

Original Research Article

<https://doi.org/10.20546/ijcmas.2018.710.129>

Prevalence and Detection of Cytomegalovirus by Polymerase Chain Reaction (PCR) in Pregnant Women

C. Revathy¹, G. Velvizhi¹ and G. Sucilathangam^{2*}

¹Department of Microbiology, Tirunelveli Medical College, Tirunelveli - 627 011,
Tamil Nadu, India

²Department of Microbiology, Government Theni Medical College, Theni - 625 512,
Tamil Nadu, India

*Corresponding author

ABSTRACT

Cytomegalovirus infections are endemic worldwide. The most frequently used methods for detecting antibodies in developing world are the Enzyme Linked Immunosorbent Assay. The polymerase chain reaction is a molecular biology technique in which the production of large amounts of specific deoxyribonucleic acid fragments is induced from very low concentrations of complex substrates allowing the detection of very low amounts of viral particles. Blood samples were collected from pregnant women with bad obstetric history attending Outpatient Department and were stored at -20°C and transported to the main Laboratory of Department of Microbiology where DNA was extracted from the serum and PCR was performed for CMV DNA amplification. A total of 47 women were screened, amongst them, 4 (8.5%) were cytomegalovirus (CMV) DNA positive by polymerase chain reaction (PCR) whereas 47 Blood donors were screened, amongst them, 1 (2.1%) were cytomegalovirus (CMV) DNA positive. Prevalence was recorded in age group 21 to 30 years. The detection of HCMV in women before pregnant should reduce miscarriage rate and also the number of congenitally infected infant. Real-time PCR is the best technique and more sensitive and effect than conventional PCR and ELISA.

Keywords

Cytomegalovirus,
Enzyme Linked
Immunosorbent Assay,
Polymerase Chain
Reaction, CMV DNA

Article Info

Accepted:
10 September 2018
Available Online:
10 October 2018

Introduction

Human cytomegalovirus is a species of virus that belongs to the viral family known as Herpesviridae or Herpes viruses. It is typical abbreviated as HCMV and is alternatively known as Human Herpes Virus 5 (HHV-5). Within Herpesviridae, HCMV belong to the Betaherpesvirina subfamily, which also includes Cytomegaloviruses from other mammals (Mocarski and Tan Courcelle,

2001). Human cytomegalovirus (HCMV) is the most common source of congenital malformation resulting from viral intrauterine infection in developed countries (Jahromi *et al.*, 2010).

Cytomegalovirus (CMV) infection during pregnancy can be transmitted to the foetus, resulting in a congenital infection and is a leading cause of hearing loss, vision loss and mental retardation (Soetens, 2008). About

58.9% of individuals aged 6 and above are infected with CMV (Staras, 2006). Women infected for the first time during pregnancy are likely to transmit HCMV to their foetuses. More children suffer serious disabilities caused by congenital HCMV than by several better-known childhood maladies such as Down syndrome or foetal alcohol syndrome. (McCarthy, 2009) Although they may be found throughout the body, CMV infections are frequently associated with the salivary glands (Koichi Yamanishi *et al.*, 2007).

Cytomegalovirus infection is typically unnoticed in healthy people, but can be life threatening for the immunocompromised, such as HIV-infected persons, organ transplant recipients, or new born infants, after infection, HCMV has an ability to remain latent within the body over long periods (Ryan and Ray, 2004). Cytomegalovirus infection is more widespread in developing countries and in communities with lower socioeconomic status and represents the most significant viral cause of birth defects in industrialized countries (Caruso *et al.*, 2009). Transmission can occur during pregnancy or after birth, from breast milk, cord blood, saliva, urine, fomites and other sources, some of infants are infected during delivery (Lazzarotto, 2004).

The development of RT-PCR technology has simplified nucleic acid quantification and is coming into more widespread use. Real-time, quantitative PCR has several advantages over older forms of quantitative PCR assays. Experience to date has reported comparable sensitivity but superior reproducibility and precision compared to previous methods, with a wide dynamic range (Ryncarz *et al.*, 1999).

No information is documented about HCMV or related disease prevalence in the southern districts of Tamilnadu. Hence, the present study was undertaken to determine the prevalence of HCMV infection in Pregnant

women by using Real-Time PCR at a tertiary care hospital.

Materials and Methods

Blood sample collection Whole blood (5ml) was obtained under aseptic conditions from each subject by a vein puncture using a disposable syringe. Blood samples were collected into EDTA tubes. They were placed in a cool box and were then transferred to the laboratory to be kept at -20°C and processed within 24hours.

