The Seroprevalence of IgM Among Iraqi Aborted Women Infected with Human Cytomegalovirus

Maysara S.Khalf, Dhammra W.Ahmad, Khalida A.Ibraheem

ABSTRACT:
BACKGROUND:
Fetal loss and abortion are responsible for significant emotional distress for couples desiring children. There are many documents which support the role of some certain asymptomatic infections such as Cytomegalovirus (CMV) in spontaneous abortion.

OBJECTIVE:
This study was aimed to evaluate the prevalence of seropositivity of specific IgM antibody for CMV by ELISA in the sera of (108) women with recurrent abortion associated with obstetric complications.

METHODS:
This study was carried out in two central hospital in Baghdad (Al-Elwea& Baghdad teaching hospitals ) during (2009-2010). A number of (108) women with definite diagnosis of previous abortion ,35women with recurrent abortion , 30 women with premature delivery and 43women with intra uterine death .compered to (50)women with history of normal full term delivery and negative history of miscarriage as control groups .Serum samples were collected and then tested by using ELISA for detection of anti-CMV IgM antibodies.

RESULTS:
Cytomegalovirus (CMV) specific IgM antibody was detected in (15.7%) of the 108 women studied ,while the women with obstetric complications were positive for CMV specific IgM antibody these comprised of(16.6%)with premature delivery , (14%) with recurrent abortion and (16.2%) with intra uterine death .these result statistically significant (P<0.05) .Our data faild to found asignificant association between the Cytomegalovirus infection with age and residence of patients (p>0.01).

CONCLUSION:
Higher seropositivity for cytomegalovirus (CMV) in women with spontaneous abortion compared to women with normal obstetric history suggests that cytomegalovirus Plays a significant role in abortion

KEY WORDS: cytomegalovirus ,fetus death ,congenital infection .

INTRODUCTION:
Human Cytomegalovirus (CMV) is the most common cause of congenital malformation in developed countries its clinical manifestations range from asymptomatic infection to severe fetal damage (1). Up to 15% of intrauterine CMV infections result in symptomatic congenital disease at birth and 10 to 15% of those born with asymptomatic congenital CMV will develop significant clinical squale in infancy (2).
The presence of CMV-specific Immunoglobulin M (IgM) may not be indicative of primary infection, since it is also produced during reactivation and re infection (3). Some researchers showed significant relation between CMV infection and spontaneous abortion (4). There are also evidences which suggest that CMV will lead to complicated pregnancies (5). It has been reported that the risk of fetal damage is greater if the primary infection occurs during the first trimester of pregnancy (6).
The prevalence of congenital infection ranges from 0.2% to 2.5% in different populations (7), in which the risk factors include particular races or ethnic groups, a low socioeconomic status, premature birth, and admission to an intensive care unit (8).
In India, serological surveys have shown the prevalence of CMV antibodies in adult population to be about 80-90%(3,4). However, the data regarding the occurrence of CMV infection in pregnant population is sparse. The aim of this study was; to determine the seropositivity of CMV IgM infection in studied group, in relation to their age and residence of patients(9).
As far as prevention is concerned, in addition to health education campaigns, the serological
screening of pregnant women has been proposed. However, there is no consensus in the scientific community concerning the implementation of screening\(^{10}\).

In Iraq, studies have revealed that the majority of women of childbearing age are seropositive for CMV and that they contract the infection either through prenatal or postnatal transmission during early childhood\(^{11}\). Sexual transmission and blood transfusion are other sources of infection.

Primary CMV infection has been found to be more prevalent in pregnant women than non-pregnant women. This difference may be attributed to the susceptibility of seronegative women at the onset of pregnancy to the first CMV infection\(^{12}\).

Transmission of CMV infection to the fetus has been identified in all trimesters of pregnancy. Abortion can result from ascending CMV endometritis and the virus has been isolated from post-abortion uterine discharge\(^{13}\).

