Serofrequency Rate of Cytomegalovirus Infection among Sudanese Aborted Women at Ibrahim Malik Teaching Hospital (Khartoum)

Alaa M. Ibrahim, Abbas B. Mohammed, Wafa I. Elhag

Al Neelain University, Khartoum, Sudan

Abstract

Background: Cytomegalovirus (CMV) is a member of the family Herpesviridae and it is the most common cause of congenital infection and pregnancy loss. Also it is the common cause of spontaneous abortion, leading to three or more consecutive miscarriages. It occurs following different etiological causes including viral infections. Most of the spontaneous miscarriages occur in the first and second trimesters of pregnancy.

Objective: To estimate the serofrequency rate of Cytomegalovirus infection among Sudanese aborted women at Ibrahim Malik Teaching Hospital (Khartoum).

Materials and methods: This was a descriptive, case-control study carried out at Ibrahim Malik Teaching Hospital (Khartoum). It was conducted during the period from December to January 2016. A total of 90 women were investigated. These patients were divided into two groups: 45 women (test group) with past history of abortion, and the other 45 women (control group) without past history of abortion. CMV IgG and IgM antibodies were tested for in serum specimens using the enzyme-linked immunosorbent assay (ELISA). Generated data were analyzed using the statistical package for social sciences (SPSS) program.

Results: The overall seropositivity of anti-CMV IgG was found more frequent (40/88.9%) among women with past history of abortion (test group) than among those (38/84.4%) without past history of abortion (control group). This difference was insignificant (p = 0.27). Regarding the seropositivity of anti-CMV IgM, only one positive case (2.2%) was found among women with past history of abortion (test group) and none was detected among those without past history of abortion (control group). This difference was insignificant (p = 0.17). Seropositivity rate for both anti-CMV IgM-IgG was (2/4.4%); while seronegativity rate for both antibodies was (7/15.6%) among the control group. Highest anti-CMV IgG seropositivity rate (15/33.3%) was found among

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women who had a past history of one abortion \( (p = 0.2) \). Also the highest anti-CMV IgG seropositivity rate \( (20/44.4\%) \) was found among patients aged 19-30 years, and during the second trimester of pregnancy \( (p = 0.1) \). Statistical analysis showed an insignificant correlation between anti-CMV IgM and IgG seropositivity rate and past history of abortion among test and control groups.

**Conclusion:** Although the serofrequency rate of anti-CMV IgG and IgM was higher among women with past history of abortions and associated with gestational trimesters, yet this difference was statistically insignificant.

**Key words:** Serofrequency rate, Cytomegalovirus antibodies, Aborted women, ELISA.

**Introduction**

Infection with human cytomegalovirus (CMV), a member of the Herpesviridae family, is very common world-wide with seropositivity rates ranging from 40% in developed countries up to 100% in developing countries. It can be transmitted vertically in utero. Following primary (40–50%) or recurrence (1%) CMV infection in a pregnant woman or perinatally from genital secretion, breast milk, or postnatally through infected saliva, sexual transmission, blood and transplanted organs. Clinical manifestations are various and symptomatic disease is rare among immunocompetent hosts\(^1\). Severe scenarios occur when the immunocompetent host is critically ill and also in immunocompromised hosts. The epidemiology and pathogenesis of infection with CMV is at least 60% of the USA population, with a prevalence rate of more than 90% in the high risk groups (e.g. homosexual males, diabetics, chronic disease patients, chemotherapy patients, and newborns). Infection by the virus can cause congenital diseases, including: microcephaly, rash, brain calcification, CNS defects and hepatosplenomegaly\(^2\).

Furthermore, 10 to 15% of the children who are asymptomatic at birth may develop late sequelae especially hearing defects, after a period of months or even years and the risk of fetal infection is greatest with maternal primary CMV infection and much less likely with recurrent infection because the virus remains latent in the host cell after initial infection. Some studies found high presence of CMV antigens in tissues from abortion, and others reported higher seropositivity\(^3\).

In Sudan, a study was conducted at Khartoum Teaching Hospital and reported the seroprevalence rate among recurrent abortion pregnant women as 55.3% and 3.2% for CMV IgG and IgM antibodies respectively\(^4\).

A recent study conducted at Omdurman Maternity Hospital reported a serofrequency rate of CMV among pregnant ladies as 74.4% for CMV IgG and 14.4% for CMV IgM\(^5\).

In Iran the seropositivity rate among women with recurrent abortion and control was 2.3% for CMV IgM antibody in each group and 90.6% and 69.8% for IgG in patients and controls\(^6\).

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The aim of this study was to determine the serofrequency rate of anti-CMV IgG and IgM using the ELISA technique. This study also aimed to correlate the seropositivity rate of CMV antibodies with other parameters such as age, number of miscarriages, and pregnancy trimesters.

