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Original Article

Seroprevalence of Cytomegalovirus Antibodies by Electrochemiluminescence Method in Young Women Referred to the Clinical Laboratory, Sanandaj, Iran

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Abstract

Background and aims: Maternal primary and recurrent infection of cytomegalovirus (CMV) may be transmitted to the fetus during pregnancy and may have complications such as death or growth, along with the development retardation of the fetus and infant. The aim of this study was to determine the prevalence of immunoglobulin G (IgG) and immunoglobulin M (IgM) antibodies against CMV in young women, Sanandaj, Iran.

Methods: To this end, 90 women (15-40 years old) referring to a clinical laboratory were randomly selected and announced their informed consent to participate in this cross-sectional study. Demographic information and women's data were collected, including pregnancy, history of abortion, and history of blood transfusion. Then, women's sera were measured for CMV IgG and IgM antibodies using the electrochemiluminescence technique. Finally, the data were analyzed by SPSS statistical software.

Results: The prevalence of IgG and IgM antibodies against CMV in women was 92.2% (95% CI = 86.5-97.8) and 0%, respectively. In addition, the average CMV IgG antibody level was about 137.52 ± 85.215 SD IU/mL. The results revealed a significant statistical association between IgG antibody and pregnancy (P value = 0.012) while there was no association between CMV IgG antibody and other demographic data.

Conclusions: In general, high percentages of women had CMV IgG antibody whereas 7.8% of them were susceptible. They are expected to acquire CMV primary infection, and therefore, the screening of antibodies to CMV is suggested for prenatal care.

Keywords: Seroprevalence, Cytomegalovirus, Antibodies, Electrochemiluminescence, Women, Sanandaj, Iran

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Introduction

Maternal primary infection with cytomegalovirus (CMV), parvovirus B19 (PB19), and rubella virus may result in pregnancy complications such as congenital infection or abortion.[1] In addition, pregnancy induces a transient immunosuppression, which appears to increase the vulnerability of pregnant women to viral infections.[2] Given the reduction in congenital rubella infections by the vaccine, CMV and PB19 are the most important causes of clinically significant intrauterine infections.[3]

Human cytomegaloviruses (HCMVs) are members of the genus CMV in the subfamily *Betaherpesvirinae* and family *Herpesviridae*, respectively. Primary CMV infections in healthy children may be asymptomatic but it may cause a mild infectious mononucleosis-like syndrome in adults. The virus is shed in multiple body fluids including urine, saliva, semen, breast milk, and cervical secretions, and is carried in circulating white blood cells. In addition, the infection is followed by the development of adaptive immune responses and results in a lifelong latent infection after several weeks. Virus shedding may intermittently continue for years as the latent virus becomes reactivated, thus exposure to CMV is common.[4]

Congenital CMV infection occurs in 0.3 to 1% of all live births worldwide.[1] This kind of infection can be due to primary infections, reinfection with a new strain of the virus, or the reactivation of a latent virus. The seroprevalence of CMV in adults is high and the incidence of congenital CMV infection is about 1 to 5% of births in developing countries. Approximately, one-third of

pregnant women with primary infections transmit the virus via the placenta to the fetus, leading to a generalized cytomegalic inclusion disease (CID), which is most often from primary maternal infections in the fetus. CMV can also be acquired by the infant through exposure to the virus in the mother's genital secretion during delivery and from the breast milk. In these cases, the infants usually have received some maternal antibody in their blood, thus perinatally acquired CMV infections tend to be subclinical. Whether CMV is acquired in the uterus or perinatally, infection is more chronic, and viral excretion is longer than when the virus is acquired later in life. A high percentage of newborns with this disease exhibits permanent developmental defects such as deafness, blindness, and mental retardation.[4]

The clinical diagnosis of the CMV infection is difficult because the disease is asymptomatic in 90% of patients and clinical symptoms are non-specific. Further, the laboratory diagnosis of primary infections is based on the detection of virus-specific immunoglobulin G (IgG) antibodies in the serum of a pregnant woman previously seronegative for the virus, associated with low avidities of IgG or the detection of virus-specific immunoglobulin M (IgM) antibodies. After primary infection, the IgM antibody is usually present for 4-8 weeks and at a low titer for years.[5]

CMV seroprevalence is significantly influenced by socioeconomic status and thus highly relies on the population under study. Some factors are known to influence the seroprevalence, including age, climate, job, race, hygienic practices, and parity.[3]

The prevalence of CMV IgG antibodies varies between countries, ranging from 40% to 100%.[6] For instance, antibody prevalence may be moderate (40%-70%) in adults in high socioeconomic populations in developed countries, high (90%-100%) in children and adults in developing countries, and low in socioeconomic groups in developed countries.[4]

Seroepidemiological data are important for estimating the risk of congenital infections. Women of childbearing age are at the risk of primary CMV infections. Such primary maternal infections are responsible for most cases of CID in infants. Prior to the evaluation of the effectiveness and cost of any interventions, the incidence of the acute infection and the proportion of childbearing women seronegative to a primary infection should be regionally evaluated by serologic methods.