**MATERIAL AND METHODS:**

During the period between January 2009 to May 2010, (108) women in Gynecology and Obstetric department of Al-Elweya hospital and Baghdad hospital were subjected for the detection of CMV specific IgM antibody by ELISA test. These included (108) women with previous history of recurrent abortions, intrauterine death (IUD), and premature deliveries. Compared with 50 women with normal delivery and negative IgM for CMV as control group, 5 mls of blood samples were collected from all patients during clinical illness and control group for detection of CMV IgM by enzyme linked immunosorbent assay (ELISA). Serum separation was done by centrifuging of whole blood samples at 2000xg for 20 min, and the serum samples were kept with -4C°.

The ELIZA technique was performed using kits intended for estimating concentration of specific CMV-IgM Markers. Cytomegalovirus IgM (CMV IgM) ELISA Kit (Cat# 1202Z: REF=1201Z: cod# 9-D3-022).

Company Sigma /USA. The ELISA technique was performed according to the instruction of the manufacturer.

Principle of Enzyme Linked Immuno sorbent Assay (ELIZA) for cytomegalovirus IgM:

- Wells of microtiter plate were coated with recombinant antigen representing epitops of CMV.
- Serum samples were added to the wells. If antibodies specific for CMV are present in the sample they will form stable complex with CMV antigen.
- After washing peroxides was added.
- If the Ag-Ab complex is present, the conjugate will bind to the complex.
- After a second wash an enzyme substrate solution containing chromogen was added. A blue color were developed that turend to yellow after H2SO4 addition as a blocking solution. The intensity of it is proportional to CMV-IgM antibodies concentration in the sample.

Wells containing negative samples remained unchanged.

**Data analysis:**

For the assessment of risk factors for CMV infection, characteristics of case patients and control group were examined using a two-sample student t-test. Cross-tabulation and chi-square were used to examine the relationship between variables using a 95% confidence interval as a measure of association.

**RESULTS:**

The age of the patients were range from 20-30 years with mean age (25.3±3SD) in the pregnant women with abortion, the mean gestational age was 8 weeks. There is no significant difference between CMV infection and age (p=0.69).

According to prevalence of seropositivity for CMV antibodies, about (15.7%) of the subjects were divided in recurrent abortion, premature delivery and intra uterine death.

The results of serological assay we can divided in three groups:

- **Group 1:** women suffering from premature delivery:
  - In table (1): out of (30/108) suffering from premature delivery, only (5/30) were positive IgM to CMV (16.6%), while (25/30) were negative IgM to CMV (83.3%).

- **Group 2:** women suffering from Recurrent abortion:
  - In table (2): out of (35/108) suffering from recurrent abortion, only (5/35) were positive IgM to CMV (14.3%), while (30/35) were negative to CMV (85.7%).

- **Group 3:** women suffering from intra uterine death:
  - Out of (43/108) women suffering from intra uterine death, only (7/43) were positive to IgM to CMV (16.3%), while (36/43) were negative to IgM to CMV.
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Our results showed the positive IgM (17/108) from rural areas (41.2%) while,10/17 from urban areas (58.8%),more over 55/108 (60.4%)negative IgM from rural and 36/108 (39.6%)from urban areas. these results showed no significant association between residence of patients and infections with virus,as shown in table (5).

Table 1: Relation between CMV infection with premature delivery according to age of patients.

<table>
<thead>
<tr>
<th>Age group/ Year</th>
<th>No.of patients with premature delivery</th>
<th>+ve IgM to CMV No.</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(20-25)</td>
<td>15</td>
<td>4</td>
<td>13.3%</td>
</tr>
<tr>
<td>(26-30)</td>
<td>10</td>
<td>1</td>
<td>3.3%</td>
</tr>
<tr>
<td>(31-35)</td>
<td>3</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>(36-40)</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>5</td>
<td>16.6%</td>
</tr>
</tbody>
</table>

P-Value =0.847.

Table 2: Relation between CMV infection with Recurrent abortion according to the age of the patients.

