Seroprevalence, transmission, and associated factors of specific antibodies against cytomegalovirus among pregnant women and their infants in a regional study

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ABSTRACT

الأهداف: لتقييم الأجسام المضادة ضد الفيروس المضخم للخلايا (CMV)، والحالة السريرية والبيانات الديموغرافية في النساء الحوامل وأطفالهن الرضع في شمال شرق إيران.

الطريقة: أجريت هذه الدراسة الاستعراضية بطريقة عشوائية على225امرأة حامل وأطفالهن حديثي الولادة الذين زاروا المستشفيات العامة في مدينة مشهد في الفترة ما بين ديسمبر 2007م حتى يناير2008م. أجري التقييم السريري من قبل اثنين من المتخصصين وكذلك الأمر تم الحصول على البيانات الديموغرافية. تم أخذ عينات الدم بعد الولادة مباشرة من الأمهات والحبل السري للأطفال لقياس وتعيين مقدار الأجسام المضادة ضد الفيروس المضخم للخلايا (IgG، IgM).

النتائج: على الرغم جميع الأمهات والأطفال حديثي الولادة كانوا إيجابين بالنسبة(100%) CMVIgG ، فقط 6 من الأمهات كانوا إيجابين بالنسبة (2.6%)IgM وكل الرضع كانت نتيجتهم سلبية. ومع ذلك لوحظ وجود عوارض سريرية لعدوى الفيروس المضخم للخلايا من خلال تقييم الإشعاعي (CT المسح) عند واحدة من الرضع (0.4%). لم يكن هناك ارتباط بين مقدارCMVIgG وعدد الولادات عند الأم، سوابق الإجهاض، فصائل دم الأمهات والأطفال حديثي الولادة، والعمر الحملي، ونوع الولادة، والحالة اقتصادية. ومع ذلك كان مقدارCMVIgG عند والأطفال حديثي الولادة ذوي الولادة الطبيعية أقل بكثير من القيصرية (p=0.03) وعند الفتيات مقارنة بالفتيان (p=0.04).

الخاتمة: انتقال المضاد للفيروس المضخم للخلايا (IgG) إلى الأطفال حديثي الولادة يرتبط بجنس المولود ونوعية الولادة. على الرغم من مكافحة المضاد للفيروس المضخم للخلايا (IgM) يبين أن الأمهات يتعرضن كثيرا للفيروس المضخم للخلايا، وقد لا ينتقل العدوى إلى الجنين.

Objectives: To assess specific anti-cytomegalovirus (CMV) antibodies, clinical status, and demographic data in pregnant women and their infants in northeast Iran.

Methods: This cross-sectional study was conducted on 225 systematic randomly selected-pregnant women and their newborns attending public hospitals in Mashhad, Iran between December 2007 and January 2008. Two specialists performed clinical assessment and obtained the demographic data. The sera from mothers and the umbilical cord of infants were then collected at the time of delivery and anti-CMV antibodies, IgG, and IgM, were measured.

Results: Although, all mothers and their neonates were positive for anti-CMV IgG (100%), only 6 were positive for anti-CMV specific IgM (2.6%), and their infants were negative. However, in one infant the clinical features of CMV infection were observed by radiological evaluation (CT scan) (0.4%). There was no correlation between anti-CMV IgG in neonates and number of parity, history of abortion, mothers' and neonates' blood groups, gestational age, and economical status. However, the concentration of anti-CMV IgG in neonates with normal delivery was significantly lower than with cesarean delivery (p=0.03), and in girls compared with boys (p=0.04).

Conclusion: Anti-CMV IgG transmission to neonates is associated with gender and type of delivery. Despite anti-CMV IgM showing active CMV infection in mothers, virus transmission to the fetus might not occur.

