Serological ELISA Test (IgM & IgG) for Prospective Study of Cytomegalovirus (CMV) Infection in Pregnant Women

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Abstract
Background: Cytomegalovirus (CMV) infection is associated with significant maternal and fetal consequences. The aim of present study was to determine the current prenatal CMV seroprevalence in Eastern Azerbaijan and evaluate the routine laboratory diagnostic techniques of anti-CMV immunoglobulin M (IgM) and immunoglobulin G (IgG).

Methods: During the present prospective cross-sectional study, 125 women referred to No. 1 Laboratory of Specialized Clinics of Tabriz University of Medical Sciences and seeking prenatal care were evaluated during 2003-2006. CMV IgG and IgM antibodies were determined with ELISA technique. Statistical analyses were performed using the SPSS statistical package version 13.0.

Results: Eighty four percent of the subjects were seropositive. Out of 20 subjects with primary seronegativity, 12 (9.6%) remained seronegative during reexaminations and follow up, but eight (6.4%) subjects showed primary infection in the second to third trimesters of gestation. In two (1.6%) of these eight subjects, IgM was persisted for more than 20 months.

Conclusion: Results showed a similar seroprevalence of CMV in Eastern Azerbaijan. Also, we found that ELISA IgM test was not an appropriate method for differentiation of past or recent CMV infections especially in the pregnant women.

Keywords: Cytomegalovirus, Congenital Disorders, Maternal-infection, Iran

Introduction
Cytomegalovirus (CMV) is a double-stranded herpes DNA virus that is transmitted by contact with infected blood, saliva, urine, or sexual contact. Vertical transmission of CMV may occur because of transplacental infection after primary or recurrent CMV infection, exposure to contaminated genital tract secretions at delivery, or breastfeeding. Primary CMV infection occurs in 0.7-4.1% of pregnancies and its transmission rate varies between 24 to 75%, averaging 40% (1). CMV established latent infections in the host that may reactivated, which results in recurrent infection and fetal transmission occurs in 0.5-1% of recurring cases (2-4). In general, prenatal infections have more severe fetal consequences. Infection in the first trimester may disrupt organogenesis and in second/third trimesters can cause neurological impairment or growth restriction. Congenital CMV infection may cause hepatomegaly, chorioretinitis, and sensorineural hearing loss in the newborns (5). So, identification of susceptible women is essential so that early treatment can be offered. Although routine qualitative anti-CMV IgM and IgG ELISA methods show seropositivity/seronegativity in patients, the kinetics of anti-CMV antibodies responses and low avidity of IgG to antigen during early primary infection vary greatly among individuals, depending substantially to the test or commercial kit (6-8). In addition, the small number of cases of CMV infection diagnosed prenatally, the consequent limited experience with prenatal diagnosis, and difficulty in determining which women should be enrolled in prenatal diagnostic programs is another important problem hampers this diagnostic aspect of CMV infection (9,10).

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The aim of present study was to determine the current prenatal CMV seroprevalence in Eastern Azerbaijan and evaluate the routine laboratory diagnostic techniques of anti-CMV immunoglobulin M (IgM) and immunoglobulin G (IgG).

**Materials and Methods**

In this prospective cross-sectional study, 125 women in childbearing age were evaluated during 2003 to May 2006. Subjects decided for pregnancy, sought prenatal care, and referred to the No. 1 Laboratory of Specialized Clinics of Tabriz University of Medical Sciences for determining CMV infection. Subjects aged between 20 and 35 yr, 80% of them were from Tabriz and 20% from other cities of East Azerbaijan Province including Ahar, Sarab, Azarshahr, Oskou. Serum samples were taken on their first attendance and examined. All participants gave informed consent, and the Ethic Committee at Tabriz University of Medical Sciences (TUMS) reviewed and approved the study protocol, which complied with the Helsinki Declaration.

At first time of attendance, the sera samples were collected and stored in -70 °C until analysis at the end of every week. Regarding the primary serologic test, subjects were divided into seropositive and seronegative groups. If the subject was seronegative, she was underwent reexamination and followed up with 2 weeks intervals until the end of gestation and delivery.

The evaluation of anti-CMV IgM and IgG was carried out with a commercial ELISA kit (Radim, Rome, Italy). The last serum sample of IgG negative subjects was analyzed for IgG to detect seroconversion. In interpretation of IgG tests, the optical density (OD) of each negative control and cut-off calibrator (10RU/ml) must be considered. The presence or absence of CMV IgG antibodies was determined in comparing of the sample absorbance with the absorbance of the cut-off calibrator. In subjects with negative IgM and IgG, the last serum sample, which was obtained during study, was assessed for IgM to detect seroconversion. In interpretation of IgM test, the OD of negative and positive controls and cut-off were considered.