Therefore, the aim of this study was to determine the seroprevalence of IgG and IgM antibodies against CMV among young women in Sanandaj, Iran.

Materials and Methods

Study Population

According to the last census done in 2011, Kurdistan province, Sanandaj, in the west of Iran had a total population of 1 493 645. The sex ratio of the population was 1.1, and regarding ethnicity, the population mostly included Kurd. The project was carried out from February to May 2015. Informed consent was obtained from all participants in writing and the principles of data privacy, based on the Helsinki Treaty, were observed by the researchers.

According to a similar study, the present cross-sectional study included 90 young women in the reproductive age or pregnant with 15-40 years old referring to a clinical laboratory in Sanandaj, Iran.[7] The simple random sampling method was used, in which one out of every three young women in the reproductive age or pregnant were randomly assigned to the required sample size. Data including age, place of residence, job, education, pregnancy, history of abortion, and history of blood transfusion were collected by a questionnaire. Then, whole blood specimens (5 to 10 mL) were taken from women. Finally, the clotted blood samples were centrifuged at 3000 rpm for 15 minutes and the sera were separated in new tubes and stored in the -20°C freezer.

Antibody Detection

Antibodies against CMV were measured for CMV IgG and CMV IgM by using electrochemiluminescence immunoassay (ECLIA, Elecsys and Cobas, Roche, Germany). The obtained results from this assay are used to diagnose recent CMV infections. The cobas ECLIAs are based on the competition of analyte in the sample with a ruthenium-labeled analogue. Then, a voltage is applied and the electrochemiluminescence signal is detected as well. Testing was carried out according to the manufacturer's instructions. Further, the ECLIA assays were calibrated 5 times during the assessment by a 2-point calibration using calibrators traceable to pure standard materials reconstituted in samples by gravimetrical methods. ECLIA is a process that is mediated by several molecules including ruthenium, osmium, iridium, and rhenium. Furthermore, ECLIA is an extremely successful technique that is used in immunoassays for clinical diagnosis. Moreover, the ECLIA is considered as a new, highly sensitive, rapid, and specific serological screening tool based on recombinant proteins for the measurement of hormones, antibodies, and markers in human investigations. Additionally, it is beneficial for the large scale screening required for epidemiology studies and the evaluation of the pathogenesis of disease states. This technique has advantages over other diagnostic techniques, including the operator's non-involvement in the testing process and the reduction of the technical error rate (close system), and high sensitivity for measuring analytes with extremely low values. It also has high repeatability and reduced reagent consumption resulting in reduced costs and time.[8-10] The analyzer automatically calculates the cut off based on the measurement of CMV IgG/M Cal1

and CMV IgG/M Cal2. The result of a sample is given either as reactive or non-reactive and a cutoff index (signal sample/cut off). Its sensitivity and specificity are 96.5% and 98.8%, respectively.

Statistical Analysis

The data were analyzed using SPSS statistical software. Then, associations between antibodies and collected variables were evaluated using statistical t-test, one-way ANOVA with Tukey post hoc, and chi-square.

Results

Demographic information and women's data, including age, place of residence, job, education, pregnancy, history of abortion, and history of blood transfusion are shown in Table 1.

The prevalence of IgG antibodies against CMV was 92.2% (95% CI: 86.5-97.8) in women who referred to the clinical laboratory. Table 1 presents the prevalence of IgG antibody according to collected demographic variables. In addition, the distribution of CMV IgG antibody levels (IU/mL) is shown in Figure 1 according to the age groups of women. The maximum level of IgG was among the age group of 26-30 years (Figure 1) and the average of the IgG antibody level was 137.52 ± 85.215 SD, IU/mL (Figure 2). Based on the results, there was a significant statistical association between IgG antibody and pregnancy (*P* value = 0.012). In other words, the prevalence of CMV IgG antibody was higher in pregnant women. However, no significant statistical association was observed between the IgG antibody and other collected demographic variables (Table 1).

