Congenital cytomegalovirus infection following first trimester maternal infection: Symptoms at birth and outcome

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Abstract

Background: The relationship between gestational age at time of maternal cytomegalovirus (CMV) infection and outcome of fetal infection is not well defined because the timing of maternal infection is usually not known.
Objective: To determine whether congenital cytomegalovirus (CMV) infection following primary maternal infection during the first trimester of pregnancy is more likely to lead to central nervous system (CNS) sequelae than fetal infection due to maternal infection later in pregnancy.
Study design: Using serum collected during pregnancy from mothers of newborns with congenital CMV infection, maternal infection was categorized as first trimester (<13 weeks) or later based on dates and results of IgG and IgM assays for CMV antibody. Outcome of congenital CMV infection was assessed by longitudinal fotlow-up of the infected cohort.
Results: Sensorineural hearing loss was found in 8/34 (24%) of children in the first trimester group, compared with 1/40 (2.5%) in the later infection group ($P = 0.01$, relative risk, 9.6). Considering any CNS sequela (hearing loss, mental retardation, cerebral palsy, seizures, chorioretinitis) 11/34 (32%) first trimester cases were affected compared with 6/40 (15%) in the later infection group ($P = 0.07$, relative risk 2.2). None of the later group had more than one sequela, compared with 4 (12%) of the first trimester group ($P = 0.04$).
Conclusions: Children with congenital CMV infection following first trimester maternal infection are more likely to have CNS sequelae, especially sensorineural hearing loss, than are those whose mothers were infected later in pregnancy. However, some degree of CNS impairment can follow even late gestational infection.
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Keywords: Congenital CMV infection; Gestational age; Birth defects; Hearing loss

1. Introduction

Although cytomegalovirus (CMV) is the leading cause of congenital infection in the United States, little is known about the relationship between gestational age at the time of maternal primary infection and clinical expression of fetal infection. Studying the effect of gestational age on the outcome of congenital CMV infection is difficult because it is difficult to define the time of onset of maternal infection. Studies of large cohorts of pregnant women have found that primary CMV infection occurs during pregnancy in around 2% of women, but over 95% of them have no identifiable illness (Ahlfors et al., 1984; Griffiths and Baboonian, 1984; Stagno et al., 1982) information on the effect of gestational age on outcome of congenital CMV infection is of practical prognostic value and is essential for considering strategies for prevention, diagnosis and treatment of infection during pregnancy. In order to determine the effect of gestational age on virulence of fetal infection, we compared the outcome of congenital CMV infection in infants born after a first trimester maternal infection with that of infants born after second or third trimester infection.

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2. Materials and methods

2.1. Study population

Criteria for inclusion of infants in this report were: (1) Congenital CMV infection proven by isolation of virus prior to 3 weeks of age. (2) Maternal primary CMV infection during pregnancy documented by seroconversion (from serum antibody negative to antibody positive) or detection of serum CMV-IgM antibody on the first prenatal sample. (3) Maternal primary infection could be categorized as occurring during the first trimester (prior to week 14 of gestation) or later ($\geq$14 weeks of gestation). Screening of newborn urine or saliva for CMV at University of Alabama at Birmingham Hospitals and at a private hospital in Birmingham between January 1980 and December 1993 identified 434 newborns with congenital CMV infection. Maternal primary infection during pregnancy was documented for 132 of the babies with congenital CMV infection. In 67 of these cases, the date of maternal infection could be categorized as either first trimester or later. Over the same time interval, 115 newborns were referred for evaluation of congenital CMV infection detected because of newborn symptoms or because of suspected maternal gestational infection. Only 12 of the referral cases had sera available that allowed them to be classified as first trimester or later. This study was approved by the UAB Institutional Review Board and mothers of participants signed an approved consent form.

2.2. Outcome of congenital CMV infection

Infants with congenital CMV infection were followed by the authors in a special clinic which provided serial age appropriate assessments aimed at identifying ocular, auditory, cognitive and neurological impairments, as previously described (Fowler et al., 1992).

