HUMORAL IMMUNOLOGICAL RESPONSE TO CYTOMEGALOVIRUS IN CONGENITALLY INFECTED NEONATES, INFANT AND REAL-TIME PCR QUANTIFICATION OF HUMAN CYTOMEGALOVIRUS DNA FOR DETECTION VIRAL LOUD IN HILLA CITY, IRAQ

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ABSTRACT

Aim of the Study. The current study was conducted to use the in vivo model of congenital CMV to examine the humoral immune responses in vertically infected neonates and infants as well as estimate CMV DNA viral loud. Methods: Both CMV-specific IgM and IgG were tested by VIDAS. Viral loud was measured for new born babies for test group and for adult as control group, the copy number of immediate early gene was estimated by real time PCR. Samples was taken from Babylon maternity and paediatric hospital from May. 2012 to Jan. 2013. Result: Study showed that all neonates and infant were CMV IgG positive while few cases show both CMV specific IgM and IgG (13%) this because IgM antibodies generally do not cross the placenta during normal pregnancy, very few cases show just IgM(9%). CMV DNA viral load was measured using real time PCR, new born babies show higher CMV DNA than Adult patients this result reflect the important of IgM for viral neutralization. Conclusion: It was very important of checking and screening women in child bearing age to prevent horizontal congenital infection. IgG cannot prevent the CMV infection in neonate and infant, CMV IgM was
detected in few newborn babies, CMV Viral loud in newborn babies was higher than in infected adult can reflect the important of CMV IgM for viral neutralization. Quantitative PCR can establish for assessment of neonate, infant damage and prognosis of infection and CMV RT PCR can use as alternative method for CMV detection.

**Keywords:** congenital, CMV IgM, CMV IgG. CMV, DNA.

**INTRODUCTION**

Many viral infections are associated with significant maternal and fetal consequences during pregnancy among which cytomegalovirus is one of the most important agent, globally. Both primary and recurrent infection due to this virus can result in fetal infection\(^{(1)}\). Cytomegalovirus (CMV) is the most common cause of congenital infection worldwide and occurs as a result of transplacental transmission of the virus. The human neonate is highly susceptible to infection due to a combination of immaturity of the immune system and antigenic inexperience.

Cytomegalovirus (CMV) is a double-stranded DNA virus and is one of the most ubiquitous human pathogens being transmitted vertically and horizontally. Primary CMV infection is always followed by an acute, often asymptomatic infection followed by lifelong latency without clinical illness. Severe symptomatic CMV infection has been shown to occur in immunosuppressed individuals such as in transplant recipients, patients with AIDS and following *in utero* infection. CMV is the most commonly acquired congenital viral infection occurring in about 0.2% to 3% of all live births. Both maternal primary and recurrent infection during pregnancy can result in congenital infection of the infant, but the rate of transmission is far higher for mothers with primary infection. Although most CMV infection are asymptomatic or cause mild disease the virus can cause serious disease in newborns and immunocompromised children. Infants born congenitally infected with CMV as a result of a primary maternal infection also are much more likely to have symptoms at birth and suffer sequelae than newborns born congenitally infected from a maternal recurrent CMV infection. Newborns with a primary immune disorder of cellular function (eg, severe combined immune deficiency or natural killer (NK) cell disorders) may also manifest severe or fatal congenital CMV infection\(^{(2,3)}\). 

*In utero* infection may result in sequelae of varying degree including mental retardation, chorioretinitis, hearing loss and neurologic problems. Since the risk of *in utero* virus
transmission and CMV related damage of the fetus is strongly increased during primary infection, reliable recognition of primary CMV infections is of high importance for pregnant women. Thus, the presence of CMV-specific IgG antibody does not assure protection from disease\(^{(4)}\).

VIDAS is an automated qualitative enzyme immunoassay used as serologic tests that detect CMV antibodies (CMV IgM and CMV IgG).

IgM antibodies generally do not cross the placenta during normal pregnancy presumably because of their large pentameric structure and the lack of a specific transporter mechanism, as for IgG\(^{(5)}\). Real-time PCR, one modulating of quantitative PCR. Is a simple reliable, cost-effective and time saving alternative strategy. Many institute have developed real time PCR assay for monitoring CMV. Real-time PCR provides an accurate means of quantifying viral DNA, with the major advantage of avoiding post-PCR handling that can be the source of DNA carryover, and several studies have reported the utility of this technique for the quantification of HCMV DNA in blood or urine \(^{(6)}\).

The present study use in vivo model of congenital CMV to evaluate humoral immune responses in vertically infected neonates and infants, both IgG and IgM as well as look at CMV DNA viral load with congenitally transmitted CMV.

**MATERIALS AND METHODS**

**Patients’ sera**

Sera were obtained retrospectively from the Laboratory of children and Delivery hospital. 40 new-borns’ sera were included in the study. Sera from ten neonate and infant were tested by RT PCR for detection viral load.

