ORIGINAL RESEARCH PAPER

Volume-7 | Issue-6 | June-2018 | PRINT ISSN No 2277 - 8179

INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

UTILITY OF REAL TIME QUANTITATIVE PCR IN THE DIAGNOSIS OF CONGENITAL AND PERINATAL CYTOMEGALOVIRUS INFECTION IN SYMPTOMATIC BABIES

Microbiology

Shabina MB Assistant Professor, Department of Microbiology, Govt Medical College,Kozhikode.

Beena Philomina J* Professor, Department of Microbiology, Govt Medical College, Kozhikode. *Corresponding Author

Priya Chandran Associate Professor, Department of Preventive Medicine, Govt Medical College, Kozhikode.

ABSTRACT

Cytomegalovirus(CMV) infection may be acquired congenitally, perinatally or postnatally in babies. Congenital and perinatal CMV infection can be diagnosed by viral culture or detection and quantitation of CMV DNA by Real Time Quantitative PCR (RT-qPCR) in blood ,urine and body fluids. The objective of this study was to diagnose and determine CMV load in infants presenting with clinical features suggestive of cytomegalovirus infection by RT-qPCR of urine. This descriptive study was done on babies admitted to the Departments of Neonatology and Paediatrics Govt Medical College, Kozhikode from January 2015 to December 2017. Urine samples from 142 babies were received and processed in the Microbiology Department. DNA isolation and amplification was performed using commercial DNA extraction kit and PCR kit for detection and quantification of CMV. Serum samples of the babies with CMV viruria were tested for CMV IgM antibodies. Of 142 babies suggestive of CMV infection CMV-DNA was detected and quantitated in urine of 25 (17.60%) (mean age 3.36 months). . CMV IgM was positive in 15/25(60%) babies with viruria .Twenty two had congenital CMV infection (cCMV) and 3 had perinatal infection.The most common clinical presentation was jaundice 13(52%). Of 8 babies started on Ganciclovir 7 responded to treatment. RT-qPCR helps in diagnosing and quantitating CMV load which helps in deciding on therapy and assessing response to treatment,and can predict risk for long term sequelae.

KEYWORDS

Congenital and perinatal CMV infection, Real time Quantitative PCR, Urine,Ganciclovir

Introduction

Cytomegalovirus belongs to the family Herpesviridae causing wide spectrum of diseases in children. The infections can be congenital, or can be acquired perinatally or postnatally. Congenital CMV infection is the most common intrauterine infection and prevalence varies from 0.2% to 2.2% of live births worldwide.[1] In India where there is high prevalence of CMV infection few studies have shown it to be responsible for birth defects in infants.[2,3]

Congenital CMV infection is less common than perinatal infection but causes serious CMV disease .Transmission is high in mothers with primary infection than with reactivated disease. Ninety percent of infants with cCMV infection are asymptomatic at birth[4] and only 10% present with features like microcephaly , intellectual impairment , sensineural hearing loss(SNHL), choriorenitis , hepatosplenomegaly , jaundice, thrombocytopenia ,low birth weight , pneumonitis and congenital heart disease. Approximately 10–15% of asymptomatic babies and and about 90% of symptomatic infants will develop neurological sequelae .[5]

Infants with symptomatic cCMV infection have a mortality rate of 20-30% and death is due to liver failure, bleeding, disseminated intravascular coagulopathy or secondary bacterial infection. It has to be diagnosed within 3 weeks of birth after which it is difficult to assess if infection occurred intrauterine or was acquired perinatally or postnatally.[6]

Perinatal CMV infection can occur on exposure to infected secretions of mother in the birth canal or via breastfeeding . Around 50% of exposed infants are infected.[7] Usually preterm babies are affected and present with lymphadenopathy, hepatitis, pneumonitis or features of sepsis after 1-6 months after birth . Postnatally CMV infection can be acquired after blood transfusion and present as cytomegalic inclusion disease in preterm infants who are born to seronegative mothers .

