International Journal of Research and Review
DOI: https://doi.org/10.52403/ijrr.20210540
Vol.8; Issue: 5; May 2021
Website: www.ijrrjournal.com
Original Research Article *E-ISSN: 2349-9788; P-ISSN: 2454-2237*

Quantitative RT PCR in the Diagnosis of Congenital Cytomegalovirus Infection with Special Reference to Sensorineural Hearing Loss in a Tertiary Care Centre in North Kerala

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ABSTRACT

Background:- Cytomegalovirus, a Herpes virus is the most common virus causing congenital viral infections. Sensorineueal hearing loss is the most common non hereditary manifestation. Majority of the babies are asymptomatic or have non specific symptoms at birth. An early diagnosis can help in starting antiviral treatment based on the clinical disease to prevent end organ damage and to intervene early in babies with hearing impairment which can reduce long term sequelae.

Objectives :- The aim of the study is to diagnose and quantitate neonatal CMV infection by qRTPCR (Quantitative Real Time Polymerase Chain Reaction) ,to evaluate the proportion and clinical profile of congenital CMV infections in a tertiary care hospital.

Materials and Methods:- This cross sectional diagnostic evaluation was done in the Dept. of Microbiology and Neonatal unit of Dept of Paediatrics, Govt Medical college .Kozhikode from August 2017 to December 2018. Details of demographic data, clinical profile, and CMV viral load in urine in various clinical infections were obtained and analyzed. Urine samples from 225 babies were received and processed in the Microbiology Department. DNA isolation and amplification was performed using commercial DNA extraction kit and CMV PCR kit for detection and quantification of CMV.

Results:- Of 225 babies with clinical features suggestive of CMV infection, CMV-DNA was detected and quantitated in urine of 27 babies (12%). The most common clinical presentation was hearing impairment, seen in 22 babies.

Conclusion:- RT-qPCR helps in diagnosing and quantitating CMV in congenital neonatal infection which helps in deciding on therapy and assessing response to treatment and can predict risk for long term sequelae. Diagnosis of congenital CMV in the newborn period is important for identifying those with neurologic abnormalities where appropriate treatment and management is essential.

Keywords: Congenital CMV, qRTPCR, SNHL, Viral load, Urine

INTRODUCTION

Cytomegalovirus (CMV) infection is a very common congenital infection occurring worldwide with an incidence of 0.6 - 0.7% of all live births [1]. The prevalence of congenital CMV (cCMV) infection varies in developing countries, with some reporting as high as 6-14% [2]. CMV is the largest and most complex member of the Herpesviridae family of DNA viruses [3]. It forms intranuclear and intracytoplasmic inclusions in infected cells and causes cytomegalic inclusion disease [4]. It is highly species specific and human are the only known reservoir.

Majority of congenitally infected babies (85-90%) are asymptomatic at birth .Clinically evident infections are seen in

(10-15%) which can lead to life threatening complications .Congenitally infected babies can present with signs and symptoms: petechiae, jaundice, hepatosplenomegaly, microcephaly, and neurologic manifestations like SNHL, mental retardation, and other neurologic deficits[5].

CMV is the leading nonhereditary cause of sensorineural hearing loss(SNHL) and neurodevelopmental delay .Among hearing-impaired children, CMV is the causative agent in 10% to 20% .Hearing impairment may be present at birth or may delayed for the first several years of life. So if an early diagnosis is made, antiviral therapy if required with additional interventions like hearing rehabilitation can reduce long term disability. Hence all babies with cCMV infection, should be monitored regularly for hearing loss for the first few years to detect possible SNHL and to intervene to reduce the functional impairment resulting from hearing loss[6].

Newborns can acquire the infection either intrauterine or postnatally. Post-natal infections are not serious and rarely cause severe illness in term infants. When transmission occurs when mother has primary maternal CMV infection it can result in symptomatic disease CMV transmission can occur either by reactivation of a previous maternal infection or by acquiring infection due to a different strain[7] .The risk to foetus increases when infection occurs in elderly mothers when there is increased rate of transmission [8]. Premature babies appear to be at particularly high risk for CMV-associated disease.

