Prenatal diagnosis and outcome of congenital cytomegalovirus infection in twin pregnancies

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Objective To study the outcome of 20 twin pregnancies with evidence of primary or recurrent cytomegalovirus (CMV) infection during pregnancy.

Design Observational study.

Setting Two tertiary perinatal departments in Israel.

Population Twenty women with twin pregnancies who were referred because of serologic investigation indicating CMV infection. Seventeen women had evidence of primary CMV infection, and three women appeared to have recurrent CMV infection.

Methods Prenatal diagnosis was made by amniocentesis of both sacs after 21 weeks of gestation. CMV isolation was performed by culture on fibroblasts, shell vial technique and polymerase chain reaction (PCR) amplification of CMV DNA. After birth, the neonatal urine and saliva were cultured for CMV.

Main outcome measures Intrauterine CMV infection defined as positive PCR at amniotic fluid analysis and congenital CMV infection defined as positive CMV cultures after birth.

Results Except for one, all women underwent amniocentesis of both gestational sacs. In 14 (70%) women, no evidence of vertical transmission to any of the 28 fetuses was found and none of the newborns had evidence of congenital CMV infection. Intrauterine infection was detected by amniocentesis in five women and by ultrasound findings with positive maternal serology in one. In three women, CMV was detected in only one amniotic sac. In five of our six total cases, both twins were found to have congenital CMV infection at birth, all of whom had dichorionic–diamniotic placentation, three fused and two separate.

Conclusions In twin gestations, as in singletons, intrauterine and congenital CMV infection occurs in about 30% of women with primary or recurrent infection. The placenta type did not predict if one or both twins would be infected. Our data do not exclude the possibility that intrauterine transmission of the virus from one fetus to the other can occur.

Keywords Congenital infection, cytomegalovirus, prenatal diagnosis, twin pregnancies.

Introduction

Cytomegalovirus (CMV) infection is the most common intrauterine infection, with an incidence of 0.2–2.2% of live births.[1,2] The incidence of congenital CMV infection is high because mothers can transmit the virus to their fetuses following either primary or recurrent infection.[3,4]

After primary infection during pregnancy, the rate of transmission to the fetuses is approximately 30–40%. Children of women who have CMV antibodies before conception are partially protected and have only a 0.5–1% risk of congenital infection.[1,5]

More than 10–15% of congenitally infected neonates have symptoms at birth, and 5–15% of the infected infants without symptoms will have long-term neurologic sequelae such as mental retardation, deafness and visual impairment.[6–8]

It has been shown repeatedly that amniotic fluid viral isolation and polymerase chain reaction (PCR) are effective in differentiating uninfected and infected fetuses.[9,10] However, positive results of amniotic fluid tests such as viral isolation and PCR do not discriminate the infants who will have symptoms at birth, although quantitative PCR might partially enable such a prediction.[11]

Prenatal counselling of the woman with evidence of primary or recurrent CMV infection is complex and becomes more difficult when the infection occurs in a twin gestation. Twin pregnancies represent an interesting model because

different fetuses are simultaneously exposed to the same maternal influences. Only few case reports of CMV-infected twin pregnancies are described in the literature.[12–16]

The occurrence of CMV infection in twin pregnancies raises a few questions:

1. Is it possible that only one of the twins will be infected, and can the pattern of infection in twins be predicted by the chorionicity and placenta type?
2. Can the virus be transmitted prenatally from one fetus to the other?
3. What is the value of prenatal diagnosis by amniocentesis, if horizontal transmission of the virus from one fetus to the other occurs?

The aim of this study is to describe our experience with 20 twin pregnancies, in which the mothers had evidence of primary or recurrent CMV infection during pregnancy.

Methods

From 1997 to 2004, 20 women with twin pregnancies were referred to one of two tertiary perinatal departments in Israel for prenatal diagnosis of CMV infection. Fourteen of the twin pregnancies were dichorionic–diamniotic (DC/DA) twins, and six were monochorionic–diamniotic (MC/DA) twins. (Demographic and obstetric data are shown in Table 1.)

Sixteen women were referred after abnormal results had been obtained on CMV screening, two women were referred after serologic investigation because of symptomatic maternal infection (fever, flu-like symptoms) and two women were evaluated because of sonographic findings suggestive of fetal infection. Fifteen women were infected during the first trimester of pregnancy, and five were infected during the second trimester.

