Original
ISSN 2413-0516

Prevalence of Cytomegalovirus Infection Among Pregnant Women with Cord Blood Examination

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(Submitted: 01 October 2022 – Revised version received: 20 November 2022 – Accepted: 18 December 2022 – Published online: 26 February 2023)

Abstract

Objectives: This study aimed at determining the prevalence of CMV infection among pregnant women at the end of pregnancy and CMV transmission to their newborns.

Methods: This is cross sectional study, 213 pregnant women at delivery and their newborn babies from the Obstetrics and Gynecology Hospital in Duhok/Iraq were enrolled. A questionnaire was prepared to be answered by participants, including age, place of residence, educational level, and obstetric history as number of births, any bad obstetric history such as abortion, still birth, intrauterine growth retardation, congenital anomalies after birth. 3–5 ml of blood was drawn from each woman and examined by ELISA kit to check for the presence of Anti CMV IgM, IgG, then IgG avidity test for those with positive (IgG and IgM). Samples of cord blood were collected from newborns after birth and checked for the presence of CMV IgM by ELISA and CMV–DNA by conventional PCR using specific primers to diagnose congenital infection and determine the rate of viral transmission from infected women.

Results: Serological examinations showed that 212 (99.5%) participants were CMV-IgG positive, 15 (7%) were positive for anti-CMV IgM and IgG antibodies, IgG avidity test for 15 women were of high avidity (>89%) which indicated non primary infections. Cord blood of newborns of those 15 women with positive IgG and IgM tested negative for Anti CMV IgM by ELISA and no CMV-DNA was detected by PCR, which revealed no transmission from those pregnant to their newborns.

Conclusion: This study demonstrated high prevalence of CMV among examined pregnant women in Duhok city which makes them prone to non-primary infection. IgG avidity test is of high efficacy to interpret the detection of IgG and IgM together in pregnant women. Cord blood examination for the existence of CMV-IgM and CMV-DNA after delivery could exclude congenital infection.

Keywords: Cytomegalovirus infections, cord blood, fetal blood, polymerase chain reaction, ELISA, IgG avidity

Introduction

Cytomegalovirus (CMV) belongs to the beta herpesvirus subfamily of herpesviruses, double stranded DNA enveloped virus. Infects 60–90% of the population worldwide, typically asymptomatic infection.[1-3] According to studies and reports the infection of Congenital cytomegalovirus (CMV) is considered one of the most common types of infections that occur inside the uterine and is considered the most common in newborns, with infection rates recorded among them from 0.4 to 2.3% all over the world.[4,5] The transmission of the Congenital cytomegalovirus (CMV) from the infected mother to the fetus is either due to primary infection or recurrent infection of the mother during pregnancy and it is believed that about 85 to 90% of children who have congenital primary infection are without signs or symptoms, but the proportion of 5 to 15% of these patients, they may show some after-effects in life.[6,7] It is possible to perform some diagnostic tests for CMV when women are at the end of their pregnancy, as this procedure and these tests help to treat the case of early diagnosis and early intervention for the newborn, especially those who do not show symptoms where they are at risk of many diseases, including sensorineural hearing loss.[8-10]

The Role of IgM, IgG and Avidity Test

A negative cytomegalovirus (CMV) IgM result suggests that the patient is not experiencing acute or active infection. However, a negative result does not rule-out primary CMV infection.

It has been reported that CMV-specific IgM antibodies were not detectable in 10% to 30% of cord blood sera from infants demonstrating infection in the first week of life. In addition, up to 23% (3/13) of pregnant women with primary CMV infection did not demonstrate detectable CMV IgM responses within 8 weeks post infection. In cases of primary infection where the time of seroconversion is not well defined as high as 28% (10/36) of pregnant women did not demonstrate CMV IgM antibody. Positive CMV IgM results indicate a recent infection (primary, reactivation, or reinfection). IgM antibody responses in secondary (reactivation) CMV infections have been demonstrated in some CMV mononucleosis patients, in a few pregnant women, and in renal and cardiac transplant patients. Levels of antibody may be lower in transplant patients with secondary rather than primary infections.[11]

