegaly, total bilirubin, aminotransferases, gamma-glutamyl transferase, alkaline phosphatase, and CMV polymerase chain reaction load, which showed a significant reduction after treatment in the intervention group (p<0.05) with oral VGCV, with very few side effects, whereas the control group showed no significant changes.
Conclusion: Oral VGCV can be used to effectively treat CMV infection with intrahepatic cholestasis without notable side effects.

Keywords: Congenital cytomegalovirus infection; Valganciclovir; Urinary cytomegalovirus polymerase chain reaction

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Funding
Planning, Monitoring, and Research (PMR) Operational Plan, Directorate General of Health Services (DGHS).

Conflict of Interest
The authors have no financial conflicts of interest.

INTRODUCTION

Cytomegalovirus (CMV) is the largest member of the herpes virus family that can cause congenital infections, resulting in multi-organ disorders in infected children [1-4]. It is the most common human congenital infection, affecting 0.6–0.7% of live births in developed countries, and 1–5% of live births in resource-poor countries [5-7]. The virus can be transmitted to fetuses, newborns, and children because of overcrowding, inadequate sanitation, poor hygiene, and nonsterile delivery practices. Newborns can contract CMV via the transplacental pathway while in the womb, during birth, or from breast milk. Congenital CMV infection is more likely to occur in female infected before and during pregnancy [2,8,9]. Early gestational infections is typically linked to poor neurodevelopmental outcomes [10-12]. The risk of perinatal and postnatal infections is directly related to the maternal viral load. The highest amount of the virus is excreted through breast milk between 2 weeks and 2 months after birth, when the risk of infection ranges from 39% to 59% [13,14].

In healthy infants and children, 90% of illnesses are asymptomatic or self-limiting. In contrast, 10% of cases are symptomatic, especially in immunocompromised hosts and infected fetuses. The involvement of multiple organs in CMV infections, especially the eyes, brain, and liver, causes a high disease burden [15,16]. With chorioretinitis, it is a major cause of sensorineural hearing loss (SNHL), epilepsy, microcephaly, neurodevelopmental disorders, and developmental delays. The traditional triad of congenital CMV infections includes hepatosplenomegaly, petechiae, and jaundice as liver symptoms and signs [17-20]. Previous studies have shown that the involvement of CMV infection in hepatobiliary disease is very high (approximately 40%), with the virus replicating in both hepatocytes and cholangiocytes. CMV hepatitis is most common in infancy and is associated with cholestasis. CMV infection during infancy is important because it can result in cirrhosis, progressive liver failure, and death [1,21,22].

Most congenital CMV-infected children are born to CMV immunoglobulin G (IgG)-seropositive mothers; therefore, prenatal maternal screening is necessary, although the international consensus discourages it as it increases anxiety, causes additional stress, and leads to unnecessary termination of pregnancies [23-27]. Amniocentesis to perform a polymerase chain reaction (PCR) for CMV DNA (deoxyribonucleic acid) is the best available prenatal diagnostic tool for predicting fetal infection because the infected fetus urinates and the virus is present in the amniotic fluid. This method has high sensitivity and specificity when performed after 20–21 weeks of gestation and 8 weeks after estimated maternal seroconversion [28,29]. Lastly, regarding newborn screening, it is said that any newborn with signs and symptoms that indicate intrauterine CMV infection should be tested [30]. Since CMV DNA PCR exhibits high sensitivity in both saliva and urine samples, post-natal diagnosis of congenital CMV is preferably performed via real-time PCR with these samples. Serological testing for IgG and immunoglobulin M (IgM) antibodies can also be performed in this age group [31,32].

In a recent guideline from Queensland, Australia (2019) regarding infants with congenital CMV disease, treatment is recommended for symptomatic focal organ diseases such as severe hepatitis, including cholestasis, severe bone marrow suppression, or central nervous system (CNS) disease (microcephaly, radiological abnormalities on magnetic resonance imaging or cranial ultrasound, chorioretinitis, or sensory neural hearing loss) [33]. Another guideline from Parma, Italy also makes this recommendation [34]. In a recent study from the Pediatric

Neurology Department of Bangabandhu Sheikh Mujib Medical University (BSMMU), Fatema et al. [35] and Ruby et al. [5] from Bangladesh observed that in symptomatic congenital CMV infection in infants, valganciclovir (VGCV) was as efficient as GCV, with fewer side effects. Saganuma et al. [36] and Morioka et al. [37] from Japan and Yang et al. [2] from China also reported that VGCV was effective, safe, and well tolerated for congenital CMV infection, and prevented the progression of SNHL without serious adverse effects.