The level of IgM antibody in all women's sera was less than the 0.7 cut-off index, thus it was considered negative, according to the instruction of the kit manufacturer. Therefore, the prevalence of IgM antibodies against CMV was 0%.

Discussion

In the present study, the seroprevalence of CMV IgG and IgM antibodies in women was 92.2% and 0%, respectively. The average CMV IgG antibody level was about 137.52 IU/mL and there was a significant statistical association between IgG antibody and pregnancy. However, no association was found between IgG antibody levels and other collected demographic data.

The prevalence of CMV IgG antibodies differs between countries, ranging from 40% to 100%.[6] Based on the results,

Table 1. Prevalence of Cytomegalovirus IgG Antibodies According to Demographic Data of Young Women Referring to the Clinical Laboratory

	Negative (IgG Antibody Level <10 IU/mL)	Equivocal (10 IU/mL< IgG Antibody Level <15 IU/mL)	Positive (IgG Antibody Level >15 IU/mL)	*P* Value
Age groups				
[≤20]	2 (2.22%)	0 (0%)	8 (8.88%)	
[21-25]	1 (1.11%)	1 (1.11%)	13 (14.44%)	
[26-30]	0 (0%)	0 (0%)	33 (36.66%)	0.164
[31-35]	1 (1.11%)	0 (0%)	17 (18.88%)	
[35≤]	2 (2.22%)	0 (0%)	12 (13.33%)	
Total	6 (6.7%)	1 (1.1%)	83 (92.2%)	
Place of residence		Urban	68 (82%)	0.079
		Rural	15 (18%)	
Job		Housewife	47 (56.62%)	0.905
		Teacher	13 (15.66%)	
		Employee	17 (20.48%)	
		Student	6 (7.23%)	
Education		Elementary	16 (18%)	0.770
		High school	27 (32.53%)	
		College	40 (48.20%)	
Pregnancy		Yes	63 (75.90%)	0.012
		No	20 (24.1%)	
History of abortion		Yes	38 (45.78%)	0.974
		No	45 (54.22%)	
History of blood transfusion		Yes	12 (13.33%)	0.943
		No	71 (85.54%)	

Note. IgG: Immunoglobulin G.

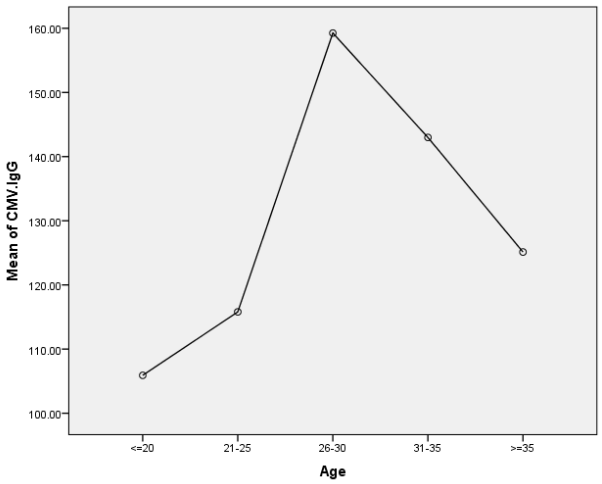

Figure 1. Distribution of Cytomegalovirus IgG Antibodies Levels (IU/mL) According to the Age Groups of Women Referring to Clinical Laboratory. *Note*. IgG: Immunoglobulin G.

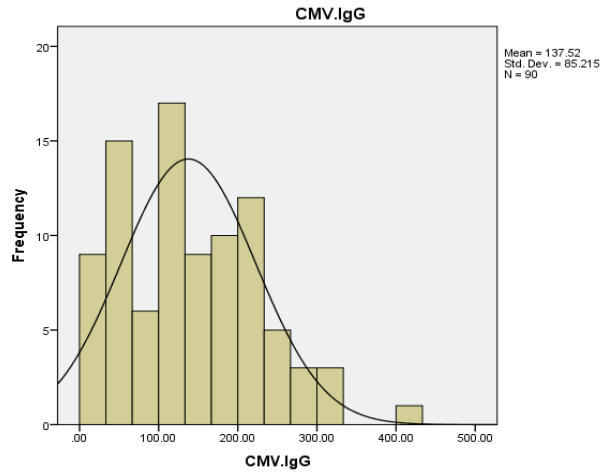

Figure 2. Histogram of Cytomegalovirus IgG Antibodies levels (IU/mL) in Women Referring to Clinical Laboratory. *Note*. IgG: Immunoglobulin G.