IgG antibodies are produced several weeks after the initial CMV infection. IgG levels rise during the active infection, then stabilize as the CMV infection resolves and the virus becomes inactive.[12,13]

Avidity is defined as the aggregate strength with which a mixture of polyclonal IgG molecules binds to multiple antigenic epitopes of proteins.[14] It gradually matures over several months, reflective of antigen-driven selection of B cells producing IgG of increasing affinity. IgG antibodies produced during the first few months following primary infection exhibit low avidity (i.e., they bind weakly to the antigen), whereas antibodies produced by 6 months post infection exhibit high avidity (i.e., they bind tightly to the antigen).[15] The basic methodology used to measure avidity capitalizes on

the weak binding of low-avidity IgG to a mixture of CMV antigens (typically viral lysate). Antigen-bound low-avidity IgG, but not high-avidity IgG, dissociates from the antigen in the presence of mild protein denaturants, such as urea, potassium thiocyanate, and guanidine chloride. The most common test format is an enzyme-linked immunosorbent assay (ELISA) utilizing urea as the dissociating agent.[16,17]

PCR for CMV DNA can be either qualitative or quantitative, in which the amount of viral DNA in the respective sample is measured. The threshold of the qualitative method needs to be carefully calibrated for preventing over-detection. The quantitative PCR (Real-Time PCR) allows for continuous monitoring of immunocompromised individuals to identify patients at risk for CMV disease for preemptive therapy and to determine response to treatment.[18] This method is generally more expensive compared to the antigenemia assay, but it is rapid and can be automated. Results are usually reported as number of copies/ml of blood or plasma.

This study was conducted in the city of Duhok/Iraq, in order to know the prevalence of CMV infection among pregnant women, where it is based on analysis and comparison of umbilical cord blood serum and blood test of the pregnant mother at the end of pregnancy.

Ethics Statement

Ethical approval was issued by the Ethics Committee of the Duhok General Directorate of Health with a number (15092021-9-9), on 15, September, 2021. Then, informed consent has been obtained from the participants. Permission was also obtained from the Ministry of Health and the Ethics Committee and approvals related to the College of Health Sciences at the University of Duhok.

Design

Cross sectional study.

Subjects and Methods

Two hundred and thirteen pregnant women at delivery participated in this study, blood samples were taken from them in the delivery room of Duhok Hospital for Obstetrics and Gynecology. Consent was obtained from the women from whom samples were collected before delivery and during delivery from the umbilical cord. The ages of the participants in the study ranged from 19 to 36 years old, mean = 25.6 year, (SD ± 5.29) years. A questionnaire was prepared and answered by participants in this study, including: age, place of residence, educational level, and obstetric history as number of births, any bad obstetric history such as abortion, still birth, intrauterine growth retardation, congenital anomalies after birth so to classify them into those with bad obstetric history and normal one.

All the participating women gave normal delivery, not by caesarean section. The samples that were taken are placed in a gel tube and classified into two types, the first before birth from the pregnant mother, where they were labeled with the letter (A, A1, A2), and the second that was taken from the umbilical cord labeled with the letter (B, B1, B2). Immediately after that, these samples were taken to the laboratory for centrifugation at 3000 rpm for 5 minutes then serum was collected and kept at a temperature of –20°C till processing. After completing the required number of samples, samples with the letter A (Mother's samples before delivery) were tested for anti-CMV IgG and IgM antibodies. Samples that revealed positive results for both anti CMV IgG and IgM together were subjected to third test which is IgG avidity test, since this test is considered as cold standard test to differentiate between recent and old infection, in other words to know whether infection has occurred before or during pregnancy. Selected umbilical cord samples (from women with positive Anti CMV IgG and Anti CMV IgM together) were examined for the presence of CMV-IgM and CMV–DNA by conventional PCR using specific primers to diagnose congenital infection and determine the rate of viral transmission from infected women.

Serological Assays

Anti-CMV IgG and IgM antibodies were determined using a CMV IgG enzyme immunoassay test kit and a CMV IgM enzyme immunoassay test kit (DIA. PRO Diagnostic Bioprobes (Milano – Italy). Serology was performed according to the manufacturer's instructions and by reading the optical density for negative control, positive control and cut-off calibrators.