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Cytomegalovirus (CMV) is the most common cause of congenital infection. Recent studies estimated the proportion of pregnancies affected with this virus is approximately 0.1-5%.[1-3] Cytomegalovirus is a member of the Herpesviridae family, which has a double stranded DNA core of 200 kilobase pairs enclosed by an icosahedral capsid.[4] The CMV is found universally throughout all geographic locations and in all socioeconomic groups, particularly in certain populations in Asia and Africa.[5] Congenital and perinatal infection rates are usually higher in developing countries than in developed countries.[6,7] The seroprevalence of CMV IgG among women of childbearing age in Iran is approximately 93%.[6] Although vertical transmission is the main route of virus dissemination within the population, CMV postnatal transmission is also common in early life. Studies have shown that CMV-associated diseases occurred in 0-87% of postnatal infected preterm infants.[3,8] Several studies have demonstrated a correlation between many clinical features and CMV infection. For instance, premature childbirth is a frequent finding associated with symptomatic, congenital CMV infection.[7,9] In addition, prenatal or postnatal CMV infection as a consequence of exposure to genital secretions can cause severe disease in infants.[10] The immunoglobulin G isotype (IgG) can actively transfer from mother to fetus during pregnancy.[11,12] Previous studies have demonstrated a correlation between IgG concentration and gestational age at the time of birth. Furthermore, a positive correlation between the levels of specific antibodies in newborn infants and their mothers was observed.[7] Approximately 0.5-2% of all live newborns are infected with intrauterine CMV.[13,14] Prematurity, low birth weight, and maternal immunoglobulin concentration can affect antibody transfer from mother to fetus.[8,12] In general, congenital CMV infection is a health threatening issue and due to controversial aspects of this virus in pregnancy, it should be considered a health threatening factor in developing countries. Khorasan province is a large area in northeast Iran, which is located at the crossroads of the East and West, and shares a long border with Afghanistan, Turkmenistan, and Pakistan. Historically, this province, particularly Mashhad, was the center of trade for the Silk Road and Eurasian trade Route. Mashhad is a very popular destination for pilgrimage tourists, and receives at least 20 million visitors per year; therefore, risk of spreading infectious diseases in this region is high.[15] Thus, studies on infectious diseases are at a high priority in such an area. The present study was conducted to evaluate the seroprevalence and transmission of CMV infection from mothers to their neonates and association of some maternal and infantile factors with anti-CMV IgG and IgM concentration among 225 mothers and their newborns.

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Methods.

Study population. To evaluate the seroprevalence and transmission of specific antibodies against CMV among pregnant women and their infants in a populated pilgrimage region with high levels of population movement (Iranian National Census 2006. Available from: http://www.sci.org.ir/englishold/Sel/jshvro84), a cross-sectional study was conducted on mother-infant pairs. The pregnant women were enrolled at the delivery time, and the clinical presentation, and frequency of congenital CMV infection in infants were also assessed. All 225 subjects were selected by systematic random sampling from pregnant women who attended the public hospitals in Mashhad, the main city of Khorasan, Iran from December 2007 to January 2008. The systematic random sampling was carried out in all of 4 main public hospitals of the city. In each hospital one from 50 pregnant women was selected according to our criteria; however, we tried to include subjects from each geographic area according to the 2006 census.

The pregnant women of gestational age under 40 years old with singleton pregnancy were eligible to participate in the study. The exclusion criteria were: CMV infection before pregnancy, particular diseases or infections, maternal immune impairment, congenital infection with rubella, syphilis, varicella, parvovirus, or toxoplasmosis. The Research Committee of the Islamic Azad University of Mashhad approved all aspects of the project with regard to ethical issues and informed consents were obtained from participants. Demographic information on maternal and gestational background including; gestational age, previous abortions, parity, mothers' and newborns' ABO blood groups, newborn's weights, and gender, economic status, and type of delivery, were collected. Furthermore, the clinical data were assessed at the time of sampling.

Serological assessment. The sera were separated from umbilical cord and maternal blood samples on the day of sampling, and aliquots were stored at -20°C until laboratory evaluation. Anti-CMV IgG assessment in

sera was carried out using a commercially enzyme-linked immune sorbent assay (ELISA) kit (RADIM, Pomezia, Roma, Italy) according to the manufacturer's instructions. An antibody level of 10 IU/ml was considered positive. For evaluation of anti-CMV IgM a commercially available ELISA kit (RADIM, Pomezia, Rome, Italy) was used and antibody levels more than 10% of cut-off were considered positive.