Each study subject had CMV infection documented by the presence of CMV-specific immunoglobulin (Ig) G or IgM (or both) in recent serologic assays. Maternal IgG or IgM assays

Table 1. Demographic and obstetric data

Characteristic	*n* = 20
Age (median)	28 (24–41)
Gravidity (median)	2 (1–7)
Parity (median)	1 (0–5)
In vitro fertilisation, *n* (%)	4 (20)
Twins' type, *n* (%)	
DC/DA	14 (70)
MC/DA	6 (30)
Primary infection, *n* (%)	17 (85)
Recurrent infection, *n* (%)	3 (15)
Gestational age at amniocentesis (median, in weeks)	22 (21–31)

were performed in various laboratories using enzyme-linked immunosorbent assay. Serologic diagnosis of primary CMV infection was documented by seroconversion (the appearance of *de novo* specific IgG and IgM antibodies in a woman who had been seronegative) or a significant rise of IgG antibody titre in the presence of specific IgM antibodies associated with low IgG avidity (<35%). Women with detectable specific IgG antibodies without IgM antibodies before pregnancy and a significant rise of IgG antibody titre with or without the presence of specific IgM antibodies and high IgG avidity (>45%) were classified as having recurrent infection. Based on the serologic tests, 17 women had evidence of primary CMV infection and 3 appeared to have recurrent CMV infection.

After written informed consent was obtained, the prenatal diagnosis was made by amniocentesis of both gestational sacs and by repeated, detailed ultrasonographic examination (every 3–4 weeks) to identify any abnormality associated with *in utero* fetal infection. Amniocentesis was performed in all cases after 21 weeks of gestation and at least 6 weeks after the first positive serologic results. Transabdominal ultrasound-guided amniocentesis was performed using a 21-gauge needle to collect 30 ml of amniotic fluid for the CMV assays and for fetal karyotype.

CMV isolation was performed by culture on fibroblasts, shell vial technique and PCR amplification of CMV DNA in amniotic fluid samples, as previously described.[17]

The twins chorionicity was established in all cases and was determined by a first trimester ultrasound scan.

After birth, during hospitalisation, the neonatal urine and saliva were cultured for CMV to determine whether congenital CMV infection was present. If positive CMV cultures resulted or if there were known abnormal ultrasound findings during pregnancy, cerebral ultrasound and hearing evaluations were performed.

Follow-up information was obtained from hospital's charts and from telephone interviews of the parents. The study was approved by the ethics committee of the Chaim Sheba Medical Center.

Results

Twenty women with twin pregnancies were evaluated, of whom 17 had evidence of primary CMV infection and 3 had evidence of recurrent infection.

With the exception of one , who elected not to undergo amniocentesis, all women underwent amniocentesis of both gestational sacs at a mean gestational age of 23.5 weeks.