Samples with positive Anti CMV IgG and IgM were tested by IgG avidity test ELISA kit (Bioactiva / Novatec - Germany-ACMV7110 Avidity Cytomegalovirus (CMV) IgG. The result of <40% index is considered as low avidity while >65 is of high avidity index.)

DNA Extraction, Primers and PCR Assays

AddPrep Viral Nucleic Acid Extraction Kit, was utilized to extract viral DNA, amplification done by nested PCR according to the manufacture's instruction using specific primers as shown below:

PCR	Primers	Sequence (5′-3′)	Product Size
Conventional n-PCR (glycoprotein B Gene)			
	Outer-1	ACAGACACAAACAGCACCCA	450 bp
	Outer-2	TAAGGTGACGACAGGTTGGC	
	Inner-1	ACACGCATACCTCAACACC	220 bp
	Inner-2	GGCCCATGGTTCCGAAGCG	
Internal Control (β-globin Gene)			
	PC03	ACACAACTGTGTTCACTAGC	110 bp
	PC04	CAACTTCATCCACGTTTCACC	

PCR mixture components (7 ul of DNA template, 12.5 ul of master mix, 2 ul external primers, and 3.5 ul sterile water) (total Volume 25 ul).
-over condition as follow:

First protocol:
Denaturation: 94°C for 2 min.
Denaturation: 94°C at 30 sec
Annealing: 58°C at 45 sec
Extension: 72°C at 60 sec (35 cycles)
Final extension: 72°C at 5 min

and after that 1 ul was taken from first amplification product mixed with master mix and amplified by the second protocol (similar to first protocol condition as mentioned above with exception of annealing temperature (55°C) and for primers (internal) and then gel electrophoresis also were done to see bands of amplified samples (product size 220).[19]

Results

The outcome of the enzyme immunoassay tests for the detection of anti-CMV IgG and IgM antibodies in 213 blood samples is shown in Table 1, 212 (99.5%) were positive for anti-CMV IgG antibodies, and 15 (7%) were positive for anti-CMV IgM antibodies The results also showed that the samples with positive IgM and IgG were subjected to the IgG avidity test, and all the results were of high avidity index, which supports the diagnosis of non-primary CMV infection. Table 1.

As shown in Table 1 there no significant association between seroprevalence of CMV-IgG and different age groups.

Results obtained in Table 2 demonstrated that obstetric history has no impact on the results of CMV-IgG and IgM.

The results of the enzyme immunoassay tests for the detection of anti-CMV IgM antibodies of cord blood samples which has positive IgM and IgG were all negative, which means that there is no transmission of infection to newborn babies.

15 samples of cord blood are suitable for PCR assay, as judged by the successful amplification of β-globin sequences. The nested PCR results demonstrated that CMV DNA was not detected in all 15 cord blood samples. Figure 1.

Discussion

CMV is considered as the main virus that may cross placenta when contracted during pregnancy, resulting in congenital anomalies and critical neurological deficits. Congenital infection with CMV is associated with irreversible hearing loss due to nerve damage.[20,21]

The current study found the prevalence of CMV among pregnant women to be 99.5%, with only 0.5% susceptible women. A survey study was conducted to demonstrate the global prevalence of CMV and was found to be 83% and 86% in general population and young women respectively, with the maximum seroprevalence of 90% in Eastern Mediterranean region and the minimum of 66% in European region.[22]

Many studies had estimated the prevalence of CMV among women which revealed high prevalence rate in different countries both developing and developed ones, including Europe, USA, Pakistan, India, Saudi Arabia and Africa with the following rates 30–90%, 58.3%, 94.4%, 80–90%, 92–100%, 60–100% respectively.[23]

In Iraq several studies had been implemented to find out the prevalence of CMV among women which range from 77.3% in Babylon to 95.7% in Kirkuk, while in Erbil the prevalence was 100%.[24-26]

