Risk factors for congenital cytomegalovirus infection in pregnant women with non-primary infection

Hideto Yamada,[1] Kenji Tanimura,[1] Shinya Tairaku,[1] Ichiro Morioka,[2] Masashi Deguchi,[1] Mayumi Morizane,[1] Satoshi Nagamata,[1] Kana Ozaki,[1] Yasuhiko Ebina,[1] and Toshio Minematsu.[3]

[1]Department of Obstetrics and Gynecology, Kobe University Graduate School of Medicine, Kobe, Japan

[2]Department of Pediatrics, Kobe University Graduate School of Medicine, Kobe, Japan

[3]Reserch Center for Disease Control, Aisenkai Nichinan Hospital, Miyazaki, Japan

Corresponding author: Hideto Yamada M.D., Ph.D., Professor and Chairman

Department of Obstetrics and Gynecology, Kobe University Graduate School of Medicine,

7-5-1, Kusunoki-Cho, Chuo-ku, Kobe 650-0017, Kobe, Japan

Tel: +81-78-382-6000; fax: +81-78-382-5756

E-mail: yhideto@med.kobe-u.ac.jp

All authors meet the ICMJE authorship criteria.

Running title: Non-primary CMV infection in pregnancy

Abstract

The aim of this cohort study was to evaluate risk factors for the occurrence of congenital cytomegalovirus (CMV) infection in pregnant women with non-primary CMV infection. In a prospective study of CMV screening for 2,193 pregnant women and their newborns, seven newborns with congenital CMV infection were identified among 1,287 pregnant women with non-primary CMV infection that was defined as negative IgM and positive IgG with IgG avidity index >45%. In the 1,287 women with non-primary CMV infection, clinical findings and complications were compared between pregnancies with and without congenital CMV infection. Risk factors for the occurrence of congenital CMV infection were evaluated. The birth weight of newborns with congenital CMV infection was less than that of newborns without congenital infection (p<0.05). Univariate logistic regression analyses demonstrated that threatened premature delivery (OR 10.6, 95%CI 2.0–55.0; p<0.01) and multiple pregnancy (OR 7.1, 95%CI 1.4–37.4; p<0.05) were associated with congenital infection. Multivariable logistic regression analyses demonstrated that threatened premature delivery (OR 8.4, 95%CI 1.5–48.1; p<0.05) was a single risk factor for congenital CMV infection in pregnant women with non-primary CMV infection. This prospective cohort study revealed for the first time that threatened premature delivery was a risk factor for the occurrence of congenital CMV infection in pregnant women with non-primary CMV infection, the pathophysiology of which may be closely associated with CMV reactivation during pregnancy.

Keywords: Congenital infection; Cytomegalovirus; Non-primary infection; Pregnancy; Screening

1. Introduction

Cytomegalovirus (CMV) is the most common mother-to-child infection in humans. The prevalence of congenital CMV infection is 0.2-2.0% in newborns [1], and 10-15% of infected newborns have symptomatic CMV infection. The clinical manifestations include fetal growth restriction (FGR), low birth weight, central nervous system and multiple organ involvement with petechiae, hepatomegaly, splenomegaly, jaundice, pneumonia and encephalitis. It can be so severe that approximately 90% of the surviving infants have a high perinatal mortality rate and major neurological sequelae [2]. In addition, 10–15% of infants with asymptomatic congenital CMV infection develop long-term sequelae, such as progressive sensorineural hearing difficulty and mental retardation [2, 3].

The risk of virus transmission to the fetus is highest in women with primary CMV infection during pregnancy [4, 5]. Maternal serological screening is considered effective for detecting primary CMV infection in pregnant women; and maternal blood tests for CMV-specific immunoglobulin (Ig) G and IgM are widely used [6]. However, pregnant women can produce CMV IgM during viral reinfection and reactivation [7]; moreover, IgM may persist for more than several months after the primary infection [8]. Therefore, CMV IgG avidity test

is employed for the detection of primary CMV infection in pregnant women with positive IgM [9], and low avidity index (AI) is considered a sign of primary infection during pregnancy [10, 11].