<table>
<thead>
<tr>
<th>Age group/ Year</th>
<th>No.of patients with recurrent Abortion</th>
<th>+ve IgM to CMV No.</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(20-25)</td>
<td>20</td>
<td>3</td>
<td>8.57%</td>
</tr>
<tr>
<td>(26-30)</td>
<td>11</td>
<td>2</td>
<td>5.71%</td>
</tr>
<tr>
<td>(31-35)</td>
<td>3</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>(36-40)</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>35</td>
<td>5</td>
<td>14%</td>
</tr>
</tbody>
</table>

P-Value =0.696.

Table 3: Relation between CMV infection with Intra uterine death according to the age of the patients.

<table>
<thead>
<tr>
<th>Age group/year</th>
<th>No.of patients with intra uterine death</th>
<th>+ve IgM CMV No.</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(20-25)</td>
<td>20</td>
<td>4</td>
<td>9.3 %</td>
</tr>
<tr>
<td>(26-30)</td>
<td>18</td>
<td>2</td>
<td>4.6 %</td>
</tr>
<tr>
<td>(31-35)</td>
<td>4</td>
<td>1</td>
<td>2.3 %</td>
</tr>
<tr>
<td>(36-40)</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>43</td>
<td>7</td>
<td>16.2%</td>
</tr>
</tbody>
</table>

P-Value =0.808 .

Table 4: ELIZA positivity for CMV IgM antibody in different group.

<table>
<thead>
<tr>
<th>Group</th>
<th>No.of sample tested</th>
<th>ELIZA positivity CMV No.</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recurrent abortion</td>
<td>35</td>
<td>5</td>
<td>14%</td>
</tr>
<tr>
<td>Intrauterine death</td>
<td>43</td>
<td>7</td>
<td>16.2%</td>
</tr>
<tr>
<td>Premature delivery</td>
<td>30</td>
<td>5</td>
<td>16.6%</td>
</tr>
<tr>
<td>Total</td>
<td>108</td>
<td>17</td>
<td>15.7%</td>
</tr>
</tbody>
</table>

P – Value = 0.085.
DISCUSSION:

CMV is the most common congenital infection with its incidence has been estimated between 0.2-2.2% of all live births in different parts of the world[14]. In 1994, a study conducted in Malaysia involving 1688 infants with congenital CMV infection and it was detected in 11.4% of the infants, which was much higher than other intrauterine infections like congenital toxoplasmosis (1%) and congenital rubella infection (2.7%).[15] Primary CMV infection in an individual can be detected by demonstration of CMV specific IgM antibody.[16] However, infected infants can be asymptomatic at birth with 10-15% of these subsequently developing late sequelae such as visual & auditory defects.[17]

Congenital intrauterine infections have been associated with congenital abnormalities, intrauterine growth retardation and intrauterine death of the fetus, as well as late sequelae such as developmental delay, blindness and deafness of the infected child.[18] Cytomegalovirus (CMV) infection during pregnancy is more complex than other infections, due to virus reactivation during the child bearing age and be transmitted to the fetus in spite of maternal immunity.[19]

Risk factors for CMV infection have been correlated with the socioeconomic status within a community.[20,21] Other studies showed that elderly persons seem to be well protected against symptomatic CMV disease due to accumulation of CD28 effector cytotoxic T lymphocytes. This is a characteristic feature of all age groups but is most pronounced in elderly persons.[22] As a consequence of placental infection, HCMV impairs cytotrophoblast differentiation and invasiveness; this could explain early abortion occurring in women with primary infection. In addition, HCMV infection impairs cytotrophoblast expression of HLA-G, thus activating the maternal immune response against the cytotrophoblast subpopulation expressing this molecule.[23]

Further more, Failure of the systemic and uterine vasculature to adapt during pregnancy leads to several complications, including preeclampsia and intrauterine growth retardation (IUGR). [24] CMV is a virus that can affect the fetal organs throughout the whole pregnancy. The damage seems to be more severe in infections occurring during the first half of the pregnancy, while infections in the second half would result in reduced morbidity.[25] Various ways of transmitting the virus to the fetus have been suggested, whereas the hematogenous spreading across the placenta with subsequent infection of placental and amniotic tissue seems to be the most common transmission way.[26] In the present study, CMV specific IgM antibody was detected in (15.7%) of all pregnant women tested indicating the substantial prevalence of infection in local population.