Statistical analysis. The Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA) software version 11.5 was used for statistical analyses in the current study. The differences between groups were determined by parametric tests if the distributions of the variables were normal and the non-parametric tests were used if it were assumed that not all data sets met a normal distribution. Briefly, Student's t-tests were used for unpaired samples to compare the mean concentration of anti-CMV IgG between groups. Pearson's test was used for testing the correlation of maternal and neonatal anti-CMV IgG. The Mann-Whitney test was used to compare mean maternal and neonatal CMV-specific IgG according to gestational age. Kruskal-Wallis test was used to compare the mean values between different groups. The Chi square test was used to evaluate the effect of different factors on seropositivity rate. The differences were considered significant if $p<0.05$.

Results. Two hundred and twenty-five mothers with the mean age of 26.8±5.6 years (X±SD) and their infants were included in this study. Twelve infants were preterm (<37 weeks) and the rest, 213 infants were full term (>37 weeks). The mean birth weight for full term newborns was 3.203±0.028 kg, and for preterm newborns was 2.431±0.145 kg. One hundred and eighty-four (81.8%) of the mothers had no abortion, and the rest had history of one (12.4%), 2 (4.4%), or 3 (1.3%) abortions. According to our evaluation, all mothers and their infants were positive for anti-CMV specific IgG. Therefore, the prevalence of anti-CMV IgG among the study groups was 100%. However, 6 mothers were positive for anti-CMV specific IgM (2.6%), but this antibody was not detected in their infants. We found clinical features of CMV infection such as microcephaly in one infant (0.4%) according to clinical findings and radiological evaluation (CT scan). The level of anti-CMV IgG in mothers and their infants were 139.2±73.6 IU/ml and 138.4±68.1 IU/ml. The mean maternal/umbilical cord ratio of anti-CMV IgG was 1.36±0.32 (range 0.030-3.9) among which, 105 pairs had ratios less than one, and 120 pairs had ratios more than one. A highly significant correlation was observed between anti-CMV IgG in mothers and their newborns (preterm and full term) (r=83.8, $p<0.0001$). The amount of anti-CMV IgG in full term neonates was 142.2±69 IU/ml, and in preterm neonates was 118.6±59 IU/ml. Statistical analyses revealed that there is no difference between anti-CMV IgG in full term and preterm neonates. However, the concentration of anti-CMV IgG in neonates of mothers with normal delivery was significantly lower than those with cesarean ($p=0.03$) (**Table 1**). In addition, the concentration of anti-CMV IgG in girls was significantly lower than boys ($p=0.04$) (**Table 1**). There was no association between anti-CMV IgG in neonates and number of parity, abortion, mothers' and neonates' blood groups, gestational age, type of delivery, neonate's gender, and economical status. The data analyses revealed a significant association between anti-CMV IgG concentration in neonates and their birth weight ($p=0.03$) (**Table 2**). The mean weight of neonates in cases with a mean cord/maternal ratio of anti-CMV IgG less than one (2.99±0.55) was significantly lower than ones with the mean cord/maternal ratio of anti-CMV IgG more than one (3.22±0.44; $p<0001$).

Table 1 - Univariate analysis of variables associated with transmission of anti-CMV IgG (IU/ml) from mothers to their neonates.

Variables	N	(%)	Concentration of anti-CMV IgG (IU/ml)	*P*-value
Delivery				
Normal vaginal	70	(31.1)	55.3±6	0.03*
Cesarean section	155	(68.9)	72.6±5.5	
Gender of newborn				
Boy	110	(48.9)	143.9±63.2	0.04*
Girl	115	(51.1)	127.8±57.7	

*Student's t test, CMV - cytomegalovirus, IgG - immunoglobulin G

Table 2 - Concentration of anti-CMV IgG (IU/ml) in neonates with different weights.

