Evaluation of Cytomegalovirus (CMV) IgM level and IgG Avidity in Detection of Primary Infection in Pregnant Women
Mohammed K. Murshid1, Wisam S.Abood1

1. Department of Medical Microbiology, College of Medicine, University of Al-Qadisiyah, Iraq.

*Corresponding author: Mohammed K. Murshid (mk.altharip@gmail.com)

ABSTRACT
Cytomegalovirus (CMV) is a leading healthcare problem associated with stillbirth and congenital abnormalities, determining the current infection along with the possible risk factors associated with cytomegalovirus infections may play a cornerstone role in preventing its complications.

The aim of this study was to evaluate the accuracy of both immunoglobulin M (IgM) and immunoglobulin G avidity (IgGA) in detection of CMV primary infection in pregnant women, to take scientific measures and procedures to reduce intrauterine transmission.

A total of 182 serum samples were collected from pregnant women with different gestational ages, their ages ranged from 16 – 44 years old attended to Al-Suwaira General Hospital, which located 60 km southwest of capital Baghdad. All samples were tested for CMV IgM antibody using Enzyme-Linked Immuno-Sorbent Assay (ELISA), CMV IgG antibody using a semi quantitative automated enzyme-linked fluorescent immunoassay (ELFA) by vidas instrument and cytomegalovirus specific immunoglobulin G avidity by vidas instrument. The result of CMV IgG was 180 (98.9)% , while the positive IgM were 5 (2.74)%. The 5 IgM positive samples and 45 IgG positive were examined with CMV IgG Avidity.

Among 180 sera form pregnant women, 5 showed the presence of Immunoglobulin M antibodies out of which two had equivocal avidity index range. The results showed that an avidity index with the presence of Immunoglobulin M antibodies is highly suggestive of a recent primary infection.

Key words: Cytomegalovirus (CMV); Congenital Cytomegalovirus; CMV IgM; CMV IgG; CMV IgG Avidity

How to cite this article: Murshid MK, Abood WS (2020): Evaluation of Cytomegalovirus (CMV) IgM level and IgG avidity in detection of primary infection in pregnant women, Ann Trop Med & Public Health; 23(S10): SP23104. DOI: http://doi.org/10.36295/ASRO.2020.23104

Introduction
Cytomegalovirus (CMV) is a member of the Betaherpesvirinae subfamily. It is an enveloped virus with a large double stranded DNA genome, the largest of all known human viral pathogens at approximately 235 kilo base pairs (1).

The burden caused by congenital CMV (cCMV) disease reportedly exceeds that caused by other childhood diseases such as Down syndrome, fetal alcohol syndrome and spina bifida, actually CMV causes more cases of congenital disease than the combination of 29 currently screened conditions in most American states (2).

Infants born with cCMV infections can suffer from severe disease and long-term consequences. Approximately 0.5-0.7% of live-birth infants are infected with CMV in-utero, and approximately 10% of infants with cCMV have severe disease manifestations including sensorineural hearing loss, cognitive impairment and retinitis (3).

Cytomegalovirus also causative agent of Spontaneous pregnancy loss which occurs commonly. Many pregnancies fail prior to being clinically recognized and approximately 15-20 % of all clinically recognized pregnancies result in spontaneous loss. Spontaneous pregnancy loss is a frustrating experience and can be physically and emotionally taxing for couples especially when faced with recurrent losses (4).

During primary infection, however CMV also establishes latent infection of long-lived myeloid precursors, which allows lifelong infection of the host and can result in periodic lytic viral reactivation. Carriage of CMV throughout life can thus alternate between two different states, either in a latent state with little to no viral replication, or in episodes of lytic replication during which CMV virions are produced and shed at mucosal surfaces and passed onto new hosts (5).

Although CMV infection is usually asymptomatic and seemingly benign, for immunocompromised individuals this is not the case. With impaired immunity, the balance between immune control of CMV and lytic virion production is disrupted which can lead to viremia and severe end-organ disease (6).