In table (1): out of (30/108) suffering from premature delivery, only (5/30) were positive IgM to CMV (16.6%), while (25/30) were negative IgM to CMV (83.3%). And out of (35/108) women suffering from recurrent abortion, only (5/35) were positive IgM to CMV (14.3%), while (30/35) were negative to CMV (85.7%). As in table (2). Further more, Out of (43/108) women suffering from intrauterine death, only (7/43) were positive to IgM to CMV (16.3%), while (83.7%) were negative to IgM to CMV. In addition, the ELISA result seropositivity of all the cases mentioned before, showed their is a significant association between recurrent abortion, premature delivery and intrauterine death and cytomegalovirus infection.

A hypothesis for the probable role of geographical influence upon CMV seroprevalence might be the route of infection. In rural areas saliva is probably the main route through which the virus is transmitted postnatally. This is likely to be the route through which the virus is transmitted early in life amongst infants and young children due to poor sanitation.[27] On the other hand in urban areas sexual transmission seems to be the major route of infection later in life during childbearing age.
In addition, our result showed that there is no correlation between CMV infections with the residence of patients. Its statistically no significant association (p>0.01) between the rural and the urban residence like.

Another factor that may contribute in human CMV infection prevalence is geographical distribution (28,29,30). Unlike previous studies data showed that CMV IgM seroprevalence had no significant correlation with geographical location, even if CMV seroprevalence had higher value in rural as compared to those of urban areas. On the other hand primary infections rate was higher through out urban areas (31).

CMV infection is endemic in Iraq in (2002); the prevalence rates of human cytomegalovirus IgM and IgG in non pregnant women have been reported to be 1% and 84% respectively, and 2.5% and 90% in pregnant women (32).

In the present study, CMV specific IgM antibody was detected in (15.7%) of all pregnant women tested indicating the substantial prevalence of infection in local population. Our result is lower than the result reported by alwam (2011); (46.6%), this different may be due to small sample size (33).

Our result was similar to result (15.98%) of Rubina et al (2004). In our study the prevalence of seropositivity for CMV was lower than western Europe, America and Australia (Munro et al., 2005). In India, the serological surveys have shown the prevalence of CMV antibodies in adult population to be about 80-90% (34).

However, our result is higher than the result of arti kapil et al that reported CMV specific IgM antibody in (12.9%) of pregnant women with complication (35). All these findings indicate that CMV infection is not uncommon in our local population. This high seroprevalence reflects the low hygienic standards and practices in our part. Also CMV can lead to substantial damage to the fetus as the damage done in utero cannot be reverted. Control of intrauterine CMV infection CMV infection is important. Hence prevention of CMV infection, especially in the pregnant women is essential by screening of pregnant women, although the measures cannot change the out come of the disease but may be useful in altering the physician for possible infection to the baby. Hence routine screening of females of child bearing age for CMV infection is desired in order to reduce the fatal out come of the pregnancy occurring due to the CMV infection.

**CONCLUSION:**
We can diagnose high risk pregnancy even with serological tests in areas with insufficient equipment. We recommend pregnant women should be attentive of disease prevention guidelines on personal hygiene during pregnancy, especially hand washing after handling diapers or oral secretions. But we recommend high risk pregnant women for example mothers that working in day care center or health care worker should be screened for CMV serological test during pregnancy. All pregnant women especially with bad obstetric history should be screened for the presence of CMV infections as well as screening against other maternal infection to exclude any congenital infection such as (TORCH) is mandatory.

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ABORTED WOMEN WITH HUMAN CYTOMEGALOVIRUS


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