CMV seroconversion in pregnants and the incidence of congenital CMV infection

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In this study, it was aimed to determine the ratio of CMV seroconversion in pregnant women, the prevalence of maternal CMV infection and also the incidence of congenital CMV infection in their newborns in the Antalya region of Turkey.

During a one-year period, CMV-specific IgG and IgM were determined in all (n: 1027) pregnant women admitted at 8 to 20 weeks of gestation, and according to the presence or absence of anti CMV-IgM and CMV-IgG, pregnant women were classified as seropositive, seronegative and having maternal CMV infection. Differentiation of primary and recurrent CMV infection in women with both CMV-IgM (+) and CMV-IgG (+) antibody was determined by the avidity index (AI) of anti-CMV IgG. Ultrasonographic examination was done and amniocentesis was performed at 21 to 23 weeks of gestation in pregnants with primary infection. CMV DNA was investigated in the amniotic fluid by quantitative polymerase chain reaction (qPCR). Pregnants with recurrent infection were followed only by ultrasonography for the presence of fetal abnormalities. Neonates born to mothers with CMV infection were examined for the findings of congenital CMV infection and screened for anti- CMV-IgM, CMV DNA and CMV antigenemia in the first two weeks of life.

The rate of seropositivity was found as 98.5% and the rate of seronegativity as 1.5% in pregnant women. The prevalence of maternal CMV infection was found as 1.2% and among these pregnant women, the incidence of primary and recurrent maternal CMV infection was 0.3% (3 women) and 0.8% (12 women), respectively. Congenital CMV infection was detected in one of the newborns born to mothers with primary infection while no infection was detected in any of the newborns of mothers with recurrent CMV infection, so the incidence of congenital CMV infection was found as 0.1% and the rate of intrauterine infection following the primary maternal infection was 33%.

In conclusion, seroprevalence rate of CMV in pregnants is high and most (66%) infections are recurrent maternal CMV infection in our region. Thus, it does not seem to be cost-effective to screen all pregnant women for CMV infection, as in the other countries with high seropositivity rate.

Key words: cytomegalovirus infection, seroprevalence.

Seroepidemiologic surveys have found that the prevalence of antibody to cytomegalovirus (CMV) in child-bearing-age women varies widely among populations, being lower (50-60%) in middle-to-high and higher (90-100%) in lower socioeconomic backgrounds[1-4]. The risk of fetal transmission is 30 to 40% in pregnancies following primary maternal infection, whereas this ratio is less than 2% (0.5-1.0%) after a recurrent maternal CMV infection[5-12].

It is generally accepted that the symptoms of congenitally infected children are more severe in primary maternal infection, with significant neurological sequelae (30-75% of infants with

symptoms at birth) and mental retardation in long-term outcome[7,13]. Infants that are asymptomatic at birth are also at risk for long-term sequelae such as sensorineural hearing loss (30% of infants with symptoms at birth, 7-13% of those with subclinical infection), visual impairment, learning disability and mental retardation[6,10,14,15]. Recurrent maternal infections involve mostly minor consequences for the fetus or newborn. However, newborns born with severe symptoms due to recurrent maternal CMV infection have also been reported in the literature. As CMV is one of the most common causes of congenital infections, there are studies from different regions of Turkey regarding the rate of seropositivity among Turkish women of childbearing age. However, we could not find any study in the literature that determines the prevalence of maternal and congenital CMV infection in Turkey.

The aim of this study was to determine the rate of CMV seroconversion and the prevalence of maternal CMV infection in pregnant women who attended the Department of Obstetrics and Gynecology of Akdeniz University Medical School and the incidence of congenital CMV infection in their newborns.

Material and Methods

Between January 2002 and May 2003, all pregnant women who attended the Department of Obstetrics and Gynecology of Akdeniz University Faculty of Medicine at 8 to 20 weeks of gestation were enrolled in this study.