Detection of HCMV specific antibodies is the most common approach used to identify HCMV infected individuals. Many types of assay are available for the determination of the anti HCMV antibody titer in serum with different degree of sensitivity, the most widely used procedure is the ELISA (7), but the presence of CMV specific IgM may not be indicative of primary infection, since it is also produced during reactivation and re-infection (8). Serological diagnosis of primary CMV infection during pregnancy can be difficult as CMV
immunoglobulin M, while suggestive of recent infection, can remain positive for many months and can also represent reactivation of past infection (9).

Detection of increasing HCMV IgG levels over time is an unreliable approach for distinguishing primary from non-primary HCMV infection (10). Another method of determining the timing of maternal CMV infections is to measure antibody avidity, that is moderate cost for low socioeconomic people, its refers to strength of antibody binding to a target antigen. As the immune response to a particular antigen mature over time, avidity increases. The IgG avidity (IgG A) assay can help distinguish primary infection from past or recurrent infection and can assist in determining when infection occurred (11).

MATERIALS AND METHODS

A cross-sectional study included 182 pregnant women with different gestational ages, with and without history of miscarriage, their ages ranged from 16 – 44 years old attended to Al-Suwaira General Hospital province during the period from January 2018 to January 2019. These specimens were stored at -20°C till tested. A detailed questionnaire was filled up for each one of them regarding their birth history and clinical signs and symptoms etc.

CMV IgM

The specimens were test for CMV for qualitative detection of CMV IgM antibodies by commercially available enzyme-linked immune sorbent assay ‘CMVIgMELISA’ kit (Foresight/USA). The assays were performed following the instructions of the manufacturer. According to the information included in the kit’s insert, the immunoassay used has 98.0% sensitivity and 98.3% specificity. The results were calculated by relating each specimen absorbance to index value.

Cut-off value = absorbance of calibrator 2 – Blank absorbance
Index value = Specimen absorbance / cut-off value
Index value > 1.1 : Positive
Index value < 0.9 : Negative.
Index value ≥ 0.9 ≤ 1.1 : Equivocal

CMV IgG (CMVG)

The determination of IgG avidity was carried out using a commercial kit VIDAS CMV IgG (Biormerieux/France). The VIDAS CMV IgG (CMVG) Assay is intended for use on the instruments of the VIDAS family (VitekImmuno Diagnostic Assay System) as a semi-quantitative automated enzyme-linked fluorescent immunoassay (ELFA). It is intended for use in determination of CMV immunological experience from a single serum sample, or as an aid in the diagnosis of current CMV infection through evaluation of paired sera for a significant increase in CMV specific IgG. The corresponding quantity of anti-CMV IgG is calculated and compared to a set of thresholds and a final result is interpreted as in the following table

<table>
<thead>
<tr>
<th>Test Value Thresholds AU/mL</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 4 AU/mL</td>
<td>Negative</td>
</tr>
<tr>
<td>≥ 4 to &lt; 6 AU/mL</td>
<td>Equivocal</td>
</tr>
<tr>
<td>≥ 6 AU/mL</td>
<td>Positive</td>
</tr>
</tbody>
</table>

NOTE: AU = Arbitrary Unit.

VIDAS CMV IgG Avidity II (CMVA)

The determination of IgG avidity was carried out using a commercial kit VIDAS CMV IgG Avidity II (Biormerieux/France). The assay uses the VIDAS CMV IgG kit. Avidity reveals the strength of the link between an antibody and a plurivalent antigen. This avidity is determined by two VIDAS CMV IgG assays: The first assay is the reference. For the second assay, the wash buffer in well 4 of the VIDAS CMV IgG strip is replaced with the urea buffer included in the VIDAS CMV IgG Avidity II kit. The assay principle combines a two-step enzyme immunoassay sandwich method with a final fluorescent detection (ELFA). The Solid Phase Receptor (SPR) serves as the solid phase as well as the pipetting device for the assay. Reagents for the assay are ready-to-use and predisposed in the sealed reagent strips, except the urea buffer.