The higher prevalence rate in the current study is in consistent with high prevalence rate in Iraq which is over 95%. Different rate of exposure to the virus hence different prevalence rates in different regions even within the same country may highlights the need of different policies for control and management of CMV infection, while screening of pregnant women for CMV antibodies remain controversial.[22]

This may highlight the unjustified routine CMV screening for pregnant women except for those with contact with someone that has confirmed acute infection or presenting with suspected CMV symptoms and immunocompromised women.[27]

There was no significant correlation between the CMV-seroprevalence and different age groups and obstetric history of enrolled women in this study which could be attributed to very high seroprevalence (99.5%). A study done by Lachmann et al. (2018) in Germany revealed that CMV seroprevalence in women aged (18–45 years) was 51.7% and age was considered as main factor significantly correlated with CMV seroprevalence.[28]

Other researches done in North America demonstrated that women over 40 years had higher seroprevalence of CMV in comparison to those <40 years. While a study done in Mexico found prevalence in pregnant women higher among those from 20 to 30 years than women aged <20 years old. Contrary to these results no association between age and infection was detected in Europe.[29-31]

Regarding seroprevalence in women with a bad obstetric history it ranged from 14.2% in Iran to 91.05% in India. Amongst Arab pregnant women with bad history, seroprevalence varies from 4.8% (Iraq) to 95% (Jordan).[32,33]

Its clear that high prevalence rate in this study will obscure the correlation between the age, and obstetric history and their impact in determining the susceptibility to CMV infection.

The risk of vertical transmission of CMV in pregnant women is higher for those who acquire primary infection than for those with previous exposure to the virus with circulating

Table 1. **CMV serological results in regard to age groups**

Age group (year)	Total no. (%)	CMV-IgG positive no. (%)	CMV-IgM positive no. (%)	CMV-IgG avidity
19–24	93	92–(98.9%)	3–(3.2%)	(86–99%)
25–30	74	74–(100%)	8–(10.8%)	(89–99%)
31–36	46	46–(100%)	4–(8.6%)	(91–99%)
Total	213	212	15	15

P value > 0.05 (no significant correlation) by Fisher exact test.

Table 2. **CMV serological results in regard to obstetric history**

Obstetric history	Total no. (%)	CMV-IgG positive no. (%)	CMV-IgM positive no. (%)	CMV-IgG avidity
Normal history	170	169–(99.4%)	11–(6.4%)	11
Bad history	43	43–(100%)	4–(9.3%)	4
Total	213	212	15	15

P value > 0.05 (no significant correlation) by Fisher exact test.

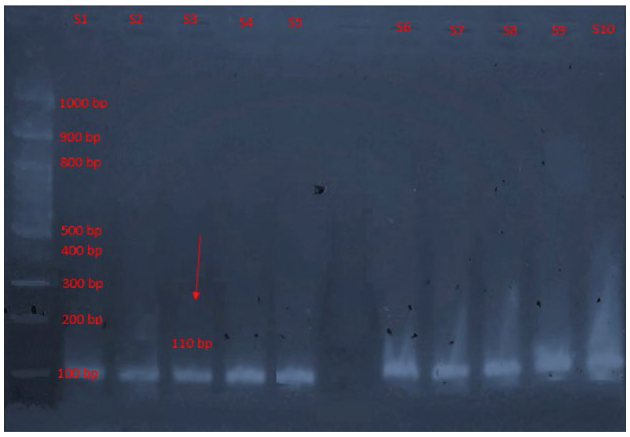

Fig. 1 **Electrophoresis of cord blood samples. All were negative for CMV_DNA, the bands with 110 bp represent the Internal Control (β-globin Gene).**

antibodies. Therefore, finding a test that help in diagnosis of new infection is very essential to put a strategy of management of suspected CMV infection in such women. Detection of CMV IgM is sensitive indicator of acute maternal infection which increases the likelihood of congenital infection and CMV DNAemia in the cord blood. Stagno et al. (1985) found detectable IgM in women with primary CMV infection and 69% of them delivered infants with congenital infections. It could be detected in combination with IgG that denotes reactivation of latent CMV or reinfection with another strain due to diversification of virus, accordingly its specificity is questionable.[34]

CMV – IgM was found in 15 (7%) of enrolled women in this study in combination with IgG, no participant was detected with IgM alone. Thus, it's not primary infection with the virus, due to presence of CMV-IgG it's reactivation of latent virus or re infection with another strain.