Among the 17 women with primary CMV infection, 12 (71%) had no evidence of vertical transmission to any of their 24 fetuses (Figure 1). These 12 twin pregnancies included 7 DC/DA twins and 5 MC/DA twins. None of these infants had evidence of congenital CMV infection and none had
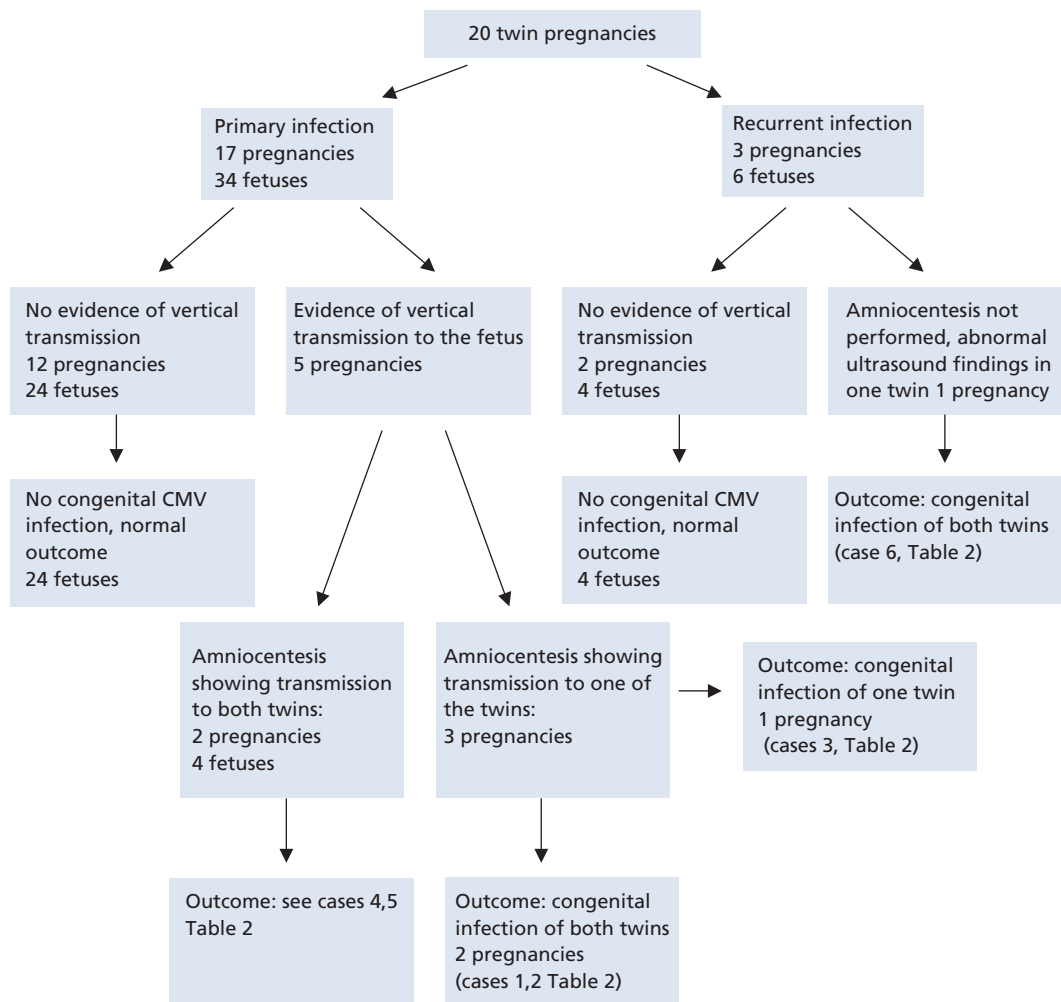
Figure 1. Prenatal diagnosis of CMV infection in twin pregnancies.

apparent disease during the neonatal course and within a median follow up of 36 months (range 4–72 months).

Five of the 17 women with primary CMV infection had evidence of vertical transmission by amniotic fluid analysis. All these five women had DC/DA twins in which two had fused placentas and three had separate placentas. In four of these women, ultrasonographic findings were normal throughout the pregnancy follow up, and in one woman, abnormal ultrasonographic findings were recorded in both the twins. The course and outcome of these pregnancies are detailed below (Table 2).

Case 1 was a woman with DC/DA twins with fused placentas, who had evidence of primary CMV infection at 18 weeks of gestation. Amniotic fluid analysis, performed at 27 weeks of gestation, demonstrated positive results of PCR, shell vial and culture in one sac, whereas the three tests were negative in the other sac. The pregnancy continued to term, and both newborns (both boys) were found to have congenital CMV

infection by positive virus isolation test in urine collected after birth. At 48 months of life, none of these infants had signs of CMV disease. No conclusive data exist to confirm the type of zygosity of these twins.

Case 2 was a pair of DC/DA twins with separate placentas and with evidence of primary CMV infection at 16 weeks of pregnancy. Amniotic fluid analysis at 24 weeks of pregnancy revealed positive results of PCR in one sac (shell vial and culture were negative) and negative results in the other sac. The pregnancy continued to term, and both newborns had congenital CMV infection confirmed by positive urine cultures, with no signs of disease at the age of 12 months.

Case 3 was a dizygotic twin pregnancy with a documented primary CMV infection at 10 weeks of gestation. The placentas were DC/DA and separated. Amniotic fluid analysis, performed at 22 weeks of gestation, demonstrated CMV infection in one sac (all three tests were positive) and no evidence of vertical transmission in the other sac. The patient

Table 2. Results of prenatal diagnostic tests and pregnancy outcome in CMV-infected twins

