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Title of the Thesis : Cytomegalovirus induced congenital/ perinatal infection in Neonates; molecular characterization of the virus

Abstract

CMV has emerged as the most important cause of congenital infection, globally in the recent years. Congenital CMV infection may lead to hearing, cognitive and motor impairment. In the US approximately 1% of all neonates excrete CMV, of which 10% are severely affected with a wide range of symptoms. In UK, 400 CMV affected neonates are born annually. 90% of the babies are asymptomatic at birth and almost 10 % develop significant complications such as deafness or neurological problems later. Congenital CMV infection is the leading cause of sensorineural hearing loss in children and leading infectious cause of brain damage. CMV is common in all socioeconomic groups but congenital infection with significant impairment is seen in lower socioeconomic strata. In-utero infection in fetus may be acquired due to primary, recurrent or re-infection with a novel strain of the cytomegalovirus infecting the pregnant mother. Vertical transmission may also occur during delivery due to contaminated secretions of mother or breast milk. Ninety percent of these newborns are asymptomatic but 10-15% may develop several sequelae during infancy.

The present study was undertaken to assess the prevalence of congenital CMV infection in symptomatic infants of Delhi and NCR with the following objectives:

- Diagnosis of congenital CMV infection in babies by detecting IgM antibodies against CMV using μ-capture ELISA.
- Molecular Diagnosis of CMV by amplification of DNA fragments from three gene regions (Immediate early, Glycoprotein B and Polymerase). Comparison between two diagnostic techniques.
- Sequence analysis to explore genetic variations and correlation of the clinical manifestations /outcome of the disease with prevalent genotypes detected during the study.

The present study encompasses the following findings:

- The high incidence of congenital CMV infection (19.4%) was detected among babies born with various birth defects, similar to our previous study and other studies conducted in India and globally
- High rate of seropositivity of CMV-IgG (83-93%) in women of child bearing age as seen in the present study supports the reactivation or re-infection of the CMV virus during pregnancy resulting in birth of symptomatic children as has been documented in many other global studies
- μ -capture, ELISA system based on recombinant technology, used in the present study is more sensitive and specific as compared to the previously used ELISA systems which showed sensitivity around 75-80% when compared with PCR assay

- Multigene molecular diagnostics using nested PCR is a very sensitive technique for detection of viral excretion in CMV infected babies.
- In the present study PCR technique employed was specific but its sensitivity was 95% when compared with ELISA. However the difference between the sensitivities of the two tests is not statistically significant ($p>0.5$)
- RFLP of gB (UL-55) PCR products for genotyping demonstrated prevalence of gB1. gB2 & gB3 genotypes in the babies. gB4 was not found.
- RFLP is a reliable technique for genotyping of gB gene as the results were concordant with sequencing of variable region of gB gene for genotyping which is a gold standard.
- The frequency of gB3 was the most dominant (49.25%) followed by gB1 (24.4%) and gB2 (22.4%).
- gB genotyping among congenital CMV infection is the unique study in India. Studies in immunocompromised adults have documented prevalence of all 4 genotypes and also mixed infection of genotypes
- Various clinical studies have suggested that gB genotypes of HCMV strains may influence the clinical outcome of acquired CMV infections. In the present study, the distribution of gB genotypes and outcome of intrauterine infections was assessed. It was found out that liver disorders were mainly associated with gB3 genotype.
- Babies who had manifestation of hearing impairment and symptoms associated with Central Nervous System had prevalence of genotype gB2 as dominant variant. It has not been reported in any other study done on congenital CMV infection. However it needs further investigation as the samples size was relatively small in this study.
- Sequences from variable region of gB gene (cod 448 to 480) including proteolytic cleavage site were compared to the published sequences of 4 gB genotypes. The result from the sequencing was concordant with RFLP results.
- The derived sequences from the 3 gene regions of CMV and deduced peptide sequences were analyzed for their heterogeneity by comparing the sequences against the prototype strain AD 169. gB gene region demonstrated the maximum variability, especially in vicinity to proteolytic cleavage site (cod460-461), as compared to variability in IE and POL gene fragments.
- Entropy plot analysis and phylogenic analysis demonstrated the similar results.

The present work acquires great significance, as it emphasizes the importance of establishing cost-effective, rapid and informative techniques in India for the diagnosis and typing of human cytomegalovirus, which is an extremely important pathogen in these clinical settings. The results from the study provide molecular epidemiological data for CMV strains circulating among congenital/perinatal CMV infected infants in India (Delhi & NCR). Genetic Variation and its correlation with disease presentation have also been documented in the present study.