The treatment of CMV hepatitis in its early stages greatly improves cholestasis and serological indicators [38]. Another recent study conducted in Indonesia [4] reported the same result. In contrast, another study conducted in Korea reported that the prognosis of patients with CMV hepatitis was good without antiviral treatment [39]. The present study has described a series of patients with hepatic CMV infections. Very few data are available regarding the treatment and outcomes of CMV-induced intrahepatic cholestasis in Bangladesh. In light of this, we suggest that this study be conducted to determine the immunological markers, clinical and biochemical profiles, and prognosis of neonatal cholestatic CMV hepatitis with or without VGCV therapy. Current evidence indicates that early awareness and prompt management of this condition leads to better outcomes in countries with limited resources.

MATERIALS AND METHODS

Study design

A randomized controlled trial was conducted at the Department of Pediatric Gastroenterology, Hepatology & Nutrition at Bangladesh Shishu Hospital & Institute, Dhaka, Bangladesh, from January 2021 to December 2021.

Inclusion & exclusion criteria

After obtaining informed written consent from their parents, 20 infants aged <6 months with intrahepatic cholestasis due to congenital CMV infection were randomly selected from the study population. Patients with biliary atresia, choledochal cysts, abdominal and hepatic surgical causes, metabolic anomalies, other viral hepatitis, liver failure, hepatic encephalopathy, coagulopathy, sepsis, renal insufficiency, neurological abnormalities, and those older than 6 months were excluded from the study. Data were collected using a structured questionnaire containing all variables pertaining to interest (**Fig. 1**).

Ethical statement

Ethical approval was obtained from the Ethical Review Committee of the Bangladesh Shishu Hospital & Institute (No. Admin/574/DSH/2021). Written informed consent was obtained from the parents of each participant upon admission.

Operational definitions

Cholestasis was defined as an increase in direct bilirubin to >20% of total bilirubin and the development of tea-colored or high-colored urine and pale feces during the icteric stage of the disease. Congenital CMV infection is defined as an active infection that can be found within the first 3 weeks of life and is diagnosed by detecting CMV DNA in the urine, saliva, or blood, along with signs of CNS involvement or any other critical illness that poses a serious risk to life, such as encephalitis, pneumonitis, or hepatitis [5,17,18]. CMV-induced cholestasis was determined using serum CMV IgM and IgG antibody positivity and urine

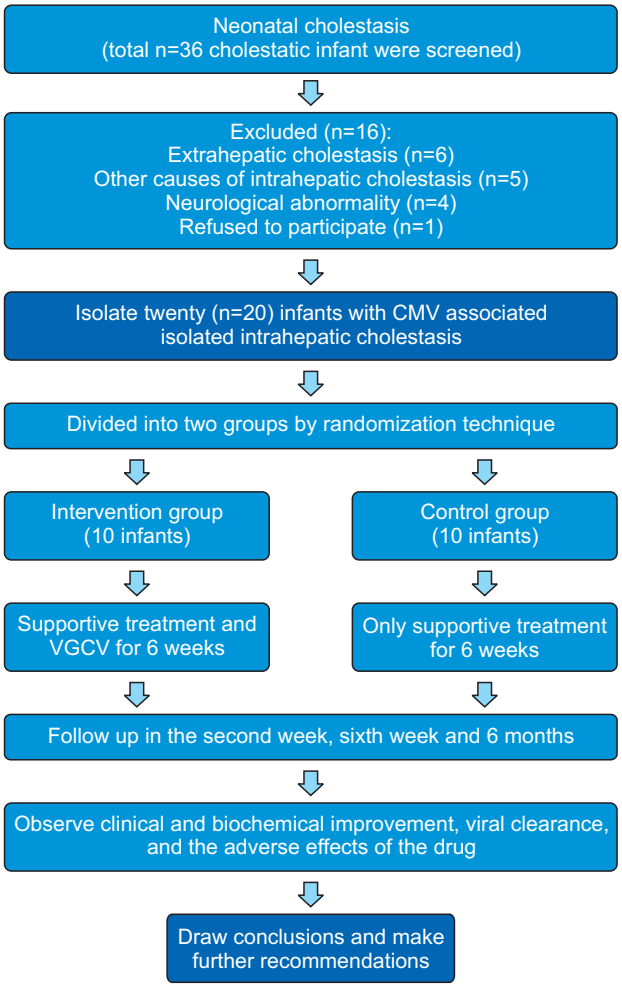

Fig. 1. Study flow chart.
CMV: cytomegalovirus, VGCV: valganciclovir.