All of the assay steps are performed automatically by the instrument. The reaction medium is cycled in and out of the SPR several times. If anti-CMV IgG are present in the sample, they form complexes with the antigen coated to the solid phase. In the strip without urea buffer, non-specific antibodies are eliminated by washing, whereas specific antibodies remain coated to the solid phase. In the strip containing urea buffer, washing with the dissociating agent changes antigen-antibody links. Only antibodies with high avidity remain bound to the solid phase, whereas antibodies with low avidity are eliminated.

Alkaline phosphatase-labeled human anti-IgG antibodies (conjugate) are then cycled in and out of the SPR and bind with any human IgG coated on the interior of the SPR. Unbound components are eliminated during the washing steps. During the final detection step, the substrate (4-Methylumbelliferyl phosphate) is cycled in and out of the SPR. The conjugate enzyme catalyses the hydrolysis of this substrate into a fluorescent product (4-Methylumbelliferone) the fluorescence of which is measured at 450 nm.

The intensity of the fluorescence is proportional to the concentration of antibodies present in the sample. At the end of the assay, the results in RFV (Relative Fluorescence Value) for each strip are used to calculate the
avidity index, determined as the RFV ratio obtained for the Test strip (with urea buffer), divided by the RFV obtained with the Reference strip. For each patient sample or control, the avidity index is calculated as follows:

\[
\text{Index} = \frac{\text{RFV Test strip}}{\text{RFV Reference strip}}
\]

### Avidity Interpretation

<table>
<thead>
<tr>
<th>Avidity Index</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>index &lt; 0.40</td>
<td>Low avidity IgG</td>
</tr>
<tr>
<td>0.40 ≤ index &lt; 0.65</td>
<td>Borderline avidity</td>
</tr>
<tr>
<td>index ≥ 0.65</td>
<td>High avidity IgG</td>
</tr>
</tbody>
</table>

### RESULTS

In this study, 182 samples of blood and urine were collected from pregnant women. After that the tests were done which included detection of CMV IgM, CMV IgG, and CMV IgG Avidity in serum.

#### Detection of CMV IgM antibodies

Cytomegalovirus IgM antibodies were detected in 182 of serum samples by ELISA technique, there were 5 samples (2.74%) gave positive, one (0.54%) equivocal, and 176 (96.7%) were negative (table 1-1).

The results commonly were corresponded or slightly difference from other studies in our country, in Diyala 1.6 %, 4.1 % in Kirkuk and in Babylon 4.1% (14).

The slightly difference between this study and others may be due to choice limited group which were just an aborted women, low eco-social conditions and geographic area.

Table (1-1) Result of IgM (Elisa technique) for detection of CMV in pregnant women.

<table>
<thead>
<tr>
<th>Test</th>
<th>Total tested samples</th>
<th>No. (%) of samples results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td>CMV IgM</td>
<td>182</td>
<td>5 (2.74)</td>
</tr>
</tbody>
</table>

#### Detection of CMV IgG antibodies

By ELFA technique (Vidas instrument), among 182 samples there were 180 (98.9%) samples gave positive results and 2 (1.09%) were negative (table 1-2).

The serological tests showed high seroprevalence of CMV IgG among pregnant women. Compared to the results of previous studies in Iraq; it was in Babylon 95.1 % while in Diyala 100 %, also in Kirkuk 98.3 % and in Al-Nas-eriya 97.3% (14).

The higher percentage of CMV IgG seropositivity are indicative of past CMV infection, specifically when IgM was negative, these women as indicated may be immunized and their primary CMV infection was considered to have occurred prior to pregnancy and were mostly asymptomatic personnel. Cytomegalovirus is endemic disease in our population. So the high prevalence proved that CMV is simply transmitted than a some other infections.

Table (1-2) Result of IgG for detection of CMV in pregnant women.

<table>
<thead>
<tr>
<th>Test</th>
<th>Total samples</th>
<th>No. (%) of samples results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td>IgG (Vidas)</td>
<td>182</td>
<td>180 (98.9)</td>
</tr>
</tbody>
</table>

#### Cytomegalovirus IgG Avidity test

After detection of CMV IgM and IgG, the serum samples were tested for CMV IgG avidity. The samples were selected according to highest titer levels, from IgM 25 (five positive, one equivocal and 19 Negative), and also 25 samples from IgG were selected for this test. Among CMV IgM samples, the result showed were no low avidity index (AI) results and two (8 %) samples with equivocal avidity index, while the other 23 (92 %) samples with high avidity index (table 1-3), in contrast among 25 of CMV IgG samples, the all gave high Avidity index (100%) (table 1-4).