Molecular detection of HCMV by Real-time PCR

Extraction

After vortexing the sample for 1-2 minutes, add 20µl proteinase K, 200µl sample, 200µAL, and 6µl RGTM IC to a 1.5ml tube and vortex for 30 seconds. 200µl of 100% ethanol was added after incubating at 56°C for 10 minutes. Vortexed at 2000rpm for 30 sec and centrifuged at 8000rpm for 1 minute. Then the sample was transferred to a collection tube with a spin column and centrifuged for 8000rpm for 1 minute. After adding 500µl of AW1 centrifuged at 8000rpm for 1 minute. Change the collection tube and centrifuge 14,000rpm for 3 minutes after adding 500µl of AW2. Change the collection tube and empty spin at 14000rpm for 1 minute. Discard the collection tube place the spin column in 1.5ml PCR tube. After adding 200µl AE and incubate at room temperature for 1 minute then centrifuge at 8000rpm for 1 minute. Discard the spin column the filtrate contain RNA.

Reaction assay

RG marker 25µl and Mg Sol 5µl to NC, Samples and standards were added.

Air bubble was removed by tapping the well and centrifuge briefly at 8000rpm or 2-3 minutes.

Amplification

Done on PCR machine as Set volume 50µ select flurochrome FAM green, VIC

- 1.95°c for 10 minutes
 - 2. 95°c for 15 seconds
 - 3.65° for 30 seconds
 - 4.72°c for 20 seconds. Go to step 2,44cycles.
- Plate read at Step 3.

Results and Discussion

A total of 47 women with Bad obstetric history were screened, amongst them, 4 (8.5%) were CMV DNA positive by PCR whereas 47 Blood donors were screened as controls, amongst them, 1 (2.1%) were CMV DNA positive (Figure 1 and 2). High prevalence was recorded in the age group of 21 to 30 years which was 11.11% (3/27), followed by the age group of >20 years which was 6.6% (1/15) and nil positive reported in the age group of 31-40 years (Table 1). From a total of 47 individuals, 34 (72.34%) showed abortion rates of 1 to 5, while 13 (27.66%) showed no abortion. Out of the 27(57.44%) pregnant women who had abortion once, only one (1.96% of this group) was CMV DNA PCR positive. Out of the 20 (42.56%) women

who had abortion thrice, three (15%) women were CMV-PCR positive. CMV is the largest and most complex member of the Herpes virus family that infects humans (Gouarin *et al.*, 2004), is named so due to the cytopathic effect resulting in enlarged cells having intranuclear and cytoplasmic inclusions (Stagno and Britt, 2006). They can be found in virtually every cell type of humans (Sinzger *et al.*, 1995).

The most common known congenital viral infection is CMV, where in different parts of the world, its incidence has been estimated to be 0.2 to 2.2% of all live births (Fisher *et al.*, 2000). PCR is the method of choice for CMV DNA identification which has been used in various clinical specimens as amniotic fluid, urine, blood and cerebrospinal fluid (Ross *et al.*, 2006). In this study, we used PCR for CMV DNA detection in blood of individuals with age range of 17 to 45 years.

CMV DNA was detected in 8.5% pregnant women. The high prevalence and force of infection indicates that CMV is easily transmitted than some other infections such as measles. In USA, more than 27,000 pregnant women experience primary CMV infection and are thus at high risk of giving birth to a child with congenital CMV infection (Stehel and Sanchez, 2005). Special care and proper vaccination coverage are required to interrupt the spread of CMV (Colugnati *et al.*, 2007).

Reaction assay

	NC	Sample	QS1, QS2, QS3, QS4
20µ l of	Nuclear free water	Corresponding sample	Corresponding standard

Table.1 Age Wise Distribution in relation to PCR

Sl no.	Age in years	Positivity of HCMV PCR
1	< 20	1(6.6 %)
2	21-30	3(11.11%)
3	31-40	0(0%)