Case no.*	Type of infection	GA at infection	Placentas	Zygosity	GA at amniocentesis	Amniotic fluid results	Ultrasound finding	Congenital CMV infection	Postnatal findings
1.A	Primary	18	DC/DA, fused	Unknown	27	+	−	+	Asymptomatic
1.B						−	−	+	Asymptomatic
2.A	Primary	16	DC/DA, separate	Unknown	24	−	−	+	Asymptomatic
2.B						+	−	+	Asymptomatic
3.A	Primary	10	DC/DA, separate	Dizygotic	22	−	−	−	Asymptomatic
3.B						+	−	TOP	
4.A	Primary	20	DC/DA, separate	Dizygotic	31	+	−	TOP	
4.B						+	−	TOP	
5.A	Primary	10–12	DC/DA, fused	Dizygotic	22	+	+	IUFD	
5.B						+	+	IUFD	
6.A	Recurrent	7–8	DC/DA, fused	Dizygotic	−		−	+	Asymptomatic
6.B							+	+	CMV disease, death**

GA, gestational age; IUFD, intrauterine fetal death; TOP, termination of pregnancy.
*A refers to twin A and B refers to twin B.
**Intra ventricular haemorrhage, hepatitis, pancytopenia.

elected to undergo selective termination after being counselled concerning the disease. At 28 weeks of gestation and after approval of the institutional committee for termination of pregnancies, the patient underwent selective termination of the infected fetus. During the procedure, an amniocentesis of the second sac was repeated, with all tests being negative for CMV infection. Pregnancy was continued to term, and at birth, urine and saliva cultures for CMV were negative. No clinical evidence of congenital infection was detected at the age of 6 months.

Case 4 was a dizygotic twin pregnancy with a documented primary CMV infection at 20 weeks of gestation. The placentas were DC/DA and separated. Amniotic fluid analysis, performed at 31 weeks of gestation, demonstrated CMV infection in both the sacs. Due to the woman's request and after approval of the institutional committee, she underwent termination of pregnancy at 32 weeks of gestation. Histopathological examination of both twins did not demonstrate features characteristic of cytomegalic inclusion disease.

Case 5 was a DC/DA twin pregnancy with documented primary CMV infection at 10–12 weeks of gestation. Abnormal ultrasonographic findings were recorded in both twins including intrauterine growth restriction (IUGR) and microcephaly. Amniotic fluid analysis, performed at 22 weeks of gestation, demonstrated positive results of all three tests in both the sacs. Pregnancy was complicated by *in utero* death of both twins at 30 weeks of gestation.

Recurrent CMV infection was documented in three women. Two of these women had DC/DA twins, and one had MC/DA twins. Two of them had no evidence of vertical transmission to any of their fetuses (one pregnancy was DC/DA twins, and one was MC/DA twins). None of these four infants had evidence of congenital CMV infection and none had apparent disease after delivery. The other woman, who had evidence of recurrent CMV infection, was referred at 28 weeks because of abnormal ultrasonographic findings in one of the twins including IUGR, ventriculomegaly and periventricular calcifications (case 6; Table 2). This was a dizygotic twin pregnancy in which the placentas were DC/DA and fused. There was serological evidence of recurrent CMV infection during the first trimester. The woman elected not to undergo amniocentesis and was delivered by caesarean section at 33 weeks of gestation due to a non-reassuring monitor of the smaller twin. The birthweights of the two male infants were 1350 and 749 g. After birth, both the twins had evidence of congenital CMV infection by repeated positive urine cultures and were treated with gancyclovir. The smaller twin (the one in whom abnormal ultrasonographic findings were noted) had symptomatic CMV disease including a grade 4 intraventricular haemorrhage, intracerebral calcifications, hepatitis and pancytopenia. He suffered from repeated episodes of seizures and died at the age of 36 days due to multiorgan failure. The other twin was asymptomatic and had a normal neonatal course. Cranial ultrasonography showed lesions in the basal ganglia. At the age of 7 months, he was healthy and developing normally without auditory impairment.

Discussion

The present study is the largest one reported so far of twin pregnancies, with maternal serologic evidence of CMV infection that was prenatally diagnosed. There have been a few case reports of CMV infection in twins, but in most of them, a prenatal diagnosis was not performed.

Among the 20 twin pregnancies in the present study, 14 had no evidence of vertical transmission of the virus and in 6 pregnancies, intrauterine infection was detected. In all 28 fetuses (14 pairs) with no evidence of vertical transmission by amniocentesis, postnatal tests ruled out congenital CMV infection, i.e. there was no false negative in the detection of intrauterine infection when both twins were negative at amniocentesis.