**VIDAS CMV IgG and CMV IgM antibody.**

VIDAS CMV IgG and IgM is an automated quantitative enzyme immunoassay for use on the VIDAS family instruments for the quantitative measurement, of anti-cytomegalovirus IgG (CMVG) in human serum. Using the technique ELFA (Enzyme Linked Florescent Assay). The assay principle combines a two-step enzyme immunoassay sandwich method with a final fluorescent detection (ELFA). Assay protocol fellow the manufacture instruction \(^{(7)}\).
DNA Extraction and purification of viral nucleic acid from serum and CMV Real-Time PCR assay for measuring viral load.

DNA was extracted from 200 µL of plasma using the QIAamp DNA Extraction kit (QIAGEN, Germantown, MD, USA). For each plasma sample, 5.5 µL (1 mg/mL) Carrier RNA and 10 µL CMV internal control were added to 200 µL of AL lysis buffer following the manufacturer’s recommendations. The sample was eluted in 50 µL and 20 µL were used for the assay. CMV viral load testing was carried out using the artus CMV PCR assay according to the manufacturer’s instructions (QIAGEN). The artus CMV™ Master Mix contains reagents and enzymes for the specific amplification of a 105-bp region of the major immediate early antigen. A standard curve was obtained from the quantitation standard (QS) CMV DNA positive controls (CMV TM QS 1-4) provided by the manufacturer (Fig-1). For the PCR amplification, 20 µL of DNA sample elute was added to 30 µL of the working master mix. The amplicons were then detected by measuring fluorescence with the following amplification conditions: 95°C for 10 min, followed by 45 cycles of 95°C for 15 sec and 55°C for 1 min. At the end of the run, the data were analyzed using the RT-PCR Run On Software Version Rotor-Gene 2.0.2.4 (Qiagen).

The artus CMV RG PCR Kit (24 Samples constitutes a ready-to-use system for the detection of CMV DNA using polymerase chain reaction (PCR) on Rotor-Gene Q Instruments. The CMV RG Master contains reagents and enzymes for the specific amplification of a 105 bp region of the CMV genome, and for the direct detection of the specific amplicon in fluorescence channels Cycling Green of the Rotor-Gene Q. "fig.1".

In addition, the artus CMV RG PCR Kit contains a second heterologous Amplification system to identify possible PCR inhibition. This is detected as an internal control (IC) in fluorescence channel Cycling Yellow of the Rotor-Gene Q. The detection limit of the analytical CMV PCR is not reduced. External positive controls (CMV QS 1–4) are supplied which allow the determination of the amount of viral DNA.
Fig-1 shows the Quantitation data for Cycling A. Yellow for the HCMV gene (data analysis was done using Software Version Rotor-Gene 2.0.2.4- Qiagen).

Result (copy/ml) = \( \frac{\text{Result (copy/ml)} \times \text{Effusion Volume (mL)}}{\text{Sample Volume (mL)}} \)

RESULTS
Age range of newborn was 2 day to 9 month. Twenty nine (78%) patients showed the presence of CMV IgG antibodies, 3 (8%) had CMV IgM, and 5 (14%) patients had IgG and IgM antibodies. Table-1 and "Fig.2". Real time PCR was done for 10 neonate and infant CMV positive patients and for three adult patients. Result shows that CMV viral load was significantly higher in neonate and infant patients than in adult patients (p=0.05) "fig.3" and table-2).

Table-1: Humoral immune response in neonate and infants.

<table>
<thead>
<tr>
<th>HumoralAb type</th>
<th>No. of cytomegalovirus seropositive patients</th>
<th>Conc. of IgG* and or IgM** (Iu/ml)</th>
<th>Range of neonate or infant age</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgM</td>
<td>3 (8%)</td>
<td>28±4</td>
<td>Neonate</td>
</tr>
<tr>
<td>IgG</td>
<td>29 (78%)</td>
<td>48.2±15.7</td>
<td>2 day-9 month</td>
</tr>
<tr>
<td>IgG &amp; IgM</td>
<td>5 (14%)</td>
<td>52.8±12.2 1.4±1.05</td>
<td>2.5-3 month</td>
</tr>
<tr>
<td>Control group</td>
<td>5 (12%)</td>
<td>0.3±0.1</td>
<td>1-30 day</td>
</tr>
<tr>
<td>IgM</td>
<td></td>
<td>4±1</td>
<td>1-30 day</td>
</tr>
</tbody>
</table>

*CMV IgG Negative <4U/ml, Equivocal ≥4 <6U/ml, Posative ≥ 6 U/ml.

**CMV IgM Negative <0.7U/ml, Equivocal ≥0.7 and <0.9U/ml, Posative ≥ 0.9 U/ml.
Fig-2 shows the percentage of presence of IgG, IgM and IgG+IgM in neonate and infant patients.

1: IgM
2: IgG
3: IgM+IgG

Table-2 shows the 10<sup>th</sup> of neonates, enfant and three control adult samples explain their age, and the viral loud which measured by quantitative Real time PCR a.