CMV DNA quantitative PCR (>500 copies of the virus) using real-time technology with intrahepatic cholestasis. Improvement of infant was defined by clinical (reduction of jaundice, dark urine, pale stool, abdominal distension, rash, hepatosplenomegaly, ascites, and bleeding manifestation), biochemical (decrease of S. bilirubin total, direct, indirect, alanine transaminase [ALT], alkaline phosphatase [ALP], gamma-glutamyl transferase [GGT], and prothrombin time [PT]), and urinary CMV PCR (when PCR copy <2,000 [totally improved], decreased compared to the previous number [partially improved], apparently not decreased or increased [Static] and increased copy number than earlier [deteriorating]) with or without VGCV administration. The age at cholestasis onset was determined as the age at which the first icteric-stage symptoms appeared. The time between the patient's diagnosis of CMV cholestasis and the start of VGCV therapy was used to characterize the duration of CMV cholestasis. The age at VGCV therapy initiation was determined as the age at which VGCV therapy was initiated. Anthropometric measurements performed prior to the administration of VGCV, which is the best technique for monitoring patients with cholestasis and categorized according to the World Health Organization's 2006 criteria, were used to determine nutritional status. Malnutrition was defined as a weight for length z score <–3. Undernutrition was defined as a z score –2 to –3 and good health was defined as a z score >–2.

https://doi.org/10.5223/pghn.2024.27.5.298

Considering the first day of the previous menstrual cycle, pregnancy was considered preterm if the gestational age was less than 37 weeks.

Study procedure

A detailed medical history, including presenting complaints, birth history, antenatal history, and medical history, was recorded, followed by a general and systemic examination. Written informed consent was obtained from guardians or caregivers. Randomization was performed using the lottery method. Only the supportive treatment group was designated as a control, and the vanganciclovir with supportive treatment group was designated as the intervention group. The terms "control" and "intervention" were written on paper strips of the same size, shape, and color. The paper strips were then folded and mixed in a container. A blinded selection was performed using the required number of slips for the desired sample size. All enrolled infants underwent baseline liver function tests (LFTs) and CMV status (antibody with number of DNA copies). Following drug treatment, clinical (jaundice, dark urine, pale stool, and liver size) and LFT (fractionated bilirubin, ALT, ALP, and GGT) tests were performed again at 6 weeks and 6 months and compared with the initial assessment. Viral clearance and adverse effects of the drugs were also recorded.

Disease specific diagnosis

Liver biopsy is an invasive procedure; therefore, perplexities arise when obtaining family consent. Due to this, biopsy was only performed in suspected cases of biliary atresia. Therefore, excluding extrahepatic cholestasis, other causes of intrahepatic cholestasis, such as endocrine, metabolic, Alagille, and progressive familial intrahepatic cholestasis were excluded using hormonal assays and genetic testing. CMV IgM and IgG antibodies were obtained from the Armed Forces Institute of Pathology, Dhaka Cantonment, Dhaka, Bangladesh. Urinary CMV DNA PCR quantification was performed by the Advanced Genomic Institute & Laboratory Medicine (Agile), New Delhi, India, through Genetic Solutions, Dhaka, Bangladesh.

Treatment protocol

All 20 subjects were administered MCT-enriched coconut oil with breast milk, phenobarbital, ursodeoxycholic acid, cholesteramine, fat-soluble vitamins (vitamins A, D, E, and K), water-soluble vitamins, calcium, zinc, and folic acid once we made our final diagnosis. The intervention group (n=10) was orally administered VGCV at a dose of 16 mg/kg 12 hourly, for up to 6 weeks. VGCV (Valgan) tablets were collected from Cipla House, Peninsula Business Park, Ganpatrao Kadam Marg, Lower Parel, Mumbai, through Genetic Solution, Dhaka, Bangladesh, which was approved by the Food and Drug Administration, India.

Follow-up

A minimum of 6 months of follow-up was provided for each case of CMV-induced intrahepatic cholestasis, 15 days and 6 weeks after treatment initiation. Baseline and weekly complete blood counts, ALT, and serum creatinine levels were measured to determine any side effects such as pancytopenia, neutropenia, anemia, infection, and hypersensitivity reactions. Side effects were observed and managed accordingly under the guidance of the respective department.