Statistical analyses were performed using the SPSS statistical package version 13.0 (SPSS Inc, Chicago, Ill, USA). The results are presented as mean±standard deviation (SD). Chi square and fisher exact tests were used to assess the differences. A P value less than .05 was considered significant.

**Results**

Eighty four percent of the subjects were CMV seropositive (Table 1). Out of 20 subjects with primary CMV seronegativity, 12 (9.6%) subjects remained seronegative during reexaminations and follow up, but eight (6.4%) subjects showed primary infection in the second to third trimesters of gestation. In two (1.6%) of these eight subjects, IgM was persisted for more than 20 months. Also, there was an indirect relationship between socioeconomic classes, and CMV IgG seropositivity (Table 2). Frequency of CMV IgG seropositivity was higher among subjects with lower socioeconomic classes (P= 0.002).

<table>
<thead>
<tr>
<th>Groups</th>
<th>IgG + IgM -</th>
<th>IgG + IgM +</th>
<th>IgG - IgM -</th>
<th>IgG - IgM +</th>
<th>Total n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seropositive</td>
<td>95</td>
<td>10</td>
<td>-</td>
<td>-</td>
<td>105 (84)</td>
</tr>
<tr>
<td>Seronegative</td>
<td>-</td>
<td>-</td>
<td>12</td>
<td>-</td>
<td>12 (9.6)</td>
</tr>
<tr>
<td>Primary infection</td>
<td>-</td>
<td>8</td>
<td>-</td>
<td>-</td>
<td>8 (6.4)</td>
</tr>
</tbody>
</table>
Table 2: Seroprevalence of CMV IgG among socio-economic classes

<table>
<thead>
<tr>
<th>Socioeconomic class</th>
<th>Seropositive (%)</th>
<th>Seronegative (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High income</td>
<td>68</td>
<td>32</td>
</tr>
<tr>
<td>Middle income</td>
<td>75</td>
<td>25</td>
</tr>
<tr>
<td>Low income</td>
<td>93</td>
<td>7</td>
</tr>
</tbody>
</table>

Discussion
In the present study, 84% of women were seropositive and the rate of seropositivity was higher in subjects with lower socioeconomic condition. The seroprevalence of CMV antibodies in the present study was in consistent with reported seroprevalence (80%-85%) in Iranian population (11-16).

Considering high seroprevalence of CMV in childbearing women during this study, we need to carry out a national study to determine prevalence of maternal and congenital CMV infections in Iranian society and design a proper plane for preventing CMV consequent sequels. Furthermore, we have found 20 (16%) seronegative cases, which were susceptible to primary CMV infection. These women should be counseled to practice good hygiene, such as avoiding direct contact with organic secretions and frequent hand washing. This is especially important if they routinely come into contact with young children, as they often excrete virus for months in the absence of clinical signs. Eight (40%) of seronegative subjects have shown seroconversion or primary infection. Therefore, anti-CMV IgG should be tested at least twice during pregnancy (at 2nd and 4th months). If seronegativity persists, it is better that subjects follow up. Also, if seroconversion is detected, a diagnosis of primary CMV infection is established and prenatal diagnosis should be offered to the mothers. The kinetics of anti-CMV IgM responses during primary infection may vary greatly among individuals depending substantially to the test or commercial kit (6, 7). However, we have used a high qualified anti-CMV IgM ELISA kit which was a qualitative procedure. For exactly determination of acute or recently acquired CMV infection, we must use a quantitative Anti-CMV IgM kit which determines increase or decrease of anti-CMV IgM levels. Regarding to these descriptions, qualitative IgM ELISA kit could not clarify the cause of long persistence of IgM. The best way for differentiation of active IgM cases from non-active/false positive IgM cases is PCR and virus detection (17-20). In addition, the most reliable serological procedure to identify primary infection is the determination of IgG avidity (8). During the early weeks of primary infection, antibodies show a low avidity for the antigen and using IgG avidity determines low functional affinity of the IgG class antibody (8).

In conclusion, there is a similar seroprevalence for CMV in Eastern Azerbaijan. Also, IgM qualitative ELISA technique could not be a suitable technique for diagnosis in different serologic situation, or useful interpretation tool for laboratory findings. Therefore, it is better to provide methods with higher diagnostic value such as quantitative ELISA and IgG avidity techniques in our laboratory.

Acknowledgments
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References