Study Population

Pregnant women

All pregnant women were tested for CMV-specific antibodies in order to identify seroconversion and also to diagnose maternal CMV infection. The women were classified according to the presence or absence of CMV-IgM and CMV-IgG antibodies. Women with:

1. CMV-IgM (-) and CMV-IgG (+) antibody were defined as CMV seropositive.
2. CMV-IgM (-) and CMV-IgG (-) antibody as CMV seronegative (They were re-studied at the third trimester and after birth to determine the seroconversion).
3. CMV-IgM (+) antibody as having infection. Those with:

   a) CMV-IgM (+) and CMV-IgG (-) antibody were defined as having a primary infection.

   b) CMV-IgM (+) and CMV-IgG (+) antibody as having either a primary or a recurrent infection. Differentiation of primary and recurrent CMV infection in women with both CMV-IgM (+) and CMV-IgG (+) antibody was determined by the avidity index (AI) of anti-CMV IgG. Pregnant women who had:

   (i) CMV-IgM (+) antibody and anti-CMV IgG of low avidity were accepted as having primary maternal infection.

   (ii) CMV-IgM (+) antibody and anti-CMV IgG of high avidity as recurrent maternal infection.

   (iii) CMV-IgM (+) antibody and anti-CMV IgG of moderate avidity as undefined group.

Women with primary and undefined maternal CMV infection were prospectively screened both by invasive (amniocentesis) and noninvasive (ultasonographic examination) prenatal diagnostic tests. These women were informed about congenital CMV infection and invasive prenatal diagnostic tests. After written informed consents were obtained, 20 ml of amniotic fluid samples were collected by transabdominal amniocentesis under ultrasound guidance, at 21 to 23 weeks of gestation. CMV DNA was investigated in amniotic fluid by quantitative polymerase chain reaction (qPCR).

Following amniocentesis, women with primary CMV infection were followed monthly by ultrasonography. A detailed ultrasonographic examination was performed for detecting fetal microcephaly, intracranial calcification, ventriculomegaly, hepatosplenomegaly, intrahepatic calcification, intestinal edema, ascites and pleural effusion.

Pregnants with recurrent CMV infection were followed only by ultrasonographic examination, as the probability of fetal injury after amniocentesis may be higher than in recurrent infection (Fig. 1).

Newborns

Neonates born to mothers with CMV infection were carefully examined for the findings of congenital CMV infection as microcephaly, hepatosplenomegaly, thrombocytopenia, chorioretinitis, and periventricular calcification, and were screened for anti CMV-IgM, anti- CMV-IgG (microELISA), CMV DNA qPCR and CMV antigenemia in the first two weeks of life.

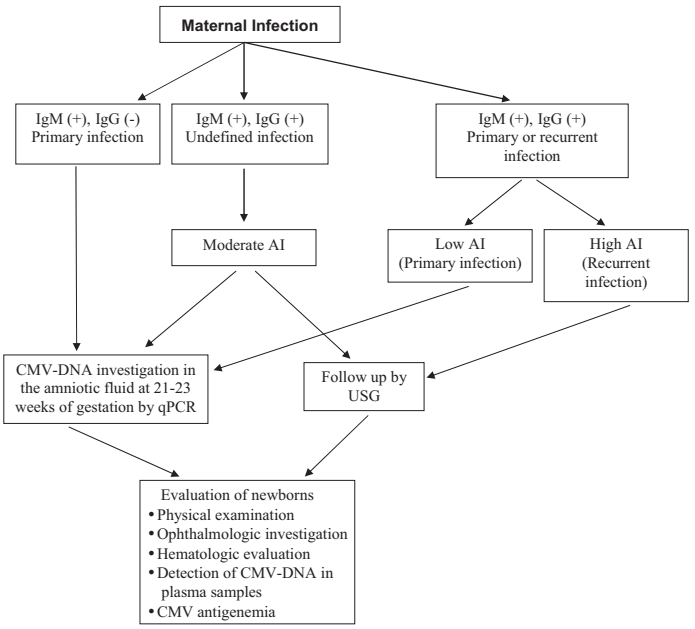


Fig. 1. Follow-up of pregnant women with maternal infection. AI: avidity index.