Outcome variables

Outcome measures were classified as improvement (clinical, biochemical, and viral clearance), deterioration with morbidity (based on clinical reviews and biochemical

findings), and mortality (death). The presence of chronic liver disease, portal hypertension (PH), liver cirrhosis, liver failure or its consequences, poor general health, failure to thrive (–2 standard deviation [SD] for weight for age), and the necessity for liver transplantation were among the morbidities. Basic clinical information was recorded during follow-up visits. These included growth parameters, an assessment of the child's development, and the current state of the liver and its complications.

Data management and statistical analysis

Data were analyzed using SPSS 23 (IBM Co.) for Windows XP. Number, percent, and mean+SD were used to express the numeric variables. Bivariate statistical analysis was used to examine the relationship between the independent and dependent variables (chi-square or Fisher's exact test). Multivariate logistic regression techniques were used to further investigate the bivariate analysis variables, with *p*-values of 0.25 or above. The findings of the multivariate analysis were presented as odds ratios (ORs) and 95% confidence intervals (CIs). Statistical significance was set than or equal <0.05, it was deemed significant.

RESULTS

Basic features of the reported cases

Twenty patients <6 months of age were included in this study. The mean age of 10 babies in the control group (4 males, 6 females) was 0.9±0.99 months; and that of the intervention group (7 males, 3 females) was 0.8±0.97 months. The 2 study groups were similar in terms of gestational age, birth weight, and neonatal presentation (**Table 1**).

Clinical characteristics

Jaundice, dark urine, pale stool, fever, rash, ascites, and bleeding manifestations were significantly reduced ($p<0.05$) in the case (VGCV) group after 6 months of intervention (**Table 2**).

Biochemical characteristics

Fractionated serum bilirubin, serum transaminases (ALT and aspartate aminotransferase [AST]), and GGT levels were significantly ($p\leq0.05$) reduced in the case (VGCV) group after 6 months of intervention (**Table 3**).

Urinary CMV PCR

Elevated quantities of urinary CMV PCR were noted in all patients; the mean value of the control group was 664,214.1±1,868,649.66 and the value of the intervention group was 576,619.42±148,869.26. After 6 weeks of VGCV treatment and at the 6-month routine follow up, the mean value in the control group was 499,834.7±101,816.10, while that in the VGCV group was 2,471.9±1,153.73. This difference was also statistically ($p\leq0.05$) significant (**Table 4**).

Outcome of CMV hepatitis with or without VGCV therapy

In terms of viral clearance, the improvement after VGCV treatment was significant; the total improved frequency was 8/10 (80%), compared to 1/10 (10%) in the control group. In contrast, the frequency was 5 (50%) in the control group with symptomatic treatment, while no deterioration (0%) was observed in the intervention group with VGCV. This difference was also statistically ($p\leq0.05$) significant (**Table 5**).

Table 1. Baseline characteristics of studied infants (n=20)

Topic	Control (n=10)	Intervention (n=10)	*p*-value
Age (mo)			0.912
<1	5 (50.0)	5 (50.0)	
1–3	4 (40.0)	4 (40.0)	
>3	1 (10.0)	1 (10.0)	
Mean±standard deviation	0.9±0.99	0.8±0.97	
Sex			0.178
Male	4 (40.0)	7 (70.0)	
Female	6 (60.0)	3 (30.0)	
Gestational age			0.136
Term	9 (90.0)	8 (80.0)	
Preterm	1 (10.0)	2 (20.0)	
Birth weight			0.301
Normal	8 (80.0)	7 (70.0)	
LBW	0 (0)	2 (20.0)	
IUGR	2 (20.0)	1 (10.0)	
Nutritional status			0.5
Good	4 (40.0)	4 (40.0)	
Undernourished	3 (30.0)	4 (40.0)	
Malnutrition	3 (30.0)	2 (20.0)	
Maternal history			
Fever	6 (60.0)	4 (40.0)	0.371
Rash	0 (0)	3 (30.0)	0.060
Pruritus	2 (20.0)	3 (30.0)	0.606
Consanguinity			0.160
Present	5 (50.0)	2 (20.0)	
Absent	5 (50.0)	8 (80.0)	
Symptoms			0.481
Jaundice	9 (90.0)	10 (100)	
Dark urine	8 (80.0)	9 (90.0)	
Pale stool	7 (70.0)	8 (80.0)	
Abdominal distension	5 (50.0)	4 (40.0)	
Rash	2 (20.0)	2 (20.0)	
Signs			0.192
Hepatomegaly	10 (100)	9 (90.0)	
Hepatosplenomegaly	5 (50.0)	6 (60.0)	
Ascites	3 (30.0)	1 (10.0)	
Bleeding manifestation	2 (20.0)	2 (20.0)	
Investigations			
Serum bilirubin (mg/dL)	14.933±2.92	14.554±2.57	0.765
Direct bilirubin	8.20±2.21	6.417±2.08	0.814
ALT (U/L)	253.5±90.98	285.4±85.84	0.435
AST (U/L)	162.8±132.92	262.4±123.04	0.093
Serum ALP (IU/L)	582.5±198.12	665.4±196.62	0.361
GGT (IU/L)	205.1±129.72	206.6±127.97	0.1296
PT (second)	17.89±12.89	23.088±11.78	0.3608
INR	1.489±1.12	1.924±0.98	0.3719
Urinary CMV PCR	664,214.1±1,868,649.66	577,610.6±1,468,649.66	0.9095