Materials and methods

This was a descriptive, case control study conducted among Sudanese aborted women attending Ibrahim Malik Teaching Hospital (Khartoum) during the period from December to January 2016. The collected data were analyzed using the Statistical Package of Social Science (SPSS) program. The serofrequency rates of the anti-CMV IgM and IgG related to age, frequency of abortions, and period of abortion were analyzed by the chi-square and p-value was calculated. Approval to conduct the study was given by Al Neelain University Research Ethical Board. Permission to collect and investigate the specimens was granted by the administration of Ibrahim Malik Teaching Hospital. Verbal consent of the patients investigated was obtained.

Data were collected using a structural questionnaire covering demographical and clinical data, e.g. age, frequency of abortion, period of abortion, and gestational age. Sampling technique employed in this study was a non-probability, convenience sampling type. The sample size was 90 aborted women. These patients were divided into two groups: 45 women (test group) with past history of abortion, and the other 45 women (control group) without past history of abortion. 3 ml venous whole blood were collected from each woman under aseptic conditions in a sterile plain container after disinfection of the skin by 70% alcohol. Serum separation was done by centrifuging at 3000 rpm for 5 minutes and then stored at -20°C. CMV IgG and IgM antibodies were tested for in serum specimens using the enzyme-linked immunosorbent assay (ELISA). ELISA technique was performed using qualitative kits to detect specific CMV IgM antibody in human serum (Fortress Diagnostic, USA); and qualitative kits to detect specific CMV IgG antibody in human serum (ACON Laboratories, USA). Techniques employed were according to the manufacturer’s instructions.

Procedure: All reagents and samples were handled at room temperature. Both anti-IgG and IgM were detected with the same method. The working wash buffer was prepared by diluting the concentrated wash buffer 1:25. Well (A1) is the blank. 100 µl of controls (negative, positive), calibrators, and specimen diluents were added into the appropriate wells. 5 µl of the specimen were added to the kit starting from well (G1), mixed gently on a flat bench for 30 seconds and the micro well plate was covered with the plate sealer to prevent evaporation and incubated at 37°C for 30 minutes. After that the fluid from all wells was removed. Then the microtiter wells were washed 5 times by automatic washer using 350 µl of working wash buffer in each well. After washing, the micro-well plate was turned upside down on an absorbent tissue for few seconds to ensure complete washing and dryness of microtiter wells. Then the conjugate was dispensed to each well, except the (A1) blank well. The plate was again incubated for 30 min. Then (as done in

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The first wash) the enzyme conjugate was washed and removed from wells. 50 µl of substrate (A) was dispensed to each well followed by 50 µl of substrate (B). All was mixed gently, then the micro-well plate was covered with the plate sealer and incubated at 37°C for 10 minutes. Next, 50 µl of the stop solution were added to each well. Then the yellow color had developed in the wells containing positive specimens. The absorbance of specimens was measured by a photometer at 450/630 NM within 30 minutes after adding the stop solution.

**Calculation of anti-CMV IgM results:** This was performed to obtain the cutoff value using the equation:

\[
\text{Cutoff value} = \frac{\text{Mean absorbance of cutoff calibrator} - \text{Blank absorbance}}{2}
\]

**Interpretation of results of anti-CMV IgM:**

a) EIA index less than 0.9 is a negative result

b) EIA greater than 1.1 is a positive result.

**Calculation of anti-CMV IgG results:** This was performed to obtain the cutoff value using the equation:

\[
\text{Cutoff value} = \frac{\text{Mean absorbance of cutoff calibrator} \times 2 - \text{Blank absorbance}}{2}
\]

**Interpretation of results of anti-CMV IgG:**

a) EIA index less than 0.9 is a negative result

b) EIA greater than 1.1 is a positive result.

**Results**

The frequency rate of abortions varied in test group patients. Anti-CMV IgG was found higher (15/33.3%) among women who had a past history of one abortion (Table 1).

<table>
<thead>
<tr>
<th>Abortions frequency Rate</th>
<th>Positive IgM</th>
<th>Positive IgG</th>
<th>Positive IgM + IgG</th>
<th>Negative IgM + IgG</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Once</td>
<td>0</td>
<td>15 (33.3%)</td>
<td>0</td>
<td>0</td>
<td>15 (33.3%)</td>
</tr>
<tr>
<td>Twice</td>
<td>1 (2.2%)</td>
<td>14 (31.1%)</td>
<td>2 (4.4%)</td>
<td>2 (4.4%)</td>
<td>19 (42.2%)</td>
</tr>
<tr>
<td>Thrice</td>
<td>0</td>
<td>11 (24.4%)</td>
<td>0</td>
<td>0</td>
<td>11 (24.4%)</td>
</tr>
<tr>
<td>Total</td>
<td>1 (2.2%)</td>
<td>40 (88.9%)</td>
<td>2 (4.4%)</td>
<td>2 (4.4%)</td>
<td>45 (100%)</td>
</tr>
</tbody>
</table>

(p = 0.2)

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45 women with past history abortion (test group) and 45 women without history of abortion (control group) were included in this study to detect CMV antibodies. 40 cases (88.9%) were found positive for anti-CMV IgG among test group patients; and 38 cases (84.4%) were found positive for anti-CMV IgG among control group patients. Only one case (2.2%) was found positive for anti-CMV IgM among test group patients. From all patients investigated, 2 cases (4.4%) were found positive for both anti-CMV IgG and IgM; and 2 cases (4.4%) were found negative. Among the control group none was found positive for anti-CMV IgG or IgM; whereas 7 cases (15.6%) were negative.