Variables	N	(%)	Concentration of anti-CMV IgG (IU/ml)	*P*-value
Weight of neonate				
<2 Kg	7	(3.1)	87.2±50.6	0.03*
2-2.99 Kg	59	(26.2)	129±60.7	
3-3.99 Kg	155	(68.9)	141.1±62.4	
≥4 Kg	4	(1.8)	163.8±77	

*Kruskal-Wallis test, CMV - cytomegalovirus, IgG - immunoglobulin G

Discussion. In this study, the seroprevalence of specific antibodies (IgG and IgM) against CMV, and the diagnostic value of these antibodies were evaluated in pregnant women and their infants. Furthermore, associations of some maternal and infantile factors with concentration of antibodies were considered among 225 mothers and their newborns, as well as the transmission mode of antibodies against CMV. According to our result, seroprevalence of anti-CMV IgG in Mashhad was 100%. The previous studies have shown that the seroprevalence of CMV IgG in Iran was approximately 93% in women of childbearing age.[6] Therefore, it seems that the prevalence of CMV infection in the present study is similar to the other regions in Iran.

In our study, the placental transfer of CMV specific antibodies was investigated in Iranian mothers to find out if infants can rely on maternal antibodies for immunity against this virus. We demonstrated that all mothers and their infants were positive for specific anti-CMV IgG. It was found that the mean maternal/cord ratio in this study is more than one (mean ±SD; 1.36±0.32) and all infants received specific anti-CMV IgG from their mothers. Furthermore, a significant positive correlation was observed between maternal and infant specific IgG antibody levels, the same as reported by other investigators.[16,17] Although, in our study a correlation between total specific IgG concentrations and gestational age in infants was very weak, in many studies this correlation has been observed.[18] Approximately 0.5-2% of all live newborns are infected with intrauterine CMV.[13,14] In the current study, 6 mothers were positive for anti-CMV specific IgM but this antibody has not been detected in their infants. Therefore, the prevalence of anti-CMV IgM in mothers was 2.6%, and in infants was 0%. These findings were in contrast to previous studies that showed a correlation between anti-CMV IgM titer in mother and child.[6]

Despite anti-CMV specific IgM showing acute CMV infection, it is more likely that none of these mothers transmitted the virus to their infants. Moreover, according to clinical and radiological (CT scan) evaluation; one of these infants showed microcephaly as a sign of CMV infection. Therefore, it seems that not only the serological assessment, but also clinical examination, and radiological evaluation, such as CT scan or MRI, should be taken into consideration.

There are controversial results of differences between full term and preterm infants for anti-CMV IgG levels. Some previous studies reported that the specific antibody levels detected in preterm infants were lower than those in term infants.[12,19] In our study, statistical analysis revealed that there is no difference between anti-CMV IgG in full term and preterm neonates, which is consistent with a study on Turkish mothers and their infants.[20]

Data analysis in this study revealed a significant association between anti-CMV IgG in neonates and neonates' weight. Low birth weight and prematurity may interfere with the maternal-fetal transport of antibodies. Taken together, it seems that low birth weight and premature infants are more vulnerable to the infection.[12] In addition, the concentration of anti-CMV IgG in girls was significantly lower than boys at the time of birth. Some studies demonstrated that sex hormones influence the immunological response, particularly immunoglobulin isotypes.[21] Our finding demonstrated that hormones during pregnancy may affect the antibody response in the fetus, or the transfer pattern of different specific antibodies from mother to the neonate. According to the results of the present study, the concentration of anti-CMV IgG in neonates of mothers with normal delivery was significantly lower than those with cesarean.

In conclusion, in contrast to some previous studies, in our study, the correlation between total specific IgG concentrations and gestational age in infants was very weak. Anti-CMVs IgG transmission to neonates was associated with gender and type of delivery. Furthermore, despite anti-CMV IgM showing active CMV infection in mothers, virus transmission to the fetus may not occur. Beside genetic factors, the impact of fetal gender on specific antibody production may associate with type of hormonal changes during pregnancy, which may affect, fetus gender too. Therefore, the impact of gender and type of delivery on fetus immune response and IgG production and transmission from mother should be further studied. To further strengthen the validity of this study, it would have been very useful if anti IgG CMV avidity tests, and CMV real time PCR quantification tests could have been carried out to confirm the active CMV infection in the mothers for CMV transmission to fetus.

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