The rapid and correct diagnosis of CMV infection in infants is very important to start appropriate treatment and to prevent the progression to severe CMV disease and sequelae . Diagnosis of congenital CMV disease in the newborn period is important for identifying those with neurologic abnormalities who may benefit from treatment with antivirals. Perinatally acquired CMV infection usually resolves on its own or may require treatment if there are symptoms of end organ damage .CMV specific IgM antibodies is commonly used to detect recent CMV infection. Its presence at birth is highly suggestive of congenital infection, although its absence does not rule out congenital infection. Moreover IgM assays lack specificity showing false positivity and can persist for months after primary infection and can be positive in reactivated CMV infections . IgM antibodies are only present in between 20 and 70% of infected babies at birth.[8]False negative results are seen in preterm babies who develop weak immune response.[9] CMV IgG could be maternal antibody which can persist up to 18 months of age. Hence serologic assays are not recommended for diagnosis of CMV infection in neonates. In the case of IgM and IgG positivity for CMV, CMV IgG avidity test can be used for determining present or past infection. CMV infected babies shed virus in urine, blood and throat for years. The best diagnostic method for congenital or perinatal CMV infection is virus isolation or detection of CMV DNA by PCR from urine or saliva of infants[10]. RT-qPCR predicts development of symptomatic disease, helps to decide on treatment and to analyse response to treatment. PCR on urine samples are more sensitive and specific for detection of congenital CMV infection . It has been considered to be the gold standard diagnostic test for cCMV since 2010.[11]

Treatment with iv Ganciclovir or oral Valganciclovir can prevent development of CNS disease in symptomatic neonates with congenital or postnatal infection .[12] A 6 week course of Ganciclovir for symptomatic infants with cCMV infection of the CNS[13] and a 2 week course for severe symptomatic perinatal CMV infection can be given.

This study was undertaken to diagnose and quantitate CMV infection in symptomatic infants by using RT-qPCR .

Materials and methods

Study setting:- This descriptive study was conducted in the Department of Microbiology Govt Medical College ,Kozhikode from January 2015 to December 2017.

Study population:- One hundred and forty two infants with symptoms suggestive of CMV infection referred from Neonatology and Paediatrics Department were included.

Inclusion criteria:- Babies with clinical signs and symptoms suggestive of CMV infection like neonatal cholestasis, microcephaly, small for gestational age , petechiae, purpura, hepatosplenomegaly, jaundice, chorioretinitis were included.

Exclusion criteria:- Babies with sepsis, inherited primary microcephaly, hepatomegaly and/or splenomegaly due to inborn errors of metabolism, and SGA due to antenatal causes .

Collection and processing of urine samples

Urine (15 ml) from 142 infants suscepted of CMV infection were received in the Microbiology Department and stored at -80°C till subjected to DNA extraction . A QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) was used to extract DNA from urine samples according to manufacturer's instructions. Primers and a fluorescence-labelled probe (CMV R-gene kit ,bioMerieux SA-France.were added to the extracted nucleic acid.. CMV DNA was amplified and quantified using Real-time PCR System ((BIORAD-California, USA). The quantification range for CMV was linear between 500 copies/ml to 10^7 copies/ml .The results were expressed as copies/ml.

Serum from babies with CMV viruria was used for detection of CMV IgM antibodies. Enzyme-linked immunosorbent assay (ELISA) was performed using a commercial kit (CMV IgM Capture Epitope diagnostics Inc, USA) following manufacturer instructions.