2.3. Laboratory methods

Newborns were screened for congenital CMV infection by isolation of virus from a urine or saliva specimen collected in the hospital nursery as previously described (Fowler et al., 1993). Standard tissue culture techniques were used prior to August 1988 (Pass et al., 1995). A rapid, centrifugation enhanced method using monoclonal antibody to immediate early antigen in infected cells was used after 1988 (Balcarek et al., 1993). Serum IgG (IMx® System, Abbott Laboratories, Abbott Park, IL) and IgM (CMV STAT M, BioWhittaker Inc., Walkersville, MD) antibody to CMV was detected using enzyme immunoassay. The IgM antibody assay was performed as recommended by the manufacturer except that an EIA value of 0.6 (instead of 0.3) was used as the cut-off for a positive IgM result. Previous experience in our laboratory showed that this modification resulted in a reduced rate of false positive results with no significant change in sensitivity (Stagno et al., 1985). In order to assess the sensitivity and specificity of the CMV-IgM antibody assay for primary infec-

tion, sera from 300 women who had past infection were tested as were sera from 43 women with proven primary infection. The past infection group were women who had serum IgG antibody to CMV as least 1 year prior to the date of the test serum. The proven primary infection group was comprised of women who converted from serum CMV-IgG antibody negative to antibody positive.

2.4. Data analysis

Serologic and clinical data were assembled on case report forms and compiled in a computer database using PC-SAS (SAS, 1987). Means were compared using Student's t-test. Frequencies were compared using Fisher's exact test or the Chi square test.

3. Results

3.1. Detection of primary maternal infection

In order to determine the specificity of the CMV-IgM antibody assay for detection of primary infection, sera from 300 women known to be seropositive for at least 1 year were tested. IgM antibody to CMV was found in 2/300 (1.5%). Among 43 women who seroconverted from CMV-IgG negative to positive, 36 (83.7%) had CMV-IgM in their first positive serum. The mean interval between the last CMV-IgG negative serum and the first positive serum was 30 weeks. When the interval between negative and positive sera was <15 weeks, 12/13 (92.3%) of sera were CMV-IgM positive.

Primary maternal infection prior to 14 weeks of gestation was proven for 35 cases of congenital CMV infection; these comprised the first trimester group. For 44 cases of congenital CMV infection, maternal seroconversion occurred between week 14 of gestation and delivery; these comprised the post-first trimester group, for convenience henceforth referred to as the *later* group. IgM antibody results were not used to time maternal infection after the first trimester because of the variable (often >6 months) persistence of IgM antibody after primary CMV infection.

3.2. Characteristics of the study population

Demographic features of mothers and infants are shown in Table 1 which compares those with first trimester maternal infection to those infected later in pregnancy. Race, maternal age, marital status, socioeconomic status (percent with Medicaid or no insurance versus private health insurance), infant's sex and gestational age were very similar in the two groups. More of the first trimester infections were in primiparous mothers, and the mean birthweight of their babies was nearly 200 g less, but these differences were not statistically significant. Comparison of the demographic features of the 67 cases of congenital CMV infection identified by virologic screening of newborns with the 12 referred cases, revealed no

Table 1
Demographic characteristics of study population by gestational age at time of maternal cytomegalovirus infection

Feature	1st Trimester $N = 35$	≥ 14 weeks $N = 44$
Mothers		
Race		
White	20 (57)[a]	21 (48)
Black	15 (43)	23 (52)
Age (years)[b]	21.7 ± 4.2	21.0 ± 5.3
No prior pregnancies	23 (66)	22 (50)
Married	16 (46)	20 (45)
Medicaid/no insurance	19 (54)	26 (59)
Infants		
Sex (male)	12 (34)	20 (45)
Gestational age (weeks)[b]	39.0 ± 1.6	39.4 ± 1.5
Birth weight (g)[b]	3045 ± 507	3233 ± 516

[a] Numbers in parentheses are percent.
[b] Mean ± 1 standard deviation.

statistically significant differences in demographic features (not shown in Table 1).

3.3. Outcome of congenital CMV infection

There were 8/35 (23%) symptomatic neonates born to mothers with first trimester infection compared with 5/44 (11.4%) born to mothers with infection after the first trimester, a difference that was not statistically significant. The frequencies of various clinical findings at birth are shown in Table 2, according to time of primary maternal infection. There were no statistically significant differences in the frequencies for petechiae, hepatosplenomegaly, jaundice or abnormal neurologic findings in the newborn, though there was a trend toward greater severity of newborn disease in the first trimester group. Three or more abnormalities were present in four of eight symptomatic newborns in the first trimester group, while none of the three symptomatic neonates in the later group had more than two clinical abnormalities.