<table>
<thead>
<tr>
<th>Neonates(N)Control(C*)</th>
<th>Age***,***</th>
<th>Calc.Conc (copies/ul)</th>
<th>No.of copy/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>N1</td>
<td>1</td>
<td>30</td>
<td>7.5*10^3</td>
</tr>
<tr>
<td>N2</td>
<td>1</td>
<td>20</td>
<td>5*10^3</td>
</tr>
<tr>
<td>N3</td>
<td>3</td>
<td>10</td>
<td>2.5*10^2</td>
</tr>
<tr>
<td>N4</td>
<td>10</td>
<td>12</td>
<td>3*10^3</td>
</tr>
<tr>
<td>N5</td>
<td>11</td>
<td>7</td>
<td>1.75*10^3</td>
</tr>
<tr>
<td>N6</td>
<td>1</td>
<td>9</td>
<td>2.25*10^3</td>
</tr>
<tr>
<td>N7</td>
<td>2</td>
<td>15</td>
<td>3.75*10^3</td>
</tr>
<tr>
<td>N8</td>
<td>3</td>
<td>10</td>
<td>2.5*10^3</td>
</tr>
<tr>
<td>N9</td>
<td>8</td>
<td>10</td>
<td>2.5*10^3</td>
</tr>
<tr>
<td>N10</td>
<td>3</td>
<td>11</td>
<td>2.75*10^3</td>
</tr>
<tr>
<td>C1</td>
<td>20***</td>
<td>2.1</td>
<td>5.25*10^2</td>
</tr>
<tr>
<td>C2</td>
<td>38***</td>
<td>5.3</td>
<td>1.3*10^3</td>
</tr>
<tr>
<td>C3</td>
<td>20***</td>
<td>4.5</td>
<td>1.125*10^3</td>
</tr>
</tbody>
</table>

* Adult

**Age by weeks

***Age by year

Mean of neonates and infant CMV DNA 3350(STDEV=1886.8)

Mean of adult patient CMV VIRAL LOUD 983.3(STDEV=406.4)

T-test show significant differences 0.05.
DISCUSSION

In the present study, the majority of newborns (78%) show higher anti-CMV IgG. (table-1, fig-2). This result is in agreement with Chen et al., who demonstrate that anti-CMV IgG can efficiently transfer from mother to their fetuses. IgG Transplacental transfer of maternal IgG to the fetal bloodstream is mediated by neonatal Fc receptor in syncytiotrophoblasts of the placenta and contributes to the passive immunity of newborns to pathogens \( ^{(8) } \)

Transplacentally acquired maternal antibodies may protect infants against diseases in the early period of life. In the present study, despite maternal anti-CMV IgG in infants, primary CMV infection occurred early in childhood this combined and or confirmed with high CMV DNA viral load indicating that maternal anti-CMV IgG cannot fully protect against CMV infection. On the other hand, in spite of being infected, the children showed no symptoms related with CMV infection. Thus, maternal anti- CMV IgG in infants may provide substantial protection against symptomatic diseases or sequelae. This is similar to the prophylactic purpose of hepatitis A vaccination among children \( ^{(8) } \).

The present study confirmed the sensitivity of RT PCR for detection viral load other study mentioned that RT PCR as plausible alternative more frequently for detection CMV. These assays, performed by dedicated instruments, carry the advantages of high sensitivity and specificity conferred by the hybridization probe, and the lack of contamination by amplification products, since the reaction tubes are never opened after amplification \( ^{(9) } \).
Our results recorded few cases of CMV IgM positive (8%) and both CMV IgG and CMV IgM positive (14%), this result in according with Gandhoke who recorded the presence of IgM in infant (\textsuperscript{10}).

IgM is a pentameric molecule and therefore proposed cannot able to cross the placenta, so positive result may be as a result of Immune complexes or other immunoglobulin aggregates present in patient samples may cause increased non-specific binding and produce false-positive results therefor I strongly suggest using RT PCR for CMV diagnosis. While other researcher suggest that the presence of IgM in the neonatal circulation at birth is indicative of intrauterine infection. This IgM is thought to be derived locally, although the potential for the presence of maternal IgM in the neonatal circulation in such cases of infection cannot be eliminated totally(\textsuperscript{11}).

Result showed that the viral load in adult was significantly lower than in neonate this may be the neonatal immune system is biased towards a Th2 response generally which is not suited to fighting intracellular pathogens(\textsuperscript{12}).

CONCLUSION
It was very important of checking and screening women in child bearing age to prevent horizontal congenital infection. IgG cannot prevent the CMV infection in neonate and infant, CMV IgM was detected in few newborn babies, CMV Viral load in newborn babies was higher than in infected adult can reflect the important of CMV IgM for viral neutralization. Quantitative PCR can establish for assessment of neonate, infant damage and prognosis of infection and CMV RT PCR can use as alternative method for CMV detection.

ACKNOWLEDGEMENTS
We are extremely thankful to the College of Nursing, Babylon University for providing all the needed facilities, which are essential for successful completion of the present work, I also thankful to the WJPR for fast reviewing and publication.

REFERENCES