Statistical analysis:-

Statistical analyses were performed using SPSS V.19.0. Quantitative variables were expressed as arithmetic mean and standard deviation SD . Qualitative data was presented as number and percentages. Association was estimated using chi square test and p value<0.05 was considered to be statistically significant

Results:- Of 142 babies 90(63.38%) were females and52(36.61%) males. CMV viruria was detected and quantitated in 25(17.6%) babies of whom 8(32%) were males and 17(68%) were females. Majority of the babies were in the age group 1-2 months 14/.25(56%). The mean age of the babies with CMV infection was 3.36 months(range 1month to 12 months). Demographic data of the 25 babies is shown in Chart 1.The highest load detected was $2.2x10^7$ and lowest being $7.2x10^2$ copies/ml.(CMV viral load distribution shown in Table-1).The mean CMV load was $3.6x10^5$.

Majority of the babies13/25(52%) presented with jaundice.. Nine (36%) were preterm .Clinical presentation of the babies shown in -Chart 2) Most of the infants presented with more than one clinical symptom. Serology for IgM was done for all the 25 babies with CMV viruria. CMV IgM antibodies. were positive in 15(60%) babies. A follow up was available for 12 babies. Intravenous Ganciclovir was started in 8 babies and all had viral load $>10^4$copies/ml and 5 were preterm. All the three babies with perinatal pneumonitis received treatment. Response to treatment was seen in 7 babies .One baby succumbed while on treatment. Four babies recovered without treatment.

Chart 1 Age and sex distribution(no) of babies with CMV infection

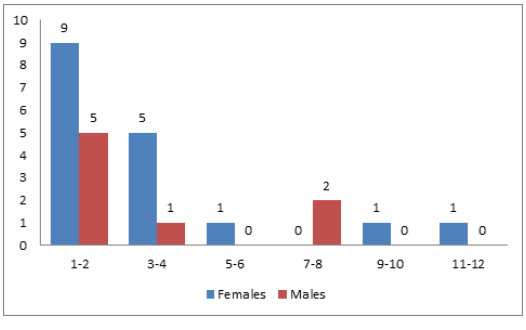

Chart 2 Clinical presentation in 25 infants with CMV infection

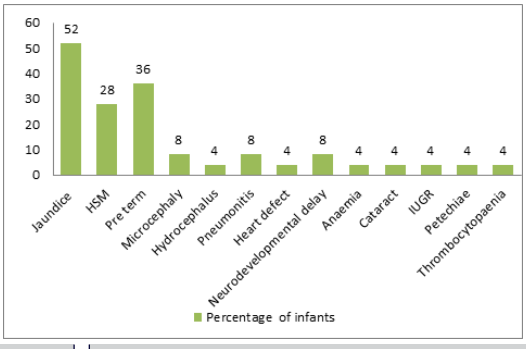

Table 1-CMV viral load in babies with CMV infection

CMV load(RANGE)	Number (%)
1.1 x 10^2 - 1 x 10^3	1(4%)
1.1 x 10^3 -1 x 10^4	3(12%)
1.1 x 10^4 - 1 x 10^5	9(36%)
1.1 10^5 - 1 x 10^6	4(16%)
1.1 X 10^6 - 1 x 10^7	3(12%)
1.1 x 10^7 - 1 x 10^8	5(20%)
Total	25

Discussion:- The main aim of the study was to diagnose and quantitate CMV in urine of symptomatic babies aged 1month to 1 year. Our data showed that females(68%) were more in the CMV infected group (P<0.05) . A study by Picone et al has shown that neurological complications are more common in females. [13]

CMV viruria was detected in 25 symptomatic babies . The urine samples were not received at birth or within 3 weeks of birth. The mean age for presentation was 3.36 months.The diagnosis of congenital CMV infection was made clinically by the Paediatricians(22/25) and confirmed with CMV viruria.Three babies aged 6,7, and 8 months who presented with pneumonitis alone were clinically diagnosed as perinatal infection and all the three were preterm.Perinatally acquired CMV infection in preterm infants may be more severe and usually present with interstitial pneumonitis.[14]