CMV Virology

CMV-specific antibodies

Blood samples obtained from neonates born to mothers with CMV infection were evaluated for the presence of specific anti-CMV IgG and IgM antibodies. CMV specific IgM and IgG antibodies were determined by microenzyme immunoassay (micro-ELISA) (RADIM, Italy). After the calculation of a cut-off value as suggested by the manufacturer, the results measured between ±10% of this limit were re-studied and values higher than 10% were defined as positive and less than 10% as negative.

CMV-avidity index (AI)

Avidity index of anti-CMV IgG (RADIM, Italy) was studied as proposed by the manufacturer. An AI of less than 35% was considered as low, higher than 45% as high and between 35% and 45% as moderate.

CMV-DNA

Quantification of CMV DNA was performed for plasma and amniotic fluid by using the Cobas Amplicor CMV Monitor System (Roche Diagnostics, Branchburg, NJ, USA) according to the manufacturer's instructions. The detection limit of this quantitative assay was 400 copies of CMV DNA/ml.

CMV-antigenemia

Cytomegalovirus pp65 was investigated in peripheral blood leukocytes according to the manufacturer's recommendations (CINAkit, Argene-Biosoft, France). Results were expressed as the number of HCMV pp65 positive cells per $2x10^5$ PBLs, and the test was considered positive when at least one fluorescent cell was observed.

Results

Between January 2002 and May 2003, 1027 pregnant women at 8 to 20 weeks of gestation attended the Obstetrics and Gynecology Department. Out of 1027 pregnants, 1000 were found as anti-CMV IgM(-), anti-CMV IgG(+); 15 as anti-CMV IgM(-), IgG(-) and 12 as anti-CMV IgM(+), IgG(+). None of the pregnants were IgM(+), IgG(-) (Table I).

Maternal Seroprevalence

Out of 1027 pregnants, 1012 were found as anti-CMV IgG (+), so the rate of seropositivity was 98.5% in pregnant women, while 15 were found as anti-CMV IgG (-), with the rate of seronegativity as 1.5%. A second determination for anti-CMV IgG was done in six seronegative pregnants at the third trimester and in four during the first weeks after birth. None of

Table I. Distribution of Pregnant Women According to CMV Antibody Status

CMV Antibody	n*	%**
CMV IgM (-), IgG (+)	1000	97.3
CMV IgM (-), IgG (-)	15	1.46
CMV IgM (+), IgG (+)	12	1.16
CMV IgM (+), IgG (-)	0	0
Total	1027	100

*Number of pregnant women.
**Percentage of CMV-specific antibodies.

them was anti-CMV IgG(+). Anti-CMV IgG could not be determined in the other five seronegative women because they did not come to follow-up (Table II).

Table II. Maternal Infection Prevalence in Follow-Up of Pregnant Women

	Positive (Maternal infection)	Negative	Total
CMV IgM	12 (1.16%)	1015 (98.8%)	1027 (100%)

Maternal Infection

Among 1027 pregnant women, none had anti-CMV IgM (+) and anti-CMV IgG (-) antibody. Positivity of both anti-CMV IgM and anti-CMV IgG was detected in 12, so the prevalence of maternal CMV infection was found as 1.2%. Type of infection was identified by the AI of anti-CMV IgG. Out of 12 pregnants, three had low AI and were accepted as primary maternal infection, and eight had high AI and were accepted as recurrent/reinfected maternal infection. Primary CMV infection incidence was found as 0.3% and recurrent maternal infection as 0.8%. One pregnant woman with moderate AI was accepted as having undefined infection (Table III) (Fig. 2).

Prenatal Diagnosis

Three women with primary maternal infection and one with undefined infection underwent amniocentesis at 21-23 weeks of gestation. A very high quantity of CMV DNA (>3.26 x 10^5 copy/ml) was detected in the amniotic fluid by qPCR in one of them with primary maternal CMV infection. At routine obstetric examination of this pregnant (at 28 weeks of gestation), it was realized that the fetus was not alive and the pregnancy was terminated. CMV DNA was not detected in the amniotic fluid obtained from the other two. Pregnancy of these two women continued normally until term. There were no pathological findings related to congenital CMV infection on routine ultrasonographic examinations of these pregnant women. Pregnancy of eight women with recurrent/reinfected maternal infection continued normally until term and no evidence of pathological findings related to congenital infection was detected on routine ultrasonographic examinations.