Symptomatic congenital CMV infection must be distinguished from other congenital infections, including toxoplasmosis, rubella and syphilis. Ultrasound scans cannot identify signs of CMV infection until towards late gestation. As most of the infections are asymptomatic at birth, we have to rely on inexpensive tests for an early diagnosis of babies at risk for appropriate management. Laboratory tests to diagnose cCMV infection should be done in the first 2-3 weeks of age .Detection after that may be either congenital or postnatal CMV infection.

The gold standard in the diagnosis of congenital CMV infection is virus isolation from urine or saliva [9]. But it is a very tedious procedure, requires tissue cultures, is expensive and cannot be employed for universal screening.

Real-time polymerase chain reaction (PCR) of urine, saliva or blood is now considered as gold standard for diagnosing cCMV[10]. The sensitivity and specificity of PCR in the diagnosis of cCMV is 97-100 % against culture. High sensitivity makes even very low number of virus detectable [11]. Estimation of CMV loads in urine using qPCR can be very useful to detect and follow up newborns with a risk of sequelae and can decide on when to start antiviral treatment[12].

Serological diagnosis is not reliable always as only 20–70% of infected neonates show specific CMV specific IgM antibodies at birth [13]. Moreover IgM assays lack specificity showing false positivity and can persist for months after primary infection and can be positive in reactivated CMV infections .False negative results are seen in preterm babies who develop weak immune response [14]. Other virus infections like Epstein Barr Virus and Herpes Simplex virus can cause false positive results. CMV IgG could be maternal antibody which can persist upto 18 months of age. Hence serologic assays are not recommended for diagnosis of CMV infection in neonates.

Treatment of congenital CMV infection is usually supportive unless indicated. Antivirals are preferred for babies with evidence of central nervous system (CNS) involvement, including SNHL and in babies with serious end-organ disease. Intravenous Ganciclovir for 2 weeks followed by oral Ganciclovir is used in treating symptomatic cCMVinfection[15].

AIM : -To detect and quantitate CMV in urine by RT-qPCR in neonates presenting

with signs and symptoms suggestive of cytomegalovirus infection.

OBJECTIVES :-

1. To assess the proportion of congenital CMV infection in clinically suspected neonates.
2. To detect CMV as the etiological agent of hearing loss in screened neonates
3. To assess the clinical profile of congenital CMV infection

This cross sectional diagnostic evaluation study was conducted in the Dept. of Microbiology and Dept of Paediatrics, Govt Medical college .Kozhikode from August 2017 to December 2018. Neonates of which 172 were asymptomatic who tested positive for hearing loss on screening and 53 babies admitted in intensive care unit of the department of Paediatrics with signs and symptoms suggestive of CMV infection like hepatitis, SGA, respiratory distress, microcephaly, hydrocephalus, hepato-megaly, splenomegaly, petechial/ purpuric rash, cataract, chorioretinitis, hearing impairment were included in the study. All babies in study group included were less than 21 days in age to distinguish congenital infection from the more common, perinatal infection.

MATERIALS AND METHODS

Study was conducted by interviewing the parent or guardian of the neonates with suspected CMV infection using a semi structured questionnaire after obtaining an informed consent. From each baby 15 ml urine was collected into a sterile screw capped disposable bottle and kept at -80°C until processed for DNA extraction.DNA was extracted using QIAamp DNA blood mini kit(Qiagen, Hilden, Germany).CMV DNA was quantitated by using CMV R-GENE Real Time PCR kit(bioMerieux SA-France) in MJ Mini Thermal Cycler, (BIO-RAD, California, USA).

ETHICAL CONCERNS

Study was conducted after obtaining clearance from Institutional Ethics Committee. Informed consent was taken from the parent or guardian of the babies before study.

DATA ANALYSIS

Data was entered in MS EXCEL and analysed using SPS software version 18.

Mean and median was calculated for quantitative variables and frequency for qualitative variables. Chi square test was done to find out association and

P value of 0.05 was considered to be significant.