Fig.1

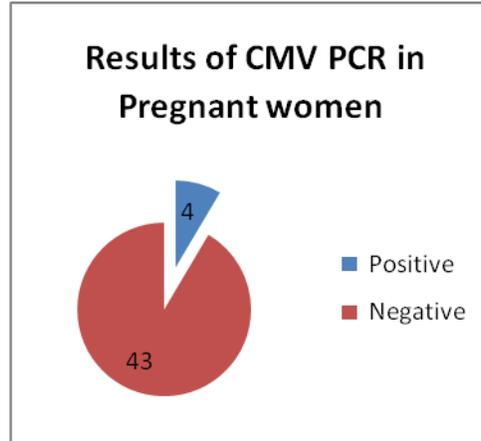
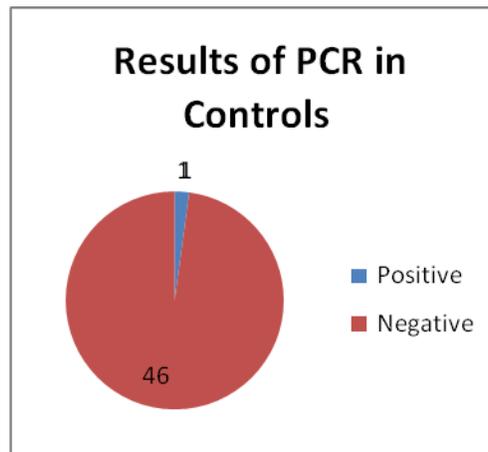


Fig.2



In the present study, High prevalence was recorded in the age group of 21 to 30 years which was 11.11% (3/27), followed by the age group of >20 years which was 6.6% (1/15) and nil positive reported in the age group of 31-40 years. Analyzing CMV infection in different age group suggested that in women that are aged 16 to 45 years, infectivity rate is independent of age (Edmunds *et al.*, 2000). This indicates that CMV transmission may be possible through these routes; same was also addressed by Jahromi *et al.*, (2010). Other sources like non sexual close contact with urine and saliva may also play a major role in CMV spread (Stover *et al.*, 2003). The transmission of CMV can

possibly be prevented by various means (Ventura *et al.*, 2001).

The screening of women before pregnancy will reduce abortion rate and also the number of congenitally infected infants. In this study Real-time PCR was run out on all samples of extracted DNA from both patient and control group belong to women that have no medical history of Hypertension, Diabetes and renal disease. In this study, out of 47 women with Bad obstetric history were screened, 4 (8.5%) were CMV DNA positive by PCR. This was lower than the results of Marwah Yousif and Ismail Hussein showed that 16 out of 70 (22.85%) patients were found to be HCMV

positive while 54 out of 70 (45.65%) patients were found HCMV negative. Real time PCR was rapid, sensitive and useful technique for diagnosis active disease and monitoring response to therapy than conventional PCR and others detection methods

Severe life threatening complications of CMV in pregnant women may not be as rare as previously considered therefore proper diagnosis must be done before pregnancy in order to reduce miscarriage rate and different congenital infant infections. The accurate diagnosis of *Cytomegalovirus* must be done by sensitive molecular methods such as Real Time PCR while ELISA should be used as screening method. Real Time PCR is the best technique and has more sensitive and specific effect than conventional PCR and ELISA.

Acknowledgement

The authors are gratefully acknowledge The Dean, Tirunelveli Medical College Hospital, Tirunelveli, Tamil Nadu and The Staff of Microbiology, Blood Bank and Obstetrics and Gynecology Department of Tirunelveli Medical College Hospital.

References

- Caruso, C, Buffa, S. and Candore, G. (2009). Mechanisms of immunosenescence. *Immun Ageing.*, 6, 10: 4933-6-10.
- Colugnati FAB, Staras SAS, Dollard SC, Cannon MJ (2007). Incidence of cytomegalovirus infection among the general population and pregnant women in the United States. *BMC Infect. Dis.* 7: 71doi:10.1186/1471-2334-7-71.
- Edmunds WJ, Gay NJ, Kretzschmar M, Pebody RG, Wachmann H (2000). The pre-vaccination epidemiology of measles, mumps and rubella in Europe: Implications for modelling studies. *Epidemiol. Infect.*, 125: 635-650.
- Fisher S, Genbacev O, Maidji E (2000). Human cytomegalovirus infection of placental cytotrophoblasts in vitro and in utero: Implications for transmission and pathogenesis. *J. Virol.* 74: 6808–6820.
- Gouarin S, Vabret A, Gault E, Petitjean J, Regeasse A, Hurault de Ligny B, Freymuth F (2004). Quantitative analysis of HCMV DNA load in whole blood of renal transplant patients using real-time PCR assay. *J. Clin. Virol.* 29: 194–201.
- Jahromi, AS, Makiani, M.J, Farjam, MR., Madani, A, Amirian, M, Eftekhri, TE and Hamidipour, S. (2010). Cytomegalovirus Immunity in Pregnancy in South of Iran. *Am. J. Infect. Dis.* 6, 1: 8-12.
- Koichi Yamanishi, Arvin Ann M, Gabriella Campadelli-Fiume; Edward Mocarski, Moore Patrick, Roizman, Bernard and Whitley, Richard (2007). Human herpes viruses: biology, therapy, and immuno prophylaxis. Cambridge, UK: Cambridge University Press. 0-521-82714-0
- Lazzarotto, T, Gabrielli, L, Lanari, M, Guerra, B, Bellucci, T, Sassi, M. and Landini, M.P. (2004). Congenital cytomegalovirus infection: recent advances in the diagnosis of maternal infection. *Hum. Immunol.* 65, 5:410-415.
- Marwah Yousif H and Ismail Hussein. (2015) Detection of Cytomegalovirus in Iraqi Recurrent Miscarriage Women. *World Journal of Pharmacy and Pharmaceutical Sciences.* 5(1):79-89
- McCarthy, FP, Jones, C, Rowlands, S and Giles, M. (2009). Primary and secondary cytomegalovirus in pregnancy. *Obstetr Gynaecol.* 11, 2: 96-100.
- Mocarski, ES and Tan Courcelle C. (2001). Cytomegaloviruses and their