Table III. Classification of Maternal CMV Infections According to AI

	Case no.	Avidity index (AI) (%)	Number of pregnant women (n=12)
Low AI (< 35%) (Primary maternal infection)	1.	26	3
	2.	23	
	3.	33	
Moderate AI (35-45%) (Undefined maternal infection)	4.	42	1
High AI (>45%) (Recurrent maternal infection)	5.	100	8
	6.	86	
	7.	56	
	8.	100	
	9.	69	
	10.	67.7	
	11.	52.8	
	12.	87	

Congenital Infection

Out of 12 women who had maternal CMV infection, 10 (1 terminated in abortus, the other moved to another city) completed their pregnancy and delivered babies with no physical

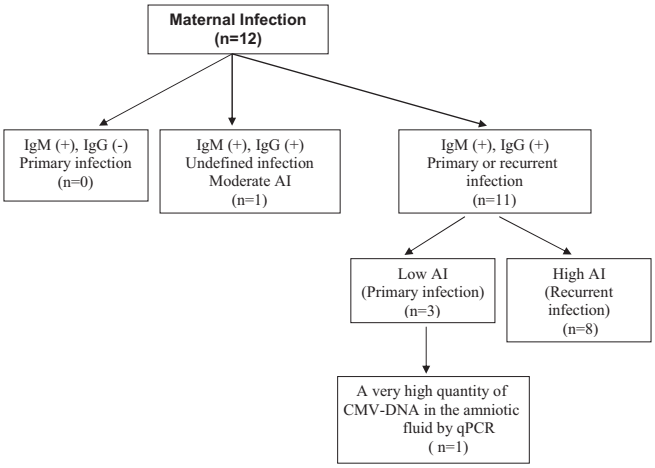


Fig. 2. Follow-up of our pregnant women with maternal infection. AI: avidity in index.

or hematological findings of CMV infection. Anti-CMV IgM positivity was not detected in blood samples of newborns (10 newborns) obtained during the first two weeks of life. CMV-DNA detection by PCR and CMV pp65 antigenemia test were also negative. Information about the newborn of the 12th pregnant, who had moved to another city, was obtained by telephone, and it was learned that she had also delivered a healthy baby whose blood samples were not eligible. Finally, the incidence of congenital CMV infection was found as 0.1% (1/1027) and the rate of intrauterine infection following the primary maternal infection as 33% (1/3). Infection was not detected in the newborns whose mothers had recurrent maternal infection (Fig. 2).

Discussion

Knowledge of the prevalence of seropositivity in pregnant women and the incidence of congenital infection in the various populations is useful in order to evaluate the socieconomic costs of this infection and to decide whether or not a screening program is necessary to identify it. Studies from different countries show that the prevalence of antibody to CMV among women of childbearing age in developing countries and in populations with low socioeconomic status is generally higher than that in developed countries[7,8,11,16-18].

Although the studies from Turkey investigating the seropositivity for CMV in women of childbearing age or postpartum women are few in number, their results have varied from 99% to 100%, and these seropositivity rates were in agreement with the rates of other developing countries[19-22]. The seroprevalence rate of pregnant women in our study was found as 98.5%, which is consistent with the rates in other parts of Turkey and in other developing countries. In a study by Ataman[23], the prevalence of CMV infection among women of childbearing age (15-49) living within the borders of the central municipality of Antalya was also found as 97.4%.

To our knowledge, this is the first study done in Turkey in which maternal and congenital CMV infection were investigated. In our study, the incidence of maternal CMV infection during pregnancy was found as 1.2%. However, blood samples were obtained from all pregnants between 8-20 weeks of gestation once, so we are not aware of pregnants that might have had a recurrent infection after 20 weeks. We suggest that the incidence might be slightly higher if we could have also screened the seropositive pregnants in the late second and third trimesters.