RESULTS

Congenital CMV was defined as urinary CMV positive by Quantitative Real Time PCR.

Of the 225 neonates in the study population 27 were diagnosed to have cCMV infection.

Characteristics of the study subjects

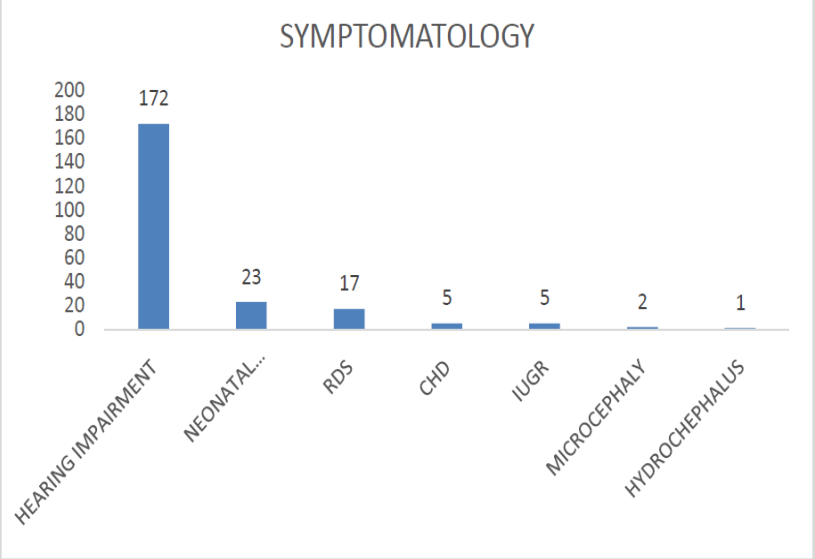
1. Age and gender distribution (n =225)

Variable	Male	Female
2-7 days	74	48
8-14 days	22	22
15-21 days	32	24
Total	128	97

Mean age of the study population was 9.2±6.5 days (range-2-21days)

Among the 225 study subjects, majority were males (128)

2. Clinical profile of the study subjects



Of 225 babies 172, screened positive for hearing impairment by Otoacoustic emission

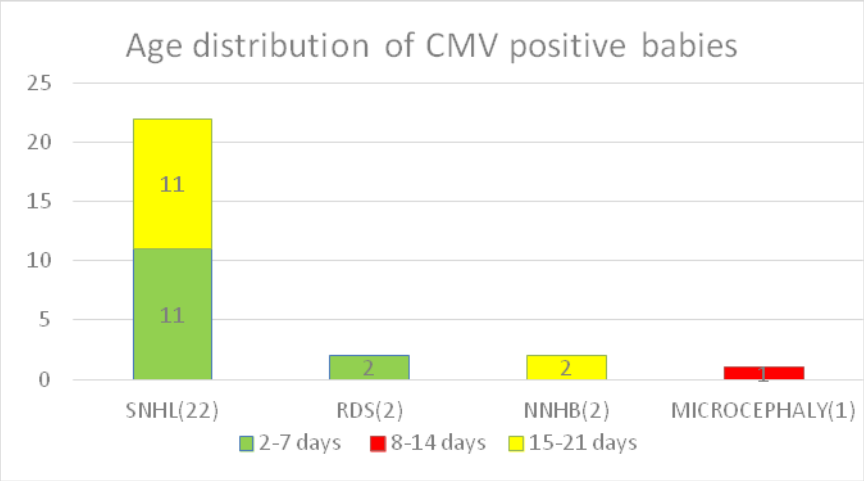
3. Birth weight and association with congenital CMV

VIRURIA	mean±SD	P value
PRESENT (27)	2.5±0.6	0.7
ABSENT (198)	2.6±0.6	

Mean birth weight was less (2.5 kg) in those with viruria when compared to subjects without viruria (2.6 kg),but this was not statistically significant.