As regard age incidence, the highest anti-CMV IgG seropositivity rate (26/57.8%) was found among patients aged 19-30 years. Also the highest seropositivity rate (23/51.1%) was found among women with more abortions in the second trimester. Statistical analysis showed an insignificant correlation between anti-CMV seropositivity rate and past history of abortion among women in test and control groups (p = 0.17 for anti-CMV IgM) and (p = 0.27 for anti-CMV IgG).

Table (2) shows the serofrequency rate of CMV antibodies among study population according to gestational trimesters.

<table>
<thead>
<tr>
<th>Trimesters</th>
<th>Positive IgM</th>
<th>Positive IgG</th>
<th>Positive IgM + IgG</th>
<th>Negative IgM + IgG</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st Trimester</td>
<td>1 (2.2%)</td>
<td>4 (8.8%)</td>
<td>0</td>
<td>0</td>
<td>5 (11.1%)</td>
</tr>
<tr>
<td>2nd Trimester</td>
<td>0</td>
<td>20 (44.4%)</td>
<td>2 (4.4%)</td>
<td>1 (2.2%)</td>
<td>23 (51.1%)</td>
</tr>
<tr>
<td>3rd Trimester</td>
<td>0</td>
<td>16 (35.5%)</td>
<td>0</td>
<td>1 (2.2%)</td>
<td>17 (37.8%)</td>
</tr>
<tr>
<td>Total</td>
<td>1 (2.2%)</td>
<td>40 (88.9%)</td>
<td>2 (4.4%)</td>
<td>2 (4.4%)</td>
<td>45 (100%)</td>
</tr>
</tbody>
</table>

(p = 0.1)

Discussion

CMV is the most common maternal infection worldwide. Its incidence has been estimated to be 0.2-2.2% of all live births in different parts of the world. This study aimed to detect the serofrequency rate of CMV antibodies among women with a past history of abortion. The present study revealed a high seroprevalence rate of CMV antibodies among women with a past history of abortion (test group) than among women without past history of abortion (control group). Anti-CMV IgG was found in 88.9% of the test group patients. Anti-CMV IgM was found in 2.2% of the positive cases and 2.2% of cases were found positive for both anti-CMV IgG and IgM. Seronegativity rate was detected in only 2.2% of cases. Among the control group patients, only 84.4% were positive for anti-CMV IgG. Also positive anti-CMV IgM was not detected among the control group cases; while 15.6% of both anti-CMV IgG and IgM were found negative.

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CMV is a widespread and common virus that can infect almost anyone. After CMV infection, the IgG antibody remains in the body for life and protects considerably against future infections. The serofrequency rate of anti-CMV IgG and IgM observed in this study was almost similar to the findings reported in western Sudan where the prevalence rate of anti-CMV IgG was 72.2% and that of anti-CMV IgM was 2.5%.

Similarly, Kafi et al. (2009) reported a frequency rate of 95%. The picture of CMV prevalence rate in different countries was also almost similar to our results. In Kashmir, Rubina et al. (2004) reported that 16.37% of women with recurrent abortion were found positive to anti-CMV IgM. Also, in Iraq Maysra et al. (2012) reported that 14% of women suffering from recurrent abortion showed positive anti-CMV IgM.

At the age range 19-30 and 31-45 years, positive anti-CMV IgG was 57.8% and 31.1% among test group patients; and it was 48.9% and 35.5% among control group patients respectively. While positive anti-CMV IgM was (1/2.2%) among test group patients at the age range 19-30 years and negative among control group patients. These findings were higher than the findings reported by Abdul Wahab et al. (2012) who found a seropositivity rate (16.7%) of anti-CMV at the age 20 years, and (21.7%) at the age range 40-45 years.

In another study, women aged more than 30 years showed a higher frequency rate of abortion. In the present context, the serofrequency rate of abortions associated with anti-CMV IgG was found higher (15/33.3%) among patients who had one abortion; while the serofrequency rate of abortions associated with anti-CMV IgM was found only one case (2.2%) aborting twice. This finding differed from that reported by Yeshwondm et al in Addis Ababa (2015) who found a frequency rate of abortions associated with anti-CMV IgG as (33/18.6%) among patients who had one abortion and anti-CMV IgM was (3/9.7%) among patients who had two abortions or more.

Furthermore, in this study the gestational age was found highly associated (20/44.4%) with anti-CMV IgG among patients during the second trimester; while anti-CMV IgM frequency rate was only one abortion case (2.2%) during the first trimester. There was no significant difference observed between the gestational trimesters (p > 0.05). Also, in this study there was no significant difference observed between age, abortion frequency rate, gestational trimesters and anti-CMV among women with past history of abortion in test and control groups (p > 0.05).

Conclusion: Although the serofrequency rate of anti-CMV IgG and IgM was higher among women with past history of abortions and associated with gestational trimesters, yet this difference was statistically insignificant.

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