Urine is an ideal non invasive sample for detecting congenital infection as viral load in blood may often be lower than in urine, or viraemia may be absent altogether in a congenitally infected baby. Studies have reported high sensitivities and specificities of PCR of urine ranging from 93-100% for diagnosis of congenital CMV infection.[15] It is highly sensitive to detect even very low viral load in specimens.[16] In our study the lowest CMV viral load was $7.2x10^2$ and highest 2.2x 10^7copies/ml. Majority of the babies 9/25(36%) were with a load in the range $1.1x10^4$-$1x10^5$ copies/ml. Urinary CMV load quantification can predict the incidence of late-onset SNHL and neurodevelopmental disorders especially in those with high load.[17]These babies will require treatment and have to be followed up regularly .

Serology for CMV IgM antibodies was done in babies with CMV viruria.CMVIgM was positive in 15 /25(60%) and could not detect all cases of CMV infection.This could be due to either undetectable amount of CMV IgM antibodies by the assay used or delayed development of CMV IgM antibodies in infected babies especially preterm babies. IgM is produced in fetus three to four weeks following CMV exposure and it remains positive for three to four months.CMV IgM alone cannot be used to diagnose cCMV infection as false positive results can arise from cross reaction with Epstein-Barr virus and Herpes Simplex virus.[11]

Majority of the babies with presented with jaundice due to neonatal cholestasis or hepatitis 13/25 52% and 22 had cCMV. In a study by Choudhary et al[18] jaundice was observed in 50% of babies with congenital CMV.

Seven babies(4 with cCMV and 3 with perinatal CMV infection) responded to treatment with IV Ganciclovir for 2 weeks followed by oral Valganciclovir for 4 weeks with symptoms subsiding and reduction in viral load seen after 2 weeks of therapy. Ganciclovir 6 mg/kg intravenously every 12 h or Valganciclovir 16mg/kg given for 6 weeks has been used to treat infants who have congenital and perinatal infection with symptoms of end organ damage . Four babies recovered without treatment as acute CMV infection is often transient and reversible. [19] One baby succumbed while on treatment .She had neonatal cholestasis with coagulopathy disorder. In a study from Japan, 12 symptomatic babies received antiviral treatment and 5 babies had normal development on 1 year follow up.[20] Early diagnosis and treatment of symptomatic congenital infection can prevent progression to severe CMV disease especially SNHL . Babies treated with Ganciclovir had a reduction in their viral load and a reversal of clinical manifestations.

Conclusion:- Quantitation by Real Time PCR helps the paediatrician to assess the severity of disease, to treat infants before end organ damage and to assess response to treatment .

RT qPCR is a sensitive, reliable, rapid, cost effective method to diagnose both congenital and perinatal CMV infection.. Detection of CMV DNA in urine can be combined with anti-CMV IgM to diagnose CMV infection in infants but not always reliable.

References.

1 Britt W. Cytomegalovirus. In: Remington JS, Klein JO, Wilson CB, Baker CJ, editors. Infectious diseases of the fetus and newborn infant. 7th ed. Philadelphia: W.B. Saunders Company; 2011. pp. 704–53.

2.. Chakravarty A, Kashyap B, Rathi K. The seroepidemiological study on cytomegalovirus in women of child-bearing age with special reference to pregnancy and maternal-fetal transmission. Indian J Pathol Microbiol. 2005;48:518-21.

3. Gandhoke I, Aggarwal R, Lal S and Khare S. Congenital CMV Infection in Symptomatic Infants in Delhi and surrounding Areas. Indian J Pediatr. 2006;73:1095-7.

4. Chakravarti A, Kashyap B, Matlani M. Cytomegalovirus infection: An Indian perspective. Indian J Med Microbiol. 2009;27:3-11.

5. Revello MG, Gerna G. Diagnosis and management of human cytomegalovirus infection in the mother, fetus, and newborn infant. Clin Microbiol Rev. 2002;15:680-715.