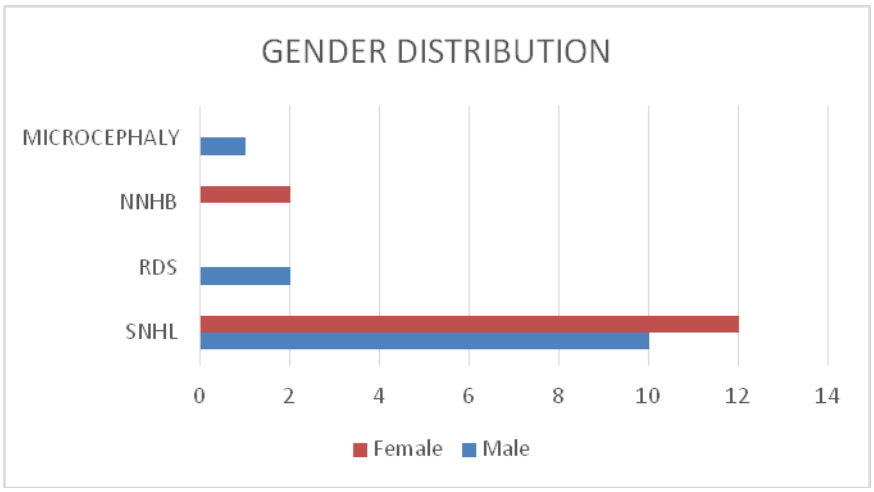
Characteristics of CMV positive babies n =27

1. Age distribution of CMV positive babies

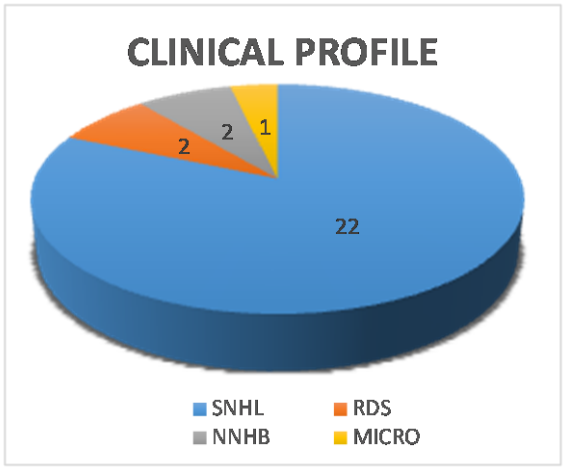


SNHL-sensorineural hearing loss
RDS-respiratory distress
NNHB-neonatal hyperbilirubinemia

2. Gender distribution in CMV positive babies n=27



3. Clinical profile of CMV positive babies n=27



Majority of the babies (22) presented with sensorineural hearing loss

4. Viral load range in CMV positive babies (N=27)

Symptoms	Range
SNHL (22)	$1.3X10^2$-$1.29\ X10^8$
NNHB (2)	$1.23\ X10^2$-$1.7X10^7$
RDS (2)	$2.98X10^3$-$5.1X\ 10^5$
MICROCEPHALY(1)	$1.6\ X10^2$

Median viral load in urine 4.5 X 1 0^3 copies/ml (range$1.23X10^2$ - $1.29X10^8$)

DISCUSSION

In our study population of 225 babies, 27(12%) were diagnosed to have congenital CMV infection by qRTPCR .In a study on prevalence of cCMV infection in Jakarta ,from a total of 411 newborns screened, congenital CMV infection was confirmed in 5.8% of the neonates[16] .

In our study hearing impairment was considered as a suspected sign of CMV infection. In our hospital, all new born babies are routinely screened with Otoacoustic emission (OAE) testing for hearing loss after 48 hrs of birth .On screening hearing impairment was seen in 172 who were otherwise asymptomatic. CMV was detected in 22(12.8%) of the babies and was the predominant manifestation. Fowler *et al* reported that congenital SNHL was seen in 7.2% of children with asymptomatic cCMV infection .[17] Children with symptomatic (22%–65%) congenital CMV infection are at greater risk for hearing impairment than those with asymptomatic infection (6-23%). In a study by Albanna et al jaundice and hepatosplenomegaly were the common manifestations of CMV infection [18] . But in this study babies with hearing impairment were not included in the study population as in our study.

