CONGENITAL CMV INFECTION; DIAGNOSIS IN SYMPTOMATIC INFANTS

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Abstract

Background: Samples from babies exhibiting clinical symptoms suggestive of congenital infection are referred regularly to NICD, New Delhi, from Government Hospitals located in Delhi and a home for abandoned children (Palna), for the diagnosis of etiological agents like toxoplasma, rubella, CMV and herpes. Blood samples of mothers of most of the affected babies are also received. Objective: Evaluation of rapid and accurate technique for the diagnosis of congenital CMV infection. Materials and Methods: One hundred and twenty five blood samples suggestive of symptomatic congenital CMV infection were selected from samples received at NICD during the period June 2005-March 2007. A request to collect and send the urine samples of the selected babies was sent to the respective hospitals. Serum samples of the babies were tested for CMV-IgM antibodies using μ-capture ELISA. Mothers’ serum samples were subjected to CMV-IgM and IgG class antibodies assay by commercial ELISA kits. DNA isolation and amplification was performed in urine samples and some of the serum samples using a commercial PCR kit for detection of HCMV. Blood and urine samples from 20 normal babies were included in the study. Results: Twenty Seven serum samples (21.6%) of infants, of the 125 tested, were positive for CMV-IgM antibodies. Twenty five samples (20%) showed amplification of CMV –DNA. All 25 samples positive for PCR were positive for CMV IgM antibodies. Sera of 73 mothers, out of 75 tested (97.3%), were positive for CMV IgG antibodies. However, none of them was positive for CMV IgM antibodies. Mothers of all 27 positive babies were positive for CMV-IgG antibodies. Serum and urine samples from 20 normal babies were negative for ELISA and PCR. Conclusion: μ-capture ELISA technique was found to be more sensitive than PCR (92.6%) for detection of congenital CMV infection. ELISA is also rapid, less cumbersome and cost effective for diagnosis of CMV infection.

Key words: Cytomegalovirus, congenital infection, enzyme linked immunosorbant assay, polymerase chain reaction

Introduction

In recent years, Cytomegalovirus (CMV) has emerged as the most important cause of congenital infection globally. Congenital CMV infection may lead to hearing, cognitive, and motor impairment. In the US approximately one per cent of all neonates excrete CMV, of which 10% will be severely affected with a wide range of symptoms.[1-4] 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.[1,2] In India the magnitude of the problem has not been adequately investigated. A few studies have shown the high prevalence of CMV as the etiological agent in babies born with birth defects.[6-8]

The rapid and correct diagnosis of congenital CMV infection in neonates/infants is very important to advocate the right therapy and proper management of the case. ELISA is the most common method employed for detection of CMV specific IgM class antibodies to establish current or congenital CMV infection but lower specificity and the sensitivity of the ELISA systems used previously have been reported in some evaluation studies.[5] CMV afflicted babies are known to shed virus in various bodily secretions especially urine, blood and throat swab, in some cases, for months and years. Detection of cytomegalovirus from clinical samples like urine and blood, by PCR, also provides important information about the excretion of virus in the infected baby and prediction of the symptomatic disease. This study was undertaken to diagnose congenital CMV infection in 125 symptomatic infants referred to NICD from a Delhi-based Government hospitals for detection of congenital CMV infection during the period June 2005 to March 2007. CMV specific IgM class antibodies were assayed by u-capture ELISA and CMV DNA was amplified in Urine and some serum samples of these infants by PCR technique using commercial kit. Samples from 20 healthy normal infants were also included. Samples from the mothers of these infants were tested for CMV-IgM by μ-capture ELISA and IgG antibodies by indirect ELISA. All the serum samples (125) of infants were also screened for Rubella and HSV IgM antibodies by μ-capture ELISA.