Our prospective study of 2,193 pregnant women and their newborns has evaluated the efficacy of maternal universal screening for the prediction of congenital CMV infection using CMV IgG, AI, and IgM. This study has demonstrated that that 30% (3/10) of newborns with congenital CMV infection are born to mothers with primary CMV infection, while 70% (7/10) are born to mothers with non-primary CMV infection, suggesting that the majority of congenital infection of newborns is caused by non-primary CMV infection during pregnancy [12]. Likewise, in the United States, 25% of newborns with congenital CMV infection are born to mothers with primary CMV infection, while 75% are caused by non-primary infection [13].

A cohort study was conducted to determine risk factors for the occurrence of congenital CMV infection in pregnant women with non-primary CMV infection.

2. Patients and Methods

The institutional review board at Kobe University Hospital approved the prospective cohort study, and written informed consent was obtained from all participants. All pregnant women who visited or were referred to Kobe University Hospital between February 2010 and April 2016 underwent maternal serological CMV screening. The screening method was

described previously [12]. Briefly, pregnant women underwent initial blood screening for CMV IgG before 22 gestational weeks (GW) or when they were referred to the hospital. CMV IgG-negative women underwent IgG measurement again at 34–36 GW. All CMV IgG-positive women were tested for serum IgG avidity. Women who had a CMV IgG AI ≤45% underwent IgM measurement. Sera form women with an AI >45% were stored at −80°C, and CMV IgM levels were later measured. All newborns received polymerase chain reaction (PCR) analyses of the urine, and congenital infection was diagnosed with the detection of CMV-DNA in the urine. Measurement methods for blood levels of CMV IgG, IgM, AI, and real-time PCR for CMV-DNA in the urine were described previously [12]. In this prospective study of CMV screening for 2,193 pregnant women and their newborns, seven newborns with congenital CMV infections were identified among 1,287 pregnant women with non-primary CMV infection that was defined as negative IgM and positive IgG with IgG avidity index >45% in their serum. Symptomatic congenital CMV infection was diagnosed when newborns with congenital CMV infections had microcephaly, hepatosplenomegaly/hepatitis, thrombocytopenia, abnormality of brain images, retinopathy, or abnormal auditory brain-stem response (ABR) [12].

In the present cohort study for the 1,287 pregnant women with non-primary CMV infection, clinical findings and pregnancy complications were compared between pregnancies with and without congenital CMV infection. Clinical findings including age, gravidity, parity,

body mass index (BMI), percentage of referrals, history of recurrent pregnancy loss, presence of maternal fever or flu-like symptoms, GW at the initial CMV IgG measurement, GW at delivery and birth weight were compared between pregnancies with and without congenital CMV infection. Risk factors for the occurrence of congenital CMV infection in pregnant women with non-primary CMV infection were evaluated. Pregnancy complications, including hypertensive disorders, thyroid diseases, diabetes mellitus/gestational diabetes mellitus, medical diseases requiring immunosuppressive therapy, threatened premature delivery, multiple pregnancy, FGR, preterm delivery and light-for-date, were assessed. Threatened premature delivery was defined as the condition that required intravenous administration of tocolytic agents such as magnesium sulfate and β-stimulant during hospitalization for one or more weeks

Differences between the two groups were analyzed using the Mann–Whitney *U* test, Fisher's exact test, and the chi-square test. Statistical significance was considered present at *p* values less than 0.05. A stepwise approach was used to evaluate risk factors for congenital CMV infection from women with non-primary infection. Variables with *p*-values less than 0.05 in univariate logistic regression analyses were subjected to multivariable logistic regression analyses, and variables with *p*-values less than 0.05 in multivariable logistic regression analyses were determined as independent factors. All statistical analyses were performed using SPSS software, version 19 (SPSS Inc., Chicago, IL, USA).