the CMV IgG prevalence rate was 66.0% in pregnant Japanese women.[11] Furthermore, CMV seroprevalence was 49%, 89%, and 98% among the white British women, South Asian United Kingdom born women, and South Asian women born in South Asia, respectively.[12] Moreover, CMV seropositivity was 57.0% among pregnant women in Paris, France.[13] In Polish pregnant women, the seroprevalence of CMV IgG and IgM antibodies was 62.4% and 2.2%, respectively.[6] Some studies focused on the seroprevalence of CMV IgG and IgM in regional countries. For instance, the seroprevalence of CMV IgG and IgM was 98.9% and 1.5% among pregnant women in Izmir, Turkey, respectively. The researchers concluded that avidity tests should be done in all IgM and IgG positive results in order to enhance the reliability of serological methods.[14] Additionally, the prevalence of CMV IgG was 84.1% in pregnant women in Istanbul, Turkey. Likewise, the IgG avidity test excluded primary infections and IgM was not detected in any women.[5] A case-control study was conducted in Kirkuk (Iraq) to determine CMV antibodies in women with a bad obstetric history in comparison to women with a normal pregnancy. Based on the findings, the CMV IgG and IgM prevalence was 96.6% and 7.2% in women with a bad obstetric history, as well as 94.7% and 5.3% in women with normal pregnancies, respectively.[15] In a total of 546 pregnant women in Egypt, all (100%) cases were seropositive for CMV IgG, and of all women, 7.3% of cases were positive for IgM.[16] In another study, the prevalence of CMV was 94.5% in pregnant women in Pakistan.[17] The seroprevalence of CMV IgM antibody was determined in 1954 Palestinian women by antenatal screening from 2000 to 2005, and CMV IgM was found in 6% of women using the ELISA.[18]

A study was conducted on 225 pregnant women and their newborns in Mashhad, Iran. All mothers and their neonates were positive for CMV IgG (100%) and only 2.6% of mothers were positive for CMV IgM.[19] In another study, 180 females were randomly selected from high schools, college students, and those who were attending marriage consulting clinics in Bushehr, Iran. IgG antibodies against CMV were detected by ELISA and 99.4% of sera were positive for CMV IgG.[20] Similarly, an investigation was conducted on the age-related seroprevalence of CMV infections in the inhabitants of Isfahan, Iran. The overall seroprevalence of CMV IgG was 98.2%. The results revealed no significant association between CMV seroprevalence and age, gender, education, family member, and place of residence.[21]

In a study, the prevalence of at-risk pregnancies was determined for congenital CMV transmission in pregnant women and their newborns in Tehran, Iran. In randomly selected specimens, ELISA and real-time polymerase chain reaction (RT-PCR) were done to screen the sera of mothers and the consecutive umbilical cord from their newborns. Out of 100 sera of mothers, 100% and 3% were positive for CMV IgG and IgM antibodies, respectively. Out of the 100 cord sera, 99% and 2% were positive for CMV IgG and IgM antibodies, respectively. CMV DNA was detected in cord blood of 4% of newborns. Out of four DNA positive newborns, one case had no IgM in the cord serum, but there was IgM in the mother's sera. Two cases were positive for IgM in the cord and the mother's sera. On the other hand, Case 3 had no CMV IgM in the cord and the mother's sera. CMV IgM positive women also had 66% and 100% of CMV IgM and CMV DNA in their delivery cord blood, respectively. These results showed increased congenital CMV infection risk in pregnancy. In addition, CMV DNA was positive in a paired sera/cord blood negative to CMV IgM. The study suggested that PCR can be used in place of IgM as a diagnostic test for

the detection of congenital CMV infections.[22]

Based on the aims of another study, 240 serum samples were collected from pregnant women in the third trimester, Gonabad, in the east of Iran. CMV IgG and IgM antibodies were detected using the ELISA. For all CMV IgG and IgM positive samples, IgG avidity test was used to differentiate primary and recurrent CMV infections. Moreover, all IgM positive pregnant women were followed up until delivery. Approximately, 72.1% and 2.5% of women were positive for CMV IgG and CMV IgM, respectively. Additionally, the positivity of IgM in primary and recurrent infections was 0.83% and 1.67%, respectively. A significant relationship was observed between the history of abortion and the place of residence with IgG. In addition, two CMV IgM positive women experienced preterm labor and low birth weight. Finally, the researchers suggested CMV IgM screening for pregnant women who were at the risk of CMV infections.[23]