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Materials and Methods

During the period of present study 125 blood and urine samples were received from children exhibiting clinical symptoms for congenital infection. Neonates or infants up to the age of six months with manifestation of clinical features associated with neurological defects or liver and/or hematological malfunction were included in the study. Various clinical features in these children reported have been compiled in Table 1. Babies above six months of age, with fever or just jaundice, HIV-positive babies or babies with a history of blood/blood product transfusion were excluded from the study.

Among the babies selected, 12 babies belonged to the age group of newborn to one month, 16 were in the age group of one plus to two months, 51 were from two plus to three months of age, 32 belonged to three plus to four months of age, nine were in age group four plus to five months and five were between five to six months.

The referred samples belonged to a mixed population of urban and rural areas but mostly from lower socio-economic strata.

Serology

Blood samples for serology, received from all the cases, were clotted and centrifuged for serum separation prior to testing. All the sera were stored at -20°C pending testing. The serum samples of babies and mothers were tested for CMV-IgM antibodies using commercially available µ-capture enzyme linked immunosorbant assay method (ELISA kits -RADIM S.p.A Via del Mare, 125-00040 POMEZIA (ROMA)-ITALIA in this study) for qualitative detection. In this test system “Biotin-Streptaviridin Complex” has been used to increase the sensitivity of the procedure. The specificity of the procedure used is also 100% with no non-specific binding due to rheumatoid or other herpatic viruses. Seventy Five sera from mothers of symptomatic babies were also subjected to CMV-IgG antibody testing, using indirect ELISA system (RADIM).

Interpretation of the results was based on controls provided with the kit. A test sample was said to be positive for IgM or IgG antibodies when its absorbance value was higher than the absorbance value of the cut-off control. Positivity of IgM antibodies against CMV in a sample indicates active infection of CMV and presence of IgG antibodies shows the exposure with CMV infection in the past.

125 serum samples from the symptomatic babies were screened for Rubella and HSV IgM antibodies by µ-capture ELISA to detect any congenital Rubella or HSV cases and to check the specificity of the ELISA technique employed for detection of CMV infection.

PCR

About 15-20 ml of urine samples were received in sterile centrifuge tubes from the 125 symptomatic and 20 normal babies. They were centrifuged at 1500 rpm for 20 minutes. Supernatant was discarded and the pallet was washed with 1XPBS, three times. The DNA was extracted from the pallet by adding 100 ul of the extraction reagent provided with the kit. Incubation was done, for 10 minutes, at 100°C. Mixing by vortex for 15 seconds was followed by centrifugation at 8000 rpm for one minute. Supernatant was treated as DNA template. From the serum samples, DNA was isolated using QIAamp Ultrasens Virus Kit. (cat. No. 53705)

PCR was conducted in the isolated samples targeting phosphorylated matrix protein (pp65) gene in CMV virus genome using the commercial kit (Bio-Core CMV PCR kit cat. No.: 11091). Thermal profile, as given in the kit literature, was used. Negative and positive controls given with the kit were used in all PCR runs to check the validity of the test.

Biocore CMV PCR kit has dUTP/UDG system to prevent any carry over contamination.

Positive PCR products were visualized on BIOMETERA gel documentation system (Bio Doc Analyze) as discrete band at 245bp in gel electrophoresis using 1.5% agarose with ethydium bromide staining. The kit instructions were strictly adhered to, while performing both the assays.

Chi square test and Fischer exact tests were used for statistical analysis of the results.
Results

Among clinical manifestations reported in the babies, hepatosplenomegaly [Table 1] was the most common feature. Other frequent symptoms were developmental delay, convulsions, pneumonitis, visual and hearing impairment. Intracranial calcification was also reported in two positive cases.

Twenty seven serum samples, out of 125 tested (21.6%), were positive for CMV IgM antibodies [Table 2] indicating serological evidence of exposure to in-utero/perinatal CMV infection. Twenty Five urine samples, out of 125 (20%), showed amplicon (245 bp) for phosphorylated matrix protein gene of CMV genome in gel electrophoresis [Figure 1], establishing the excretion of CMV virus in urine of the affected babies. No amplification was seen in the control samples.