Results

In the prospective study of CMV screening for 2,193 pregnant women and their newborns, three newborns with congenital CMV infection were born to mothers with primary CMV infection that was defined as IgG seroconversion, or positive/borderline IgM and positive IgG with AI <35% [12]. Seven (0.54%) newborns with congenital CMV infections were also identified in 1,287 women with non-primary CMV infection that was defined as negative IgM and positive IgG with AI >45% (Table 1). Three (42.9%) of the seven newborns had symptomatic infection. Case 4 had bilateral abnormal ABR and ventriculomegaly; case 6 had low platelet counts and liver dysfunction; and case 7 had low platelet counts. Only case 4 received valganciclovir therapy (16 mg/kg/day, 6 weeks), after which the abnormal ABR was restored to normal. Case 5 was classified as asymptomatic congenital infection, because this case had only a symptom of small for gestational age.

Table 2 shows clinical characteristics of seven pregnancies with congenital CMV infection and 1,280 pregnancies without congenital infection in women with non-primary CMV infection. The birth weight of newborns with congenital CMV infection was less than that of newborns without congenital infection (p<0.05). The proportion of threatened premature delivery in pregnancies with congenital CMV infection of fetuses was higher than

that in pregnancies without congenital infection (p<0.01). Other clinical findings or pregnancy complications were not significantly different between the two groups.

Table 3 shows the results of univariate and multivariable logistic regression analyses of risk factors for the occurrence of congenital CMV infection in pregnant women with non-primary infection. Univariate logistic regression analyses demonstrated that threatened premature delivery [odds ratio (OR) 10.6, 95% confidence interval (CI) 2.0–55.0; p<0.01] and multiple pregnancy (OR 7.1, 95% CI 1.4–37.4; p<0.05) were associated with the occurrence of congenital CMV infection. Multivariable logistic regression analyses of these two factors revealed that threatened premature pregnancy (OR 8.4, 95% CI 1.5–48.1; p<0.05) was a significant risk factor for the occurrence of congenital CMV infection in pregnant women with non-primary CMV infection.

Discussion

This cohort study demonstrated that 7 (0.54%) of 1,287 women with non-primary CMV infection during pregnancy delivered newborns with congenital CMV infection. The transmission rate of CMV to a fetus was consistent with that reported previously, showing that 0.5-1.0 % of women with non-primary CMV infection delivered newborns with congenital CMV infection [14, 15]. It has been thought that the majority of symptomatic congenital CMV infection is caused by primary CMV infection of women either during or just before pregnancy

[16]. However, other retrospective studies report that disease manifestations, severity and sequelae are similar between congenitally infected newborns born to mothers with primary and those with non-primary CMV infection during pregnancy [17, 18]. The present prospective study demonstrated for the first time that three (42.9%) of the seven newborns born to mothers with non-primary CMV infection had symptomatic congenital CMV infection, and discovered that not only women with primary CMV infection during pregnancy but also those with non-primary CMV infection carry a high risk of delivering newborns with symptomatic congenital infection. A recent retrospective study also reported that non-primary CMV infection was responsible for the majority of symptomatic congenital infection and resulted in significant morbidity of newborns in Finland [19].

It is reported that low birth weight and preterm delivery are risk factors for symptomatic congenital CMV infection in women with primary CMV infection during pregnancy [20, 21]. No prospective studies have evaluated risk factors for the occurrence of congenital CMV infection in women with non-primary CMV infection during pregnancy. In the present study, the seven pregnancies with congenital CMV infection caused by non-primary maternal CMV infection had a variety of pregnancy complications. Clinical findings and pregnancy complications were compared between pregnancies with and without congenital CMV infection. As a result, pregnancies with congenital CMV infection had lower birth weights ($p<0.05$), higher frequencies of threatened premature delivery ($p<0.01$), multiple

pregnancy (p=0.05) and preterm delivery (p=0.07) than pregnancies without congenital infection. These pregnancy complications are closely associated with prematurity and/or inflammation. Univariate and multivariable logistic regression analyses revealed for the first time that threatened premature delivery was a single risk factor for the occurrence of congenital CMV infection in pregnant women with non-primary CMV infection. The presence of threatened premature delivery may reflect the conditions in which CMV is reactivated; alternatively, it may give rise to the necessary conditions for CMV reactivation in pregnant women. The pathophysiology underlying threatened premature delivery may be closely associated with CMV reactivation during pregnancy.