In several previous studies, revealed 90% or more of CMV low IgG avidity were CMV IgM positive (18); therefore, away from the result of CMV IgM, the avidity test can used as a diagnostic method to determine if the infection in past or current.

The results of this study were consistent with reports from other parts of the world including Iraq and some neighboring countries, such as study done by Ikuta et al., in 2019 who found among 15 IgM Positive samples, 11 (73.3 %) were high AI and 4 (26.6%) equivocal (7).

The study also agreed with the study which done by Kamelet et al., which showed a high and intermediate CMV IgG avidity index without low IgG avidity index (19), other research done by Neirukh et al., that found among 40 women had IgM positive and were tested for IgG avidity, there are two (5 %) women were in the equivocal AI and the other 38 (95 %) women presented with high IgG AI (20).
Lazzarotto et al., concluded that among 12 women who had IgM positive did not show a low avidity index (21), a study done by Seo et al. showed the avidity test for 12 samples from pregnant women who gave positive results for both CMV IgG and CMV IgM tests, all of them showed high CMV IgG avidity index (22).

In contrast, the results of study was disagreement with a study done by Chakravart et al., in 2007 have found among 18 positive IgM pregnant women, 12 (66.6%) low Al, 2 (11.1%) equivocal Al and 2 (11.1%) high Al (23). Of out 142 woman who their CMV IgM Positive, only 34 (23.9%) gave low Al (24). In 1997, Grangeot-Keros et al., have reported low IgG avidity index in all of the 41 (100%) pregnant women that CMV IgM of them positive (25).

The detection of CMV IgM has a powerful sensitivity but a low specificity for the identification of CMV infections, because the variability in the persistence or appearance of IgM antibody after primary infection (65).positive CMV IgM yielded 20–25% sensitivity, also CMV IgM may persist for 6 – 9 months, even for one or more years following primary infection (26).

The specificity for IgM test in primary infection gave false-positive results, these results maybe come from cross reaction with Epstein-Barr virus (EBV) and Herpes Simplex Virus (HSV) (27). In 2018, Sohn et al. mentioned there are a controversy in those cases as to whether dual positivity of EBV IgM and CMV IgM antibodies represents a co-infection of the two viruses or a false-positive finding due to the cross-reaction of serum antibodies (28). Furthermore many studies have shown poor correlation of results obtained with different commercial kits for IgM testing (7), therefor Ackermann-Gäumann et al. (29) have Saied that requires a test for IgM antibody to specific viral proteins that has been used to enhance the specificity of CMV IgM results.

Accordingly, the conclusions above has decreased the importance of CMV IgM test as a final diagnosis, so should be find confirmed tests as a diagnosis of maternal primary CMV. The data in this study, with combination in the timeline for CMV avidity maturation that mentioned previously, indicates that patients with IgM persistence will have an IgM positive but high CMV avidity outcome, because the persistence of IgM level more than 5 to 6 months after primary infection. Thus, some pregnant women with an IgM positive and high IgG avidity result may actually have non recent primary CMV infection rather than viral reactivation or reinfection.

In any case when the results have indicates to high IgG avidity index, that mean a low risk of vertical CMV transmission. So IgM Positive testing alone don’t enough to classify pregnant women as having increased risk for intrauterine CMV transmission.

Equivocal IgG avidity findings were regarded hard to interpret for risk assessment purposes (30). However, a remarkably coherent finding in the literatures is that 10% to 20% of patients in the at-risk group have equivocal avidity index (CMV IgG and IgM are positive but an unknown time of infection) (31). A group of researchers from Bologna were analyzed the proportion of transmitted mothers infection to their fetus, there is relation with their CMV IgG avidity index, and they found the transmission rates of 30% had the low avidity, 4% equivocal and 2% high avidity index (32). After years, approximately the same results were obtained by Leruez-Ville et al. in 2013 about transmission levels. Thus, those investigators from a counseling standpoint have suggested that an equivocal avidity index was more like a high avidity index result (33).