Mothers of 73 babies (97.3%), including all positive babies, were positive for CMV IgG antibodies and negative for CMV IgM antibodies indicating their past exposure to CMV infection. All the 25 PCR positive samples were also positive for CMV IgM antibodies. None of the sample negative for serology was positive by PCR. [Table 2] Serum and urine samples of asymptomatic and normal babies were all negative for ELISA and PCR tests suggesting that both the techniques were 100% specific. Both the tests were reproducible.

None of the samples positive for CMV were positive for Rubella or HSV-IgM antibodies, supporting the specificity of the µ-capture ELISA.

Discussion

In our retrospective study, samples from most of the babies, in the age group of one to three months, with reported multiple congenital anomalies, were referred to the laboratory, as 90% of babies with congenital CMV are asymptomatic at birth and some of them develop sequelae later.[1,2,4] The presence of CMV specific IgM antibodies and CMV DNA amplification in babies with manifestation of at least two clinical features were termed positive for congenital CMV infection. Any child, up to the age of 12 months, in whom CMV is isolated in urine or other body fluids and/or positive serum IgM is found and in whom clinical features exist that may be due to intrauterine CMV infection is termed as a suspected congenital CMV infection case.

The high incidence of congenital CMV infection detected among babies born with various birth defects is similar to our previous study and other studies conducted in India and globally.[7,8,12] Seropositivity in mothers of positive babies, for CMV IgG antibodies suggest that they were exposed to cytomegalovirus infection, possibly during early pregnancy which could have been primary infection or reactivation of the virus or re-infection with some other strain of CMV. High rate of seropositivity of CMV IgG (80-95) % in the child-bearing age supports the re-activation or re-infection of the virus in pregnancy resulting in birth of symptomatic children.[6,7,9]

ELISA procedure used in our study is more sensitive and specific based on recombinant technology (µ -capture) as compared to the previously used ELISA systems which showed sensitivity around 75-80% when compared with PCR assays.[5] In the present study, PCR technique employed was specific but its sensitivity was 92.6%.

![Figure 1: Amplification of phosphorylated matrix gene of CMV. Positive samples show discrete band at 245bp. Sample 1: positive control. 2: negative control. 3,4 &5: patient's samples and 6: 100bp ladder.](image)

<table>
<thead>
<tr>
<th>Test performed</th>
<th>Subjects</th>
<th>Number of samples tested</th>
<th>No. positive</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>ELISA for CMV-IgM</td>
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<td>125</td>
<td>27</td>
<td>21.6</td>
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<tr>
<td></td>
<td>Mothers of symptomatic babies</td>
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<td>0</td>
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<tr>
<td></td>
<td>Normal babies</td>
<td>20</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>ELISA for CMV-IgG</td>
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<td>75</td>
<td>73</td>
<td>97.3</td>
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<td></td>
<td>Mothers of positive babies</td>
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<td>100</td>
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<tr>
<td>ELISA for HSV-IgM</td>
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<tr>
<td>PCR</td>
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<tr>
<td></td>
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when compared with ELISA. The difference between the sensitivities of the two tests was significant (p<0.05) Our findings about PCR sensitivity are in accordance with some earlier studies.[10,11]

Trials regarding anti-viral treatment of the symptomatic babies with Ganciclovir have shown that the infected babies’ show positive response to the drug.[13] The rapid and correct diagnosis may help the pediatrician carry out the appropriate therapeutic treatment and case management. In our country many symptomatic infants are unreported and thereby remain unscreened for CMV infection and the true impact of the congenital CMV infection still remains under-appreciated. Screening of pregnant women by ELISA could further be emphasized which would help the obstetricians make informed decisions regarding management of the pregnancy complicated by CMV infection.

Conclusion

The µ-capture ELISA is a sensitive and specific technique. Being more cost effective, less cumbersome and less time consuming, it may be used in detection of current/congenital CMV infection in a country with limited resources, like India.

References


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