Values are presented as number (%) or mean±standard deviation.
LBW: low birth weight, IUGR: intrauterine growth retardation, ALT: alanine aminotransferase, AST: aspartate aminotransferase, ALP: alkaline phosphatase, GGT: gamma-glutamyl transferase, PT: prothrombin time, INR: international normalized ratio, CMV PCR: cytomegalovirus polymerase chain reaction.
A chi-square test was considered statistically significant if the *p*-value was <0.05.

https://doi.org/10.5223/pghn.2024.27.5.298

Table 2. Distribution of cytomegalovirus hepatitis infants by clinical status initial, 6 weeks and 6 months of intervention (n=20)

Point	Control (n=10)				Intervention (n=10)			
	Initial condition	After 6 weeks	After 6 months	*p*-value	Initial condition	After 6 weeks	After 6 months	*p*-value
Symptoms								
Jaundice	9 (90.0)	7 (70.0)	6 (60.0)	0.012	10 (100)	0 (0)	0 (0)	0.001
Dark urine	8 (80.0)	5 (50.0)	4 (40.0)	0.067	9 (90.0)	0 (0)	0 (0)	0.001
Pale stool	7 (70.0)	5 (50.0)	4 (40.0)	0.177	8 (80.0)	0 (0)	0 (0)	0.001
Abdominal distension	5 (50.0)	4 (40.0)	4 (40.0)	0.652	4 (40.0)	3 (30.0)	1 (10.0)	0.121
Fever	8 (80.0)	5 (50.0)	4 (40.0)	0.067	5 (50.0)	0 (0)	0 (0)	0.009
Rash	2 (20.0)	1 (10.0)	0 (0)	0.136	2 (20.0)	0 (0)	0 (0)	0.136
Signs								
Hepatomegaly	10 (100)	7 (70.0)	7 (70.0)	0.060	9 (90.0)	5 (50.0)	2 (20.0)	0.001
Hepato-splenomegaly	5 (50.0)	3 (30.0)	2 (20.0)	0.158	6 (60.0)	3 (30.0)	1 (10.0)	0.019

Values are presented as number (%).
Chi-square test is statistically significant, if *p*-value <0.05.

Table 3. Distribution of cytomegalovirus hepatitis in infants according to liver function tests and urinary cytomegalovirus polymerase chain reaction before and after 6 weeks and 6 months of intervention (n=20)

Test	Control (n=10)				Intervention (n=10)			
	Initial condition	After 6 weeks	After 6 months	*p*-value	Initial condition	After 6 weeks	After 6 months	*p*-value
Serum bilirubin (mg/dL)	14.933±2.92	14.968±2.14	12.782±2.26	0.97	14.74±3.57	9.81±2.13	7.19±1.93	0.001
Direct bilirubin	8.20±2.21	7.62±2.97	6.63±2.13	0.123	7.17±2.10	3.721±2.98	1.27±2.79	0.001
ALT (U/L)	253.5±90.9	271.7±123.0	283.7±111.2	0.514	286.4±86.1	105.9±90.16	66.9±54.4	0.001
AST (U/L)	162.8±132.9	191.3±114.5	196.7±98.5	0.525	261.4±121.1	112±105.61	87.1±66.65	0.009
Serum ALP (IU/L)	582.5±198.1	578.8±221.9	556.4±178.9	0.760	667.4±195.16	442.2±203.13	312.2±101.3	0.001
GGT (IU/L)	205.1±129.7	222.6±140.1	244.3±190.3	0.599	207.6±128.19	114.12±136.11	94.1±54.2	0.01

Values are presented as mean±standard deviation.
ALT: alanine aminotransferase, AST: aspartate aminotransferase, ALP: alkaline phosphatase, GGT: gamma-glutamyl transferase.
A *t*-test was used to identify the mean difference.