On other hand, study was done by Lazzarotto et al. 2011 about the correlation between pregnancy trimesters and CMV infection, it was showed there was considering between of it, in second trimester, 23% of CMV IgG and IgM positive, their results for CMV IgG avidity index were equivocal, while in first trimester, only 3% CMV IgG and IgM positive, their results for CMV IgG avidity index were equivocal. Also the pregnant women who that in second trimesters have transmitted the infection to their offspring(26). The authors concluded that equivocal CMV IgG avidity index is a reliable marker of recent primary CMV infection in women initially tested in the second or third trimester. Stated a different way, from the standpoint of counseling, an equivocal avidity index in the first trimester is comparable to a high avidity index, whereas an equivocal avidity index in the second or third trimester is comparable to a low avidity index result.

Table (1-3) Comparison of highest titer level for CMV IgM and IgG Avidity results for diagnosis of CMV from 25 pregnant women.

<table>
<thead>
<tr>
<th>Avidity results</th>
<th>Positive N = 5</th>
<th>Equivocal N = 1</th>
<th>Negative N = 19</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low avidity</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Equivocal</td>
<td>2 (40)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>High avidity</td>
<td>3 (60)</td>
<td>1 (100)</td>
<td>19 (100)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10.72 (HS)</td>
</tr>
</tbody>
</table>

References:
2. Lazzarotto et al., 2019.
5. Sohn et al., 2018.
7. Leruez-Ville et al., 2013.
8. Lazzarotto et al., 2011.

For more information, please visit: [Annals of Tropical Medicine & Public Health](http://doi.org/10.36295/ASRO.2020.23104)
Table (1-4) Comparison of highest titer level for IgG and IgG Avidity results for diagnosis of CMV from 25 pregnant women.

<table>
<thead>
<tr>
<th>Avidity results</th>
<th>No. (%) of samples with high IgG results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive (N = 25)</td>
</tr>
<tr>
<td>Low avidity</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Borderline</td>
<td>0 (0)</td>
</tr>
<tr>
<td>High avidity</td>
<td>25 (100)</td>
</tr>
</tbody>
</table>

**Conclusions**

Congenital CMV is a major cause of disability in children and it is still neglected due to the absence of clinical signs of CMV at birth in the majority of newborn children. Incorporation of the CMV IgG avidity test was beneficial to determine the CMV infectious status with the presence of Immunoglobulin M antibodies is highly suggestive of a recent primary infection. This might possibly reduce the healthcare expenses incurred. Moreover, women with high CMV IgG avidity indices could maintain their pregnancy without the concerns of transmitting CMV infection to their offspring. This study concluded that there was high prevalence rate of human cytomegalovirus infections among pregnant women.

**References**

4. Bendavid, Jesse. 2019. 'Psychological outcomes of those experiencing early pregnancy loss'.
18. Chakravarti, Anita, BineetaKashyap, and AnupriyaWadhw. 2007. 'Relationship of IgG avidity index and IgM levels for the differential diagnosis of primary to recurrent cytomegalovirus infections', Iranian Journal of Allergy, Asthma and Immunology: 197-201.
23. Sohn, Min Ji, Jin Min Cho, Jin Soon Moon, Jae Sung Ko, and Hye Ran Yang. 2018. 'EBV VCA IgM and cytomegalovirus IgM dual positivity is a false positive finding related to age and hepatic involvement of primary Epstein–Barr virus infection in children', Medicine, 97.
25. Delforge, Marie-Luce, JoëlleEykmans, Deborah Steensels, Elena Costa, Catherine Donner, and Isabel Montesinos. 2019. 'Combination of line immunoassays MikrogenrecomLine CMV IgG and recomLine CMV IgG Avidity helps to date the onset of CMV primary infection', Diagnostic microbiology and infectious disease, 93: 208-12.

http://doi.org/10.36295/ASRO.2020.23104