Most of the maternal infections are recurrent in underdeveloped and developing countries with high seropositivity rates. In our study, we also found the incidence of recurrent maternal CMV (66%) infection higher than primary (25%). Our results are consistent with the fact that high seropositivity is parallel with high recurrent versus primary maternal infection.

It is generally accepted that the incidence of congenital CMV infection varies from 0.2 to 2.0% of live births, depending on

socioeconomic conditions[24]. The incidence of congenital CMV infection seems to be lower (0.1%) in our study, probably due to the higher percent of seropositivity in our region. It is also probable that we skipped some of the congenital infections that might have occurred in the late second and third trimesters, as we screened the pregnants for CMV-specific antibodies only in the first trimester. However, if prevalence is evaluated on the basis of primary or recurrent maternal infections, it is not different from that of other studies. Although the rate of transmission to the fetus is accepted as 40% following primary maternal infection, results of studies on the basis of transmission rates vary widely, like 18% in the series of Fowler[25], 29% in the study of Bodeus[26] and 50% in Griffiths' study[27]. The large difference between these studies may be related not only with the different socioeconomic status of the study populations, but also with the definition of primary maternal infection. In Fowler's series, the pregnants were defined as having primary maternal infection with only CMV IgM antibody positivity, while determination of the avidity of anti-CMV IgG was also used in addition to anti CMV-IgM positivity in others. It is well known that anti-CMV IgM can become positive during recurrences, and maternal primary and recurrent infection cannot be identified unless the AI of anti-CMV-IgG is determined. The incidence of CMV infection following primary maternal CMV was 33.3% in our study, which is in agreement with the studies in which AI was used for the identification of primary and recurrent maternal CMV infection. We did not find any congenital infection among pregnants with recurrent infections. This result is not surprising because the number of pregnants with recurrent CMV infection was only 12 in our study and the reported incidence of congenital infection is 0.5-1% during maternal recurrence.

We performed amniocentesis at 21-23 weeks of gestation, as in the other studies, because it is well known that the fetus excretes CMV into the amniotic fluid by way of urine and fetal diuresis becomes established only after 20-21 weeks of gestation. It has been reported that CMV-DNA load by qPCR $>10^3$ genome/ml in amniotic fluid sample is predictive of intrauterine infection with 100% probability and $>10^5$ genome/ml is predictive of symptomatic infection. In our study, we performed amniocentesis in four women for prenatal diagnosis and three of them did not have CMV genome in their amniotic fluid. One of them had a very high quantity of CMV DNA ($>3.26x10^5$ copy/ml) and her fetus died in the intrauterine period. Termination of this pregnancy was done in another hospital, so histopathologic confirmation of diagnosis could not be established. Although sample size was limited in our study, presence of CMV DNA load $>10^5$ genome/ml in the amniotic fluid of the pregnant whose fetus died in utero confirmed the suggestion that CMV qPCR method seems to be a reliable marker for the diagnosis of symptomatic infection and also fetal damage.

In conclusion, routine antenatal screening seems to be reliable in countries where the incidence of maternal primary CMV infection is high, as it would be cost-effective considering the costs of care and treatment of CMV-afflicted infants. In countries with a high seroprevalance rate such as Turkey, it does not seem to be necessary to screen all pregnant women for CMV infection. According to the results of our study, seroprevalence of CMV in pregnants is high and most (66%) infections are recurrent maternal CMV infection, so it also does not seem to be cost-effective to screen all pregnant women for CMV infection in our region. However, it is generally accepted that high seropositivity is parallel with high recurrent maternal infection and, though not frequent, severe congenital infection may occur with recurrent maternal infections. Thus, before deciding not to screen women of childbearing age in developing countries, more studies are necessary to determine the extent to which reactivation or reinfection is responsible for congenital infection in the fetuses of women who are seropositive for CMV.

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