All but five patients (one from the first trimester group and four from the later group) had follow-up evaluations for determination of outcome. The most frequent abnor-

Table 2
Clinical findings at birth in newborns with congenital cytomegalovirus infection according to gestational age at time of maternal infection

Finding	1st Trimester	≥ 14 Weeks
N	35	44
Petechiae	5 (14.2)[a]	3 (6.8)
Hepatosplenomegaly	4 (11)	1 (2.3)
Jaundice	3 (8.6)	4 (9.1)
Abnormal neurologic[b] exam	3 (8.6)	0 (0)
Total symptomatic[c]	8 (23)	5 (11)

[a] Numbers in parentheses are percent.
[b] Includes patients with microcephaly, lethargy, hypotonia, or poor suck.
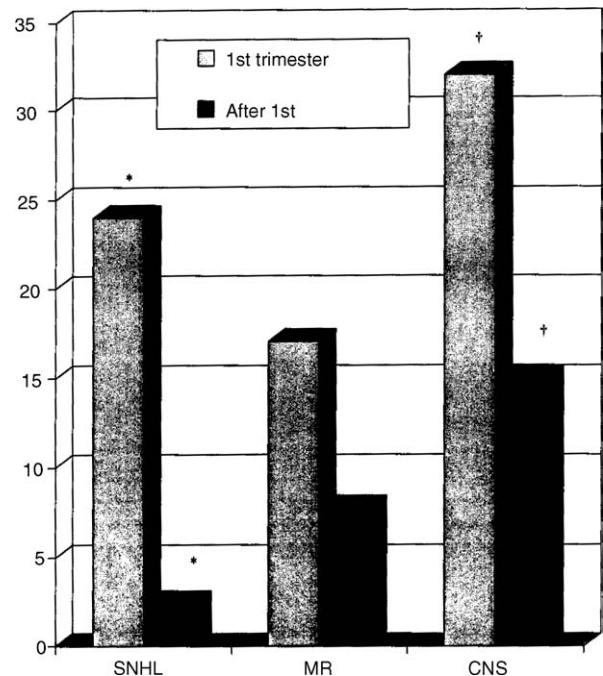[c] Includes patients with any of the above newborn findings.

Fig. 1. Percent of patients with central nervous system sequelae of congenital cytomegalovirus infection according to gestational age at time of maternal infection. SNHL, sensorineural hearing loss. MR, mental retardation (IQ < 70). CNS, central nervous system, indicating patients with any CNS sequelae. *$P = 0.01$; RR = 9.6. †$P = 0.07$; RR = 2.2.

mality on follow-up was sensorineural hearing loss; 8/34 (24%) patients in the first trimester group had sensorineural hearing loss compared with 1/40 (2.5%) in cases with later infection ($P = 0.01$, RR = 9.6). Neuropsychologic examinations were completed on 24 patients in the first trimester group and 26 in the later group; 4 (17%) of the former and 2 (7.7%) of the latter were mentally retarded (IQ < 70) (not statistically significant). Seizure disorder, cerebral palsy and chorioretinitis were seen respectively in 3, 2 and 1 patient from the first trimester group; seizures and chorioretinitis were each seen in one patient in the later group. The overall rate of CNS sequelae was 11/34 (32%) for the first trimester group compared with 6/40 (15%) for later infections ($P = 0.068$, RR = 2.2). Four (12%) of the first trimester cases had more than two CNS sequelae, compared with none of the infants born to women who were infected after the first trimester ($P = 0.038$). Frequencies of the major CNS sequelae are shown for comparison in Fig. 1.

3.4. Congenital CMV infection foliowina third trimester maternal infection can produce CNS sequelae

Nine infants with congenital CMV infection were born to mothers who were seronegative at 27 weeks gestation or later. Two of these were symptomatic as newborns and one has sensorineural hearing loss.

4. Discussion

Approximately 20–30% of infants with congenital CMV infection due to a first trimester primary maternal infection will have CNS sequelae. With maternal infection later in pregnancy, the rate of sequelae in infected offspring is lower. However, even third trimester maternal infections are capable of causing hearing loss, a result that is not surprising because progressive deterioration in hearing after birth is a characteristic feature of congenital CMV infection (Dahle et al., 1979; Fowler et al., 1997; Williamson et al., 1992). An association between outcome for the fetus, and newborn and gestationat age at time of maternal infection was suggested in reports in the 1980s by Ahlfors et al. (1983) and Stagno et al. (1986). These studies included very few cases of congenital infection that could be categorized as first trimester, though they did conclude that earlier gestational infections were more likely to have poor outcome and they noted cases with sequelae following late gestation maternal infection. Two more recent studies of outcome in relation to prenatal diagnostic results have reached similar conclusions based on a date of fetal infection established by testing of amniotic fluid and/or fetal blood. Liesnard studied 55 cases of congenital CMV infection from 237 pregnancies undergoing prenatal evaluation (Liesnard et al., 2000). Based on prenatal ultrasound and autopsy results in 26 fetuses from terminated pregnancies and clinical evaluations in 29 infected newborns they found that 10/38 (26%) cases infected before 20 weeks gestation had severe disease compared with only 1/16 (6.2%) infected after 20 weeks. In a similar study of prenatal diagnosis which included cases in which maternal infection occurred prior to conception, Daiminger et al. (2005) reported an association between periconceptional, and earlier gestation maternal or fetal infection and poor outcome for the fetus (Daiminger et al., 2005) However, 7 of 12 pregnancies with an infected fetus were terminated and thus outcome data was limited. Since IgM antibody to CMV persists from one to over 6 months after primary infection, it is possible that some of our first trimester infections occurred months prior to conception. Because of the persistence of IgM antibody for months after primary infection, IgM antibody results cannot be used to define maternal infection as second or third trimester. For example, if the first prenatal serum available is at the 28th week of gestation, IgM antibody to CMV could indicate first, second or third trimester infection. All of the maternal infections that occurred after the first trimester in this report were timed based on conversion from seronegative to seropositive.