Table 4. Distribution of cytomegalovirus hepatitis in infants by urinary cytomegalovirus polymerase chain reaction before and after 6 weeks and 6 months after intervention (n=20)

Test	Control (n=10)				Intervention (n=10)			
	Initial count	After 6 weeks	After 6 months	*p*-value	Initial count	After 6 weeks	After 6 months	*p*-value
Urinary CMV PCR copies/mL	664,214.1± 186,869.66	542,736.6± 108,126.10	499,834.7± 101,816.10	0.86	576,619.42± 148,869.26	6,519.9± 2,859.21	2,471.9± 1,153.73	0.001

Values are presented as mean±standard deviation.
CMV: cytomegalovirus, PCR: polymerase chain reaction.
A *t*-test was used to identify the mean difference.

Table 5. Outcome of cytomegalovirus hepatitis infants by urinary cytomegalovirus polymerase chain reaction after 6 weeks intervention with valganciclovir and 6 months surveillance (n=20)

Clearance of virus	Control (n=10) (no treatment)	Intervention group (n=10) (VGCV)	*p*-value
Totally improved	1 (10.0)	8 (80.0)	0.001
Partially improved	3 (30.0)	2 (20.0)	0.603
Static	1 (10.0)	0 (0)	0.303
Deteriorating	5 (50.0)	0 (0)	0.009

Values are presented as number (%).
VGCV: valganciclovir.
Chi-square test is statistically significant, if *p*-value <0.05.

Predictive factors for improved CMV cholestasis after VGCV therapy

When cholestasis was diagnosed within 1 month, all patients (n=5) showed complete improvement compared to those diagnosed within >1 month (n=3). When the duration of CMV cholestasis was 3 months, all patients (n=8) improved completely compared to those treated for >3 months. No statistically significant improvement was observed in premature infants with CMV cholestasis (p=0.022). Malnourished cholestatic infants did not improve compared to those with good or undernourished nutritional status. All infants (n=8) showed complete improvement when VGCV therapy was initiated within 3 months of age (**Table 6**).

Table 6. Bivariate analysis of predictive factors in cytomegalovirus cholestasis patients after 6 weeks of valganciclovir therapy (n=10)

Predictor	Totally improved (n=8)	Not totally improved (n=2)	RR	Bivariate 95% CI	*p*-value
Age at cholestasis diagnosis					
<1 mo	5	0	1.667	0.81–3.40	0.14*
≥1 mo	3	2			
Duration of CMV cholestasis					
<3 mo	8	1	0.11	0.0175–0.7052	0.19†
≥3 mo	0	1			
Prematurity					
No	8	0	0.83	0.5827–1.19	0.022*
Yes	0	2			
Nutritional status					
Good	4	0	0.67	0.029–3.21	0.71†
Undernourished	4	0	0.91	0.3–4.87	0.92†
Malnutrition	0	2			
Age at VGCV therapy					
<3 mo	8	1	1.28	0.54–3.02	0.2*
≥3 mo	0	1			

RR: relative risk, CI: confidence interval, CMV: cytomegalovirus, VGCV: valganciclovir.
*Chi-square. †Fisher exact test.
Chi-square/Fisher's exact test was considered statistically significant if *p*-value <0.05.

Table 7. Bivariate analysis of predictive factors in cytomegalovirus cholestasis patients after 6 weeks of valganciclovir therapy (n=10)

Predictor	OR	95% CI	*p*-value
Age at cholestasis diagnosis	0.6	0.02–13.582	0.1
Duration of CMV cholestasis	1.667	0.07–37.72	0.01
Age at VGCV therapy	3.01	0.12–27.72	0.22

OR: odds ratio, CI: confidence interval, CMV: cytomegalovirus, VGCV: valganciclovir.

We performed a multivariate analysis to analyze the relationships between independent variables (predictors) and CMV cholestasis outcomes. All variables with a *p*-value <0.25 from the bivariate analysis were included in the multivariate backward logistic regression analysis. Significant improvement of CMV cholestasis was observed with shorter duration (OR, 1.667; 95% CI, 0.07 to 37.72; *p*=0.01) (**Table 7**).

Adverse effects of drugs

In the intervention group, the treatment was well tolerated, with evidence that no side effects were observed in 7 (70%) patients ($p<0.05$). Neutropenia was observed in 2 (20%) patients, and diarrhea was seen in 1 (10%) patient. Therefore, overall drug tolerability was satisfactory (**Fig. 2**).