In five of the six twin pairs with evidence of infection, both twins were infected according to postnatal tests. All these five twin pairs were DC/DA, in which three had fused placentas and in two, the placentas were separate. Therefore, the presence of separate placentas does not eliminate the possibility that both twins will be infected. There are four case reports in the literature of DC/DA twins with fused placentas and evidence of congenital CMV infection. In three of them, both twins were infected, suggesting that the presence of fused placentas might increase the risk of infection of both twins.[12–14] However, Ahlfors et al.[15] described dizygotic twins with DC/DA and fused placentas, in which only one twin was infected. Segui and Cho[16] described MC/DA twins in which only one fetus was infected. Based on our observations, we conclude that the placenta type cannot predict the pattern of infection in twins.

Case 6 in our study and previous case reports show that even when both twins are infected, their clinical outcome might be completely different. Saigal et al.[12] reported on a case of twins in which both siblings were asymptomatic at birth. Although infection occurred in both twins, one member was normal at follow up, while the other had bilateral deafness. Lazzarotto et al.[14] showed that the clinical outcomes of congenital infection differed in dizygotic infants even if both placentas were infected. They suggested that viral load in the amniotic fluid was correlated to pregnancy outcomes, a high viral load being linked with congenital infections symptomatic at birth.

Congenital CMV infection is almost invariably manifested by positive histopathological findings in the placenta, indicating that the placenta is the main entrance for CMV to the fetus and that maternal viraemia is the initiating cause of congenital infection.[18] According to our study and previous case reports, if vertical transmission occurs in DC/DA twins with fused placentas, both fetuses are likely to be infected. We postulate that initially maternal viraemia causes infection of the least resistant placenta. Placenta fusion and local increase of the amount of virus in the infected placenta/fetus lead to viral transfer from the infected fetus to the more resistant twin. The hypothesis is strengthened by our observation that in two twin pairs, amniotic fluid analysis revealed intrauterine infection of only one sibling, while postnatal tests showed CMV infection of both twins. In one of these cases, the placentas were separated, which implies that prenatal horizontal acquisition of the infection could happen even in the absence

of fusion of the placentas. Lazzarotto et al.[14] described a similar case of DC/DA twins with fused placentas, in which amniocentesis at 21 weeks of gestation showed infection of only one twin. After delivery, congenital infection of both newborns was diagnosed by positive virus isolation in urine collected 1 day after birth. We believe the possibility that our observation attributable to a late intrauterine viral transmission from the mother to the second fetus is unlikely because the viraemia phase in immunocompetent subjects is very short (<30 days).[14,19] We cannot, however, rule out the rare but existing possibility that our observation is due to false-negative laboratory results due to the limits of detection of laboratory testing. Therefore, prenatal transmission of the virus from one fetus to the other is the best explanation for the discrepancy between the prenatal amniotic fluid tests and the postnatal urine cultures in these two pregnancies.

Our study also included a case of dizygotic twins with separate DC/DA placentas, in which only one member of the twins was congenitally infected with CMV. There are four case reports of twin pairs with separate DC/DA placentas, in which only one member in each pair was congenitally infected with CMV.[15,20–22] Best[23] suggested that women, who transmit CMV to their fetuses, might have defective immunological response and therefore are unable to limit replication of the virus. However, our findings and previous case reports, which showed that only one out of a pair of twins exposed to the same maternal influences becomes infected, contradict this idea.

In summary, serological evidence of maternal primary or recurrent CMV infection in twin pregnancies should prompt prenatal diagnosis by amniocentesis. In our study, the placenta type could not predict if one or both twins would be infected, and in DC/DA gestations, separate placentas do not guarantee infection of only one twin. However, even in cases in which both twins are infected, one can be completely asymptomatic, while the other suffers from generalised CMV disease. In addition, this study suggests that prenatal transmission of the virus from one fetus to the other is possible. Because of the possibility of prenatal horizontal acquisition of the infection, prenatal diagnosis by a single amniocentesis has a limited value. If amniocentesis demonstrates infection of only one twin, a second amniocentesis at the third trimester should be considered in order to rule out late intrauterine transmission of the virus from one fetus to the other. This option of repeating amniocentesis at the third trimester should be reserved for women who consider termination of pregnancy once intrauterine infection is detected. More data concerning CMV infection in twins are needed in order to evaluate the necessity of this option and to define the right timing for performing a second amniocentesis.

Prenatal counselling of women with evidence of CMV infection in twin pregnancies is a very complicated task. Moreover, accurate prenatal diagnosis is essential, especially

in countries where the option of late termination of pregnancy is available.

Acknowledgements

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