Presence of CMV IgG in pregnant women does not exclude the possibility of congenital infection of fetus, since there is chance for reactivation of latent CMV or reinfection with another strain during pregnancy. Many studies demonstrated increase in rate of congenital CMV alongside increase in prevalence of CMV antibodies in women which is due to nonprimary rather than primary infection. The outcome of congenital infection in infants born to women with non-primary CMV infection is nearly similar to that in newborn babies delivered by mother with primary infection in regard to severity of clinical manifestation including sensory-neural hearing defect.[17]

To solve this dilemma CMV IgG-avidity test is considered gold standard test to differentiate between recent infection and old one. Many researches revealed that CMV avidity test as sensitive and specific technique to diagnose pregnant women with recent primary infection with CMV so with high risk of intra uterine transmission. This test detects the strength of binding of IgG to specific antigens on any protein, which is enhanced through several months after primary infection. When the test is implemented low CMV IgG avidity indicates recent primary infection within previous 3 to 4 months while high avidity result means no infection in the preceding 3–4 months.[16,35]

In several researches, avidity indices (AI) higher than 60% during pregnancy could reasonably be explained a true indicator of past CMV infection, whereas in women with low AI less than or equal to 50%, there was a risk of intrauterine transmission with congenital CMV infection.

The avidity test was done in this study and involved 15 samples with CMV-IgG and CMV IgM positivity, but all 15 participants showed high avidity with more than 89%. This indicates that all were not primary infections within preceding months. The presence of IgM with IgG of high avidity may denote that there is reactivation of latent CMV or reinfection with another strain.

There is routine clinical use of CMV avidity tests in many developed countries. However, there is deficiency of information in underdeveloped and developing countries regarding use of CMV IgG avidity tests in the diagnosis of maternal and congenital CMV infections.[36]

To diagnose congenital CMV infection there should be isolation of virus by tissue culture from urine samples or detection of viral DNA by PCR, or by demonstration of CMV-IgM in blood samples. However, -CMV IgM test alone is no more useful to diagnose congenital CMV infection, since PCR is highly sensitive it has been considered as gold method for diagnosis.[37]

We have collected cord blood samples at delivery and were used to detect transplacental transmission of CMV by demonstrating the CMV IgM in these samples taken from those 15 pregnancies with both CMV IgM-IgG, but all were negative for IgM which indicates that no viral transmission has happened since IgM does not cross placenta and upon detection in cord blood indicates its production in response to fetal infection.

Shin et al. (2017) studied the serological markers in cord blood of women with suspected CMV infection at delivery and concluded that cord blood unit with positive CMV-IgM intended to use for transplantation, should be discarded regardless the result of CMV nucleic acid test.[38]

Nucleic acid detection by PCR for those 15 samples showed negative results, which confirm the results of ELISA and prove no transmission has happened. Al-Awadhi et al. (2013) found 89 out of 983 cord blood serum positive for anti-CMV IgM antibodies; while PCR result showed only 4.5% positive for CMV DNA.[39]

Its recommend to use PCR test in blood, urine, and saliva in neonates with suspected congenital infection followed by treatment of positive cases with valganciclovir.[40]

Fowler et al. (2018) suggested that the risk of CMV transmission during pregnancy following non primary type of infection in seropositive women should not be underestimated and needs to be explained.[20]

Conclusion

High sero-prevalence of CMV-IgG in pregnant women in Duhok, make them more susceptible to non-primary infection. Detection of CMV-IgG and IgM together should be taken in consideration and analyzed by IgG avidity test which is of high efficacy to differentiate between recent infection and non-primary maternal infection to elucidate the impact of infection on the rate of intrauterine transmission. Testing of cord blood immediately after delivery by CMV IgM and PCR is very useful to exclude congenital infection.

Conflict of Interest

Authors declare no conflict of interest.

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