

Immunological Study of Cytomegalovirus Infection in Women with Recurrent Fetal Loss in Ramadi City, West of Iraq

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Abstract

Objective: The aim of this study was to detect the role of anti-cytomegalovirus IgM and IgG antibodies in the diagnosis of CMV infection in women with recurrent fetal loss.

Patients and Methods: Eighty-seven sera were obtained from women suffering from recurrent fetal loss admitted to Maternity and Children Hospital in Ramadi during the period from September 2007 to June 2008. Anti-Cytomegalovirus IgM and IgG antibodies were detected by ELISA Technique. The positive cases were tested again two weeks later to prevent seroprevalence conversion.

Results: Out of the 87 asymptomatic pregnant women (33.3%) tested positive for the CMV specific IgM antibody and 28.5% positive for IgG antibody revealing primary infection during the first trimester and increasing the possibility of transmission of infection in uterus to the fetus.

Conclusion: The study concluded that screening of pregnant women, although, cannot change the outcome of the disease but may be useful in alerting 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 outcome of the pregnancy occurring due to the CMV infection.

Keywords: Anti-CMV IgG, Anti-CMV IgM, recurrent Fetal loss.

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Introduction

It is well known that congenital intrauterine infection has 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 child ⁽¹⁾. Cytomegalovirus (CMV) infection during pregnancy is far more complex than other infections, due to the ability of the virus to be frequently reactivated during the child bearing age and be transmitted to the fetus in spite of maternal immunity ⁽²⁾.

Cytomegalovirus is a virus that is transmitted through many body fluids. It is usually spread during casual contact, and it can also be transmitted sexually; it is present in both semen and cervical secretions. It can also be transmitted during blood transfusion and organ transplants ⁽³⁾. Cytomegalovirus (CMV) is one of the most common causes of congenital abnormalities in developed countries with reported incidences varying between 0.15% and 2.0% ^(4, 5). The effects of congenital CMV infection may vary from a congenital syndrome to an asymptomatic course. Infants that were asymptomatic at birth may still remain handicapped at a later age. It was generally accepted that symptoms of congenitally infected children were more severe after primary infection than after recurrent infection.

This study has been undertaken to detect the role of anti-CMV IgM and IgG antibodies in the diagnosis of CMV infection in women with recurrent fetal loss.

Patients and Methods

Eighty-seven sera were obtained from women suffering from recurrent fetal loss admitted to Maternity and Children Hospital in Ramadi during the period from September 2007 to June 2008. Anti-cytomegalovirus IgM and IgG antibodies were detected by ELISA technique (Biocheck, Inc., Germany) ⁽⁶⁾.

The positive cases were tested again two weeks later to prevent seroprevalence conversion. A four fold increase in the antibodies level indicates and confirms the activity of infection.

Five ml of serum sample was collected from all patients and centrifuged at 3000 rpm for 15 minutes.

Methods

The procedure was performed according to those mentioned by (Voller, 1985⁽⁷⁾ Cremer, 1985⁽⁸⁾).

1-The desired number of coated wells was placed into the holder.

2-1:40 dilution of test samples were prepared, Negative Control, Positive Control, and Calibrator by adding 5 µl of the sample to 200 µl of sample Diluent. Mix well.

3-100 µl of diluted sera were dispensed, Calibrator, and Controls into the appropriate wells. For the reagent blank, dispense 100 µl Sample Diluent in 1A well position. Tap the holder to remove air bubbles from the liquid and mix well and incubate at 37 °C for 30 minutes.

4-At the end of the incubation period, remove liquid from all wells. Rinse and flick the microtiter wells 4 times with diluted wash Buffer (1x) and then once with distilled water.

5-100 µl of Enzyme conjugate to each well was dispensed. Mix gently for 10 seconds and incubate at 37 °C for 30 minutes.

6- Enzyme Conjugate from all wells was removed. Rinse and flick the microtiter wells 4 times with diluted Wash Buffer (1x) and then once with distilled water .
 7- At 37 °C for 30 minutes was incubated.
 8- Enzyme Conjugate from all wells were removed . Rinse and flick the microtiter wells 4 times with diluted Wash Buffer (1x) and then one time with distilled water.
 9-100µl of TMB reagent into each well were dispensed . Mix gently for 10 seconds and incubate at 37 °C for 15 minutes.
 10-At 37 °C for 15 minutes was incubated.
 11-100µl of Stop Solution (1N HCL) was added to stop reaction.
 12- Gently for 30 seconds were mixed. It is important to make sure that whole the blue color changes to yellow color completely .
 13-The O .D. at 450 nm within 15 minutes was measured with Stat Fax 3200 ELISA reader .