In our study both high and low viral load were seen in babies with hearing impairment. The highest viral load was $1.29X10^8$ and the lowest was$1.3X10^2$ copies/ml. An increased CMV load in infancy can identify those asymptomatic

children who are at increased risk for hearing loss These babies may require treatment with Ganciclovir and should be monitored regularly for progression of hearing loss and for timely intervention to prevent further sequelae. In our hospital babies are followed up regularly for progressive hearing loss for timely intervention. However, further studies are needed to conclude if viral load measured in congenitally infected infants significantly predicts hearing loss.

The other clinical manifestations seen in our babies who were positive for CMV DNA were CMV hepatitis characterised by hyperbilirubinemia and respiratory distress seen in 2 babies each. Congenital CMV infection may present as conjugated jaundice on the first day of life with hyperbilirubinemia. Such babies need evaluation for congenital CMV infection as treatment with Ganciclovir can result in symptomatic improvement and also prevent long-term neurodevelop-mental sequelae. Pneumonitis is usually a rare manifestation of congenital CMV infection. CMV pneumonitis should be suspected in babies presenting with respiratory distress.

In this study we found that CMV-infected neonates had low birth weight (2.5 ±0.6) when compared to non-infected babies. A study by Morgan et al [19] reported that CMV infections are more prevalent in premature and low birth weight neonates. Mussi-Pinhata et al[20] reported that congenital CMV infection can result in significantly lower mean birth weights. The reason could be that CMV infects the uterine wall and/or the placenta, and impairs syncytio-trophoblast and cyto-trophoblast differentiation, that interferes with the capacity to provide oxygen and nutrients to the developing fetus[21], In contrast to these results Al-Hareth et al [22] reported that that low birth weight and small head circumference at birth failed to predict congenital CMV infection.

The rapid diagnosis of CMV infection is important to start appropriate treatment which can prevent the progression to severe CMV disease and sequelae. Diagnosis of congenital CMV disease in the new born period is important for identifying those with neurologic abnormalities who may benefit from treatment with antivirals. Infected babies can shed virus in urine, saliva and CMV DNA can be detected in these specimens. PCR based methods[23] are used now and it has been standardised and is more sensitive and specific for diagnosing cCMV especially in urine samples .Studies have reported high sensitivities and specificities of PCR of urine ranging from 93-100% for diagnosis of congenital CMVinfection[24]. It has been considered as the gold standard diagnostic test for diagnosis of cCMV since 2010 [25]. The high sensitivity of PCR makes even very low amounts of viral particles to be detected in the specimens. In many studies urine samples have been used as the specimen for detection of CMV-DNA in neonates, as it can be collected in a non-invasive way, and can detect even inactive virus [26]. All of these characteristics have made the results of PCR comparable with those from tissue culture[27].

CONCLUSION

CMV viral load determination in urine could help in evaluating newborns with cCMV infection. It can help in identifying babies who require appropriate management with antiviral therapy to prevent development of complications. CMV screening of neonates with hearing loss can be a reasonable strategy in identification of symptomatic congenital CMV infection. This has to be followed by detection of CMV in blood which can predict the development of SNHL. Thereby CMV-DNA detection in urine by PCR is a sensitive, reliable, rapid, cost effective and convenient method to diagnose congenital CMV infection. It helps in the correct management of those babies at risk where an early diagnosis would not have been possible clinically.

Acknowledgement: None

Conflict of Interest: None

Source of Funding: None

Ethical Approval: Approved

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How to cite this article: Manal K, Shabina MB, Suresh Baboo VK et.al. Quantitative RT PCR in the diagnosis of congenital cytomegalovirus infection with special reference to sensorineural hearing loss in a tertiary care centre in North Kerala. *International Journal of Research and Review*. 2021; 8(5): 316-323. DOI: *https://doi.org/10.52403/ijrr.20210540*

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