6. Marin LJ, Santos de Carvalho Cardoso E, Bispo Sousa SM, Debortoli de Carvalho L, Marques Filho MF, Raiol MR, et al. Prevalence and clinical aspects of CMV congenital infection in a low-income population. Virol J. 2016;13:148.

7. AAP committee on infectious diseases. Cytomegalovirus infection. In: Pickering LK, Baker CJ, Kimberlin DW, Long SS, editors. Red book. 28th ed. IL:American Academy ofPediatrics, 2009:275-80.

8. Revello MG, Zavattoni M, Baldant F, Sarasini A, Paolucci S, Gerna G. Diagnostic and prognostic value of human cytomegalovirus load and IgM antibody in blood of congenitally infected newborns. J. Clin. Virol.1999; 14: 57–66.

9. Prateek B, Narang A , Minz RW, Neonatal cytomegalovirus infection: Diagnostic modalities available for early disease detection The Indian Journal of Pediatrics. 2010; 77: 77–79 .

10. Stagno S, Britt W. Cytomegalovirus infections. In: Remington J, Klein J, Wilson C, et al. (eds). Infectious Diseases of the Fetus and Newborn Infant. Sixth edn. Philadelphia: Elsevier, 2006, 739–81.

11. NasirIA, Babayo A, and Shehu MS .Clinical Significance of IgG Avidity Testing and Other Considerations in the Diagnosis of Congenital Cytomegalovirus Infection: A Review Update ., Med. Sci. 2016; 5: 1-11.

12. Oliver SE, Cloud GA, Sanchez PJ,Demmler GJ, Dankner W,Shelton M et al. Neurodevelopmental outcomes following ganciclovir therapy in symptomatic congenital cytomegalovirus infections involving the central nervous system. J Clin Virol. 2009;46:Suppl 4:S22-S26 .

13. Picone O, Costa JM, Dejean A, Ville Y. Is fetal gender a risk factor for severe congenital cytomegalovirus infection? Prenat Diagn. 2005 Jan;25(1):34-8.

14. Hirsch MS. Cytomegalovirus and human herpes virus types 6,7,8. In: Kasper DL, Braunwald E, Fauci AS, Hauser SL, Longo DL, Jameson JL, editors. Harrison's Principles of Internal Medicine. 16th ed. New York, NY: McGraw-Hill; 2005. p. 1049.

15. de VriesJJ ,van der Eijk AA,WolthersKC, Rusmana LG , Pas SD, Molenkamp R,et al. Real-time PCR versus viral culture on urine as a gold standard in the diagnosis of congenital cytomegalovirus infectionJ Clin Virol. 2012; 53: 167-70.

16. Albanna EAE, El-latif RSA, Sharaf HA, Gohar MK, Ibrahim BM. Diagnosis of congenital cytomegalovirus infection in high risk neonates. Mediterr J Hematol Infect Dis. 2013;5:1–7.

17. Yamaguchi A, Oh-ishi T, Arai T Sakata H, Adachi N, Asanuma S et al, Screening for seemingly healthy newborns with congenital cytomegalovirus infection by quantitative real-time polymerase chain reaction using newborn urine: an observational study BMJ Open. 2017; 7(1): e013810.

18. Choudhary A, Pati S K, Patro R K, Deorari A K, Dar L. Comparison of conventional, immunological and molecular techniques for the diagnosis of symptomatic congenital human cytomegalovirus infection in neonates and infants. Indian J Med Microbiol. 2015;33 :15-19.

19. Yadav SS, Narula G, NarayanS, BiswasS, GuptaG. Cytomegalovirus infection in Six Neonates. Indian Pediatrics .2010;47:174-75.

20. Nishida K, Morioka I, Nakamachi Y, Kobayashi Y , Imanishi T, Kawano S et al. Neurological outcomes in symptomatic congenital cytomegalovirus-infected infants after introduction of newborn urine screening and antiviral treatment. Brain and Development . 2016; 38: 209–216.