Calculation of Results

1-The mean of duplicate calibrator value X_c was calculated .
 2-The mean of duplicate positive control (X_p) , negative control (X_n) and patient samples (X_s) were calculated .
 3-The CMV IgM or IgG index of each determination by dividing the mean values of each sample (X) by calibrator mean value , X_c were calculated.

Results

Out of 87 patients suffering from fetal loss, 29 (33.3%) were diagnosed as Cytomegalovirus positive cases depending on the positive ELISA test for both of anti-CMV IgG and IgM.

The mean age of the patients was 24.3 years; the mean gestation was 17.0 weeks. To determine the influence of age, the patients were divided into the two age groups; those less than or equal to 23 years and those more

than 23 years. However, seropositivity with regard to age was not found to be statistically significant ($p = 0.66$). Seropositivity of CMV IgM antibody was seen in asymptomatic pregnant. Out of the 87 asymptomatic pregnant women (33.3%) tested positive for the CMV specific IgM antibody and 28.5% positive for IgG antibody revealing primary infection during the first trimester and increasing the possibility of transmission of infection from uterus to the fetus.

Discussion

CMV was the most common congenital infection and its incidence has been estimated to be between 0.2-2.2% of living births in different parts of the world ⁽⁹⁾. Primary CMV infection in an individual could be detected by demonstration of CMV specific IgM antibody ⁽¹⁰⁾ . Also primary infection in pregnancy women has a higher incidence of symptomatic congenital infections and fetal loss ⁽¹¹⁾ . However, infected infants could be asymptomatic at birth with 10-15% of these subsequently developing late sequelae such as visual and auditory defects ⁽¹²⁾ .

The public health impact of congenital CMV infection was largely due to its ability to damage the central nervous system , including the auditory system. Although few population-based studies of the etiology of hearing loss in infants have been performed, such studies have included assays for congenital CMV infection, which are sensorineural hearing loss in children.

CMV could be transmitted across the placenta in women who are CMV seropositive before pregnancy . Although mother-to-fetus transmission of CMV does occur in women with preexisting immunity , previous studies have suggested that the incidence of sequelae in children born to

women with non-primary infection (women with pre-existing immunity) is significantly lower than those infants born following primary maternal CMV infection⁽³⁾. These observations led to the strategy that an effective vaccine for the prevention of primary CMV infection during pregnancy could prevent or significantly reduce the neurologic damage from congenital CMV infection. However, more recent studies, demonstrated that symptomatic congenital CMV infection is also seen following a non-primary maternal infection. In addition, congenitally infected children born to immune mothers have been shown to be at significant risk for an adverse outcome.

With advances in CMV serology, the presence of anti CMV-specific immunoglobulin-M(IgM) detected by a screening test such as the enzyme linked immunosorbent assay (ELISA), can be confirmed by immunoblot, identifying pregnant women undergoing an active or recent infection. Furthermore, primary infections observed or suspected due to the presence of CMV IgM in mothers' blood, can now be readily diagnosed by disclosing the presence of anti-CMV low avidity immunoglobulin-G (IgG) antibody in IgM-positive mothers, greatly reducing the number of women who should be considered at risk of transmitting the virus to the fetus. Of great significance is the finding that only 30% of primarily infected mothers transmit the virus. Identifying these women is a major diagnostic problem that has been solved by moving to in utero diagnosis⁽¹³⁾.

Kapil and Broor⁽¹²⁾ have reported CMV specific IgM antibody in 12.9% of pregnant women with complications. Also, CMV specific IgM antibody was detected in 15.98% of all pregnant women tested in a study published by (Lone and others, 2004)⁽¹³⁾, indicating the substantial prevalence of infection in the local population while in the present study, 18.27% obstetric patients

showed the CMV specific IgM antibodies. 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 and as the damage done in utero cannot be reverted, control of intrauterine CMV infection is important. Hence prevention of CMV infection, especially in the pregnant women is essential. Screening of pregnant women, although, cannot change the outcome of the disease but may be useful in alerting 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 outcome of the pregnancy occurring due to the CMV infection.

In the present study, CMV specific IgM antibody was detected in 29 of 87 pregnant women tested, indicating the substantial prevalence of infection in the local population. Out of the 87, asymptomatic pregnant women (33.3%) tested positive for the CMV specific IgM antibody and 28.5% positive for IgG antibody revealing primary infection during the first trimester and increasing the possibility of transmission of infection in uterus to the fetus.

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