Similarly, a case and control study was done in Hormozgan, Iran during 2003-2004, including 250 women with the history of previous abortion and 200 matched women with normal full-term delivery. All serum samples of women were tested using ELISA. Based on the results, CMV IgG was positive in 94% and 75% of case and control groups, respectively. In the case group, 5.2% of women were positive for CMV IgM, but no CMV IgM positive result was detected in the control group. The researchers concluded that there was higher seroprevalence of CMV in women with spontaneous abortions in comparison to women with a normal delivery and suggested that CMV plays a role in abortion.[24] The assessment of risk factors on CMV prevalence among young women in Hamadan, Iran showed a significant association between job and CMV infections. Similarly, there was a significant association between CMV IgM and job, pregnancy, and referring years.[25]

In the present study, the seroprevalence of CMV IgG antibody in women was higher, which is inconsistent with the results of other studies in developed countries.[6,11,13] while it is compatible with the results of other studies conducted in regional and neighboring countries and other cities in Iran.[5, 14-17,19-22] This high immunity is acquired probably in childhood. Likewise, the result of the seroprevalence of CMV IgM antibody in our study corroborates with the results of some other studies[5,6,14,19,22-24] whereas it contradicts the results of other studies.[15-18] This discrepancy could be due to different geographical and health conditions. In addition, the method of testing in this study was different from those of the above-mentioned studies. As previously mentioned in our study, there was a significant statistical association between IgG antibody and pregnancy, which is consistent with the results of other studies.[15,24,25]

Herpesviruses such as CMVs cause primary infections that can be converted to latency and latent CMV can reactivate during pregnancy. This reactivation produces viremia and can cause a congenital infection of the fetus. Evidence indicates that a part of these CMV infections includes new infections with different CMV strains. Therefore, maternal seropositivity does not protect the fetus against intrauterine CMV infections.[3] In developing countries, with a high prevalence of CMV antibodies, the reactivation of maternal latent CMV during pregnancy may be transmitted to the fetus and cause adverse pregnancy outcomes although the majority of infected infants are asymptomatic.[26]

A meta-analysis indicated that CMV infection during pregnancy was a risk factor for spontaneous abortion, premature birth, and stillbirth.[27] The results of a study showed that CMV was shedding in seropositive pregnant women.[28] The presence of CMV IgM antibody in the serum indicates a recent active infection and is a useful marker for transmission.[29] Screening for CMV during pregnancy is recommended for managing the complications caused by this virus.[17] There are debates over the implementation of antibody screening for CMV infections in pregnant women.[5]

Systemic CMV serologic screening is not warranted in Iran, where CMV would be a public health problem among pregnant women. The prevalence of IgM antibody in our study was 0%. Therefore, CMV transmission will be low in our population. High percentages of women in the study population had CMV IgG antibody although 7.8% of women were susceptible. Accordingly, we recommend serologic testing to determine the immune status of young women.

In most studies, the ELISA method was used to measure antibodies and the presence of CMV IgM antibody in the serum was diagnostically-clinically important in pregnant women. Therefore, in some studies,[5,22] molecular tests such as PCR and the avidity test in addition to ELISA were used to confirm the presence of this antibody. ELISA is an older method compared to ECLIA, which has a higher technical error because it is not a closed system and the operator is involved in the testing process. In addition, the ELISA method has lower repeatability in comparison to the ECLIA method. However, ECLIA is a modern, rapid, highly sensitive, and accurate method with a closed system and high repeatability.

It should be noted that in this study, an advanced and accurate electrochemiluminescence technique was used to measure antibodies, which is one of the differences and innovations of this study over other studies.

Conclusion

In general, a high percentage of young women in the present study had CMV IgG and were immune to this infection. However, 7.8% of women were susceptible, thus the screening of antibodies to CMV is recommended for

prenatal care.

Ethical Approval

Ethical issues including plagiarism, data fabrication, and double publication were completely observed by the author. Similar to other studies involving human participants, all procedures of this study were performed in accordance with the Ethical Standards of the Institutional and National Research Committee and the 1964 Helsinki declaration and its later amendments. In addition, all participants provided written and informed consent. Eventually, the project was in accordance with the ethical principles and the national norms and standards for conducting medical research in Iran.

Conflicts of Interest

On behalf of all authors, the corresponding author declares that there is no conflict of interest.

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