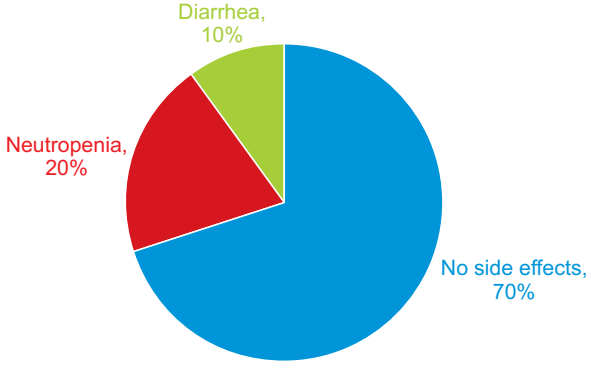

Fig. 2. Adverse effects following valganciclovir treatment.

https://doi.org/10.5223/pghn.2024.27.5.298

DISCUSSION

Non-hepatotropic CMV is crucial for the development of CMV cholestasis. The most frequent cases of CMV cholestasis occur during infancy and are asymptomatic. Normal hosts infected with the CMV enter a lifelong latent phase and expel the virus from their saliva and urine for extended periods. However, hepatomegaly and PH can be long-term consequences of CMV cholestasis [40,41]. Because to the potential side effects of injectable ganciclovir, oral VGCV has been introduced for the treatment of congenital CMV infection and has been proven to be effective with fewer adverse effects [42-46].

The mean age of the control group was 0.9±0.99 months, while that of the intervention group was 0.8±0.97 months. All infants were younger than 6 months with symptoms since the neonatal period. The average age of patients in a similar study from Indonesia was 3.32±2.76. This study included 3-year-old children, which explains the large differences. In this study, we focused on CMV infection. In this study, CMV-induced cholestasis was more common in male infants. The male:female ratio was 1.2:1. Similar results were observed by Hasosah et al. [1] (1.2:1), Ozkan et al. [38] (1.4:1), and Puspita et al. [4] (1.7:1). Therefore, CMV-induced cholestasis is more common in male infants than in female infants.

The prevalence of preterm birth was approximately 15% in both CMV cholestasis groups. Similar results were reported in Saudi Arabia (14.3%) [1], Türkiye (14.2%) [22], and Korea (6.6%) [39]. Other studies in Bangladesh (21.6%) [35], Indonesia (29.3%) [4], and Türkiye (25%) [38] have reported high rates of CMV cholestasis. The frequency of occurrence may have been influenced by the children' various socioeconomic circumstances.

In our study, 35 percent of the undernourished infants and 25 percent of the malnourished infants had CMV cholestasis. An Indonesian study reported nearly identical results. Among the children studied, 41% were undernourished, and 12.2% were malnourished. These results suggest that malnutrition is prevalent in children with liver problems at rates ranging from 9.1% to 71.1%, depending on the severity of the liver condition. Nutritional status is affected by the malabsorption of macronutrients such as lipids, carbohydrates, proteins, and vitamins [47]. In the present study, a maternal history of fever (50%) and rash (15%) were the dominant features of CMV cholestasis. Other studies from Bangladesh [48] have found nearly identical results associated with CMV infection.

According to a study in Indonesia [4], the 3 most common clinical signs of CMV cholestasis were hepatomegaly (90.2%), tea-colored urine (97.6%), and jaundice (100%). Additionally, we discovered that all patients had increased serum AST (100%), hypoalbuminemia (43.9%), total and direct bilirubin, and ALT (87.8%) levels. Hasosah et al. [1] from Saudi Arabia, Ozkan et al. [38] from Türkiye, Na [39] from Korea, and our study used the same mirror image.

Liver biopsy is the gold standard for identifying CMV cholestasis. Multinucleated large cells, an "owl's eye"-shaped cytomegalic inclusion, granuloma development, mild hepatocellular necrosis, or sinusoidal infiltration of mononuclear cells are the main biopsy findings [3,49,50]. As this is an invasive procedure, obtaining familial consent was difficult, and biopsies were not routinely performed in this study. Positive CMV IgG and IgM serology as well as positive urine CMV antigenemia were used to confirm the diagnosis of CMV infection. IgM has variable sensitivity for identifying CMV infections, although it has a maximum sensitivity

of 72.97% and a maximum specificity of 62.06%. Urinary CMV PCR >500 copies/mL was positive and more specific than antibody testing [51,52].