The relationship between first trimester infection and outcome is much less distinct with congenital CMV infection than with congenital rubella. There are several notable differences between these infections. Rubella infection is highly contagious; during epidemics, it is often possible to identify maternal exposure to someone with an acute rash illness as the source of infection. Maternal rubella infection is usually symptomatic which facilitates determining the timing of maternal infection. Rubella is an acute viral infection, with

viral shedding lasting around 2 weeks or less; in contrast, the source of maternal CMV infection is usually unknown, but is likely to be a close contact such as a child or sex partner who is asymptomatic. CMV infection in the contacts and in the mother is a chronic infection with viral shedding for months to years (Jordan et al., 1973; Pass et al., 1982; Revello et al., 1998; Zanghellini et al., 1999). It is possible that transmission of CMV to the fetus occurs weeks or even months after maternal acquisition of CMV. Indeed, studies of prenatal diagnosis of fetal CMV infection have noted that the ability of virus detection in amniotic fluid to predict congenital infection in the newborn is diminished if the amniotic fluid studies are performed close to the time of maternal infection, suggesting that transmission to the fetus can occur weeks after maternal primary infection (Donner et al., 1993; Enders et al., 2001; Gourain et al., 2001; Revello et al., 1995).

It is well known that transplacental transmission of CMV can occur even when maternal infection occurs a year or more prior to conception. Congenital CMV infection in a newborn of a mother with past infection is usually attributed to a maternal *recurrence,* a term that encompasses the possibility of reactivation of endogenous latent virus, reinfection with a new strain or persistent active infection. A recent study provided molecular evidence that some of these congenital infections in offspring of immune mothers are associated with reinfection by a strain of CMV that differs from the original strain in a key antigenic site of envelope glycoprotein H (Boppana et al., 2001).

The effect of gestational age on transmission of recurrent maternal infection to the fetus and on the outcome of fetal infection remains unexplored because of difficulty in determining the timing of these maternal recurrences.

Although we did not evaluate transplacental transmission rates in relation to gestational age in this report, previous studies have reported that around 35–40% of primary maternal CMV infections are transmitted to the fetus (Stagno et al., 1982). Whether this rate is influenced by gestational age at the time of maternal infection has not been fully investigated. Stagno et al. found no difference in transmission rate comparing pregnancies with maternal CMV infection at 4–22 weeks, 16–27 weeks and 23–40 weeks (Stagno et al., 1986). Griffiths et al. suggested that the rate of transmission was lower with first trimester maternal infection (Griffiths and Baboonian, 1984). More recently, Bodeus reported an overall rate of transmission of 57.5% with maternal seroconversion during pregnancy; however, the transmission rate was lower, 36.0% with first trimester infection than with third trimester infection, 77.6% (Bodeus et al., 1999).

It is important to note that the current report is based on study of mothers after their newborn was found to have congenital CMV infection. Although the IgM antibody test we used allowed us to categorize maternal infection with reasonable accuracy in this population, it would not perform as well if used to screen prenatal patients. In prenatal patients, the rate of true positives (recent primary maternal infection) would likely be very similar to the rate of false positives (for

recent infection) of 1.5% that we noted in women known to have been infected for more than 1 year. Thus any positive screening result would as likely be a false positive as a true positive, and further laboratory evaluation would be required to determine whether the result was due to primary infection. Determination of IgG antibody avidity and CMV IgM reactivity with specific viral proteins has been used successfully for this purpose, and to identify candidates for prenatal fetal monitoring and diagnosis of fetal infection (Lazzarotto et al., 2000).

Acknowledgements

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