- replication. In *Fields Virology*. 4, 2: 2629–2673.
- Ross DS, Dollard SC, Victor M, Sumartojo E, Cannon MJ (2006). The epidemiology and prevention of congenital cytomegalovirus infection and disease: Activities of the centers for disease control and prevention workgroup. *J. Women's Health*, 15: 224-229. PMID: 16620180.
- Ryan, KJ and Ray, C.G. (2004). *Sherris Medical Microbiology* (4th ed.). McGraw Hill. 556:566–9.
- Ryncarz, AJ, Goddard, J, Wald, A, Huang, ML, Roizman, B. and L. Corey. (1999). Development of a high-throughput quantitative assay for detecting herpes simplex virus DNA in clinical samples. *J. Clin. Microbiol*; 37:1941–1947.
- Sinzger C, Grefte, A, Plachter B (1995). Fibroblasts, epithelial cells, endothelial cells and smooth muscle cells are major targets of human cytomegalovirus infection in lung and gastrointestinal tissues. *J. Gen.Virol.* 76: 741–750.
- Soetens, O, Vauloup-Fellous, C, Foulon, I, Dubreuil, P, Saeger, BD, Grangeot-Keros, L. and Naessens, A. (2008). Evaluation of Different Cytomegalovirus (CMV) DNA PCR Protocols for Analysis of Dried Blood Spots from Consecutive Cases of Neonates with Congenital CMV Infections. *J. Clin. Microbiol.* 46, 3: 943-946.
- Stagno S, Britt W (2006). Cytomegalovirus infections. In: Remington J, Klein J, Wilson C *et al.*, eds. *Infectious Diseases of the Foetus and Newborn Infant*, Sixth Edition. Philadelphia: Elsevier, pp. 739–781.
- Staras, SA, Dollard, SC, Radford, KW, Flanders, WD, Pass, RF and Cannon, M.J. (2006). "Seroprevalence of cytomegalovirus infection in the United States, 1988–1994". *Clin. Infect. Dis.* 43, 9: 1143–51.
- Stehel EK, Sanchez PJ (2005). Cytomegalovirus Infection in the Foetus and Neonate. *NeoReviews*; 6; e38-e45 DOI: 10.1542/neo.6-1-e38.
- Stover CT, Smith DK, Schmid DS, Pellett PE, Stewart JA, Klein RS, Mayer K, Vlahov D, Schuman P, Cannon MJ (2003). Prevalence of risk factors for viral infections among human immunodeficiency virus (HIV)-infected and high-risk HIV-uninfected women. *J. Infect. Dis.* 187: 1388-1396.
- Ventura, SJ, Mosher WD, Curtin SC, Abma JC, Henshaw S (2001). Trends in pregnancy rates for the United States (1976-1997): An update. *Natl. Vital Stat. Rep.* 49(4): 1-9.

How to cite this article:

Revathy, C., G. Velvizhi and Sucilathangam, G. 2018. Prevalence and Detection of Cytomegalovirus by Polymerase Chain Reaction (PCR) in Pregnant Women. *Int.J.Curr.Microbiol.App.Sci.* 7(10): 1160-1165. doi: <https://doi.org/10.20546/ijcmas.2018.710.129>