Patients in the intervention group (n=10) treated with VGCV showed significant clinical and biochemical improvements. Before treatment, jaundice, dark urine, and pale stool were present in 100%, 90%, and 80% of the patients, respectively, whereas after 6 months of treatment, these symptoms were absent in all patients (p<0.001). Four (40%) patients presented with abdominal distension, which reduced to 1 (10%) after 6 months of treatment. Hepatomegaly was observed in nine (90%) patients before declining to 2 (20%). However, these parameters did not differ significantly in the control group. The pre-treatment mean direct bilirubin level was 7.17±2.10, which decreased to 1.27±2.79 (p=0.001) after 6 months. The patients also showed significant improvements in liver enzyme levels. Pre-treatment ALT was raised; the mean value was 286.4±86.1, while it normalized in most of the patients after treatment and declined to 66.9±54.4 (p=0.001). The mean pre-treatment AST was 261.4±121.1, while the post-treatment AST was 87.1±66.65 (p=0.001) after 6 months. The pre-treatment mean value of GGT was 207.6±128.19 and significantly (p=0.01) decreased to 94.1±134.1. Similarly, urinary CMV PCR values decreased to the desired levels after 6 weeks of treatment in the intervention group (p=0.001) and persisted for up to 6 months of follow-up; however, these changes in the control group were inappreciable. Hasosah et al. [1] from Saudi Arabia, Ozkan et al. [38] from Türkiye, and Na [39] from Korea reported similar results following ganciclovir therapy. The primary endpoint of these trials was viral clearance from urine 6 weeks after treatment. In the current study, 80% of the VGCV-treated infants showed clearance of the virus from urine at 6 months which was statistically significant (p=0.001), while only 2 infants (20%) showed partial clearance of the virus. In contrast, 5 infants (50%) in the control group deteriorated because of virus persistence. However, few clinical trials have been conducted to date. In a related investigation, Lombardi et al. [53] found that when oral VGCV was administered at a dose of 15 mg/kg every 12 hours for 6 weeks, 8 out of 12 neonates with symptomatic CMV demonstrated virus clearance, whereas 33.3% did not.

Long-term hospitalization is necessary when intravenous GCV is administered. Hematological complications of GCV, such as leukopenia, neutropenia, and thrombocytopenia, are the main adverse effects. Bone marrow suppression, elevated liver enzyme levels, hypokalemia, and renal impairment are common adverse effects [54]. There are no clear recommendations regarding the administration of IV or oral VGCV in infants with symptomatic cCMV infection. There is little research on this topic, especially in situations with scarce resources where the issue is more common. The Bangladeshi researchers Fatema et al. [35] and Ruby et al. [5] concluded that VGCV and GCV are equally effective in treating infants with symptomatic CMV infections. Both medications have comparable efficacies. However, with the option of oral administration, VGCV appeared to produce a better effect with fewer side effects than GCV. Yang et al. [2] from China also stated that VGCV is similar to GCV in the treatment of congenital CMV infection; however, VGCV has advantages over GCV owing to its low price, drug delivery, short hospital stay, reduced toxicity, and side effects caused by GCV [37,55].

In the present study, when infants with CMV cholestasis were term with good or undernaurished nutritional status, the introduction of VGCV therapy within <3 months of age with a shorter duration of cholestasis led to improvements observed clinically and biochemically and decreased the number of DNA copies. Puspita et al. [4] from Indonesia reported a similar result.

The 2 most commonly reported adverse events were neutropenia (20%) and diarrhea (10%). Kimberlin et al. [56] observed similar adverse events, such as neutropenia, which occurred in approximately 20% of infants treated with VGCV at 6 months. This rate is almost the same as that observed in our study [41]. Other side effects reported in another study included anemia, neutropenia, thrombocytopenia, renal or liver dysfunction, phlebitis, and hypersensitivity reaction [36].

Limitations of this study

This study has some limitations. This was a single-center study with a limited sample size because few health centers provide these treatment facilities. Other common neurological features, such as SNHL, visual status, cognitive status, and psychological status, were not included in this study.

Conclusions

Compared with the control group, the VGCV group showed significant clinical and biochemical improvements. Even after 6 months of treatment, urinary CMV PCR was undetectable in the majority of the cases in the intervention group, whereas the CMV virus raising trait by RT-PCR was noticed in the other half of the samples of the control group. Early diagnosis with prompt introduction of VGCV, good nutritional status, and shorter duration of cholestasis are good predictors of improvement following VGCV therapy. Oral VGCV is highly effective and well-tolerated in the treatment of CMV-induced intrahepatic cholestasis in infants, with no major undesirable side effects. The number of studies on the treatment of CMV hepatitis with VGCV is currently insufficient.

Recommendations

We recommend that researchers conduct a nationwide study over a longer period with a larger number of samples to help determine how to use oral VGCV to treat CMV-induced cholestasis.

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