Physical stimulation, bleeding and bacterial infection at the decidua and villi i.e., the fetomaternal interface may activate the latent virus in the uterus, and subsequently cause congenital CMV infection of fetuses. CMV reactivates from latently infected macrophages and dendritic cells in different organs [22, 23]. The inflammatory response associated with the cause or effect of threatened premature delivery is likely to reactivate CMV from latently infected cells in the uterus, leading to the mother-to-fetus CMV transmission. Recently, we reported that the presence of CMV-DNA in the uterine cervical secretion was closely associated with CMV transmission to fetuses in pregnant women with positive CMV IgM [24]. Whether the presence of CMV-DNA in the uterine cervical secretion is a risk factor for congenital CMV infection in pregnant women with non-primary CMV infection should be

further studied.

A high risk for congenital CMV infection is thought be indicated by CMV IgG seroconversion, positive IgG with AI <35%, and/or positive IgM in pregnant women with primary infection. However, our prospective study revealed that 70% (7/10) of newborns with congenital CMV infection were born to mothers with non-primary CMV infection, and 42.9% (3/7) of these newborns were symptomatic. Maternal serological screening for the detection of primary CMV infection during pregnancy is insufficient to effectively identify all newborns with congenital CMV infection. Universal screening of PCR tests for CMV-DNA in the newborn urine will identify all newborns with congenital infection.

Kobe University Hospital has a maternal-fetal center where many pregnant women with a variety of complications are referred from local clinics and hospitals. If high-risk pregnancies for threatened premature labor and premature delivery were excluded from the study subjects, the risk factor for the occurrence of congenital CMV infection would differ. Clinics and hospitals treating low-risk pregnancies for prematurity may experience fewer newborns with congenital CMV infection.

Acknowledgments

We are grateful for participation of the subjects and care provided by staff at Kobe University Hospital. We thank the clinical and laboratory personnel who supported this study at Kobe University Hospital and Aisenkai Nichinan Hospital.

This work was supported by the Ministry of Health, Labour and Welfare of Japan (grant nos. H23-Jisedai-Ippan-001, AM55708030).

Potential conflicts of interest

All authors report no potential conflicts of interest.

References

1. Pass RF. Cytomegalovirus. In: Knipe DM and Howley PM, editors, 4th. Vol 2. LIPPINCOTT WILLIAMS & WILKINS, Philadelphia, PA; 2001, p. 2675-2705.

2. Stagno S, Whitley RJ. Herpesvirus infections of pregnancy. Part I: Cytomegalovirus and Epstein-Barr virus infections. N Engl J Med 1985;313:1270-4.

3. Stagno S, Pass RF, Cloud G, Britt WJ, Henderson RE, Walton PD, et al. Primary cytomegalovirus infection in pregnancy. Incidence, transmission to fetus, and clinical outcome. JAMA 1986;256:1904-8.

4. Gaytant MA, Steegers EA, Semmekrot BA, Merkus HM, Galama JM. Congenital cytomegalovirus infection: review of the epidemiology and outcome. Obstet Gynecol Surv 2002;57:245-56.

5. Fowler KB, Stagno S, Pass RF, Britt WJ, Boll TJ, Alford CA. The outcome of congenital cytomegalovirus infection in relation to maternal antibody status. N Engl J Med 1992;326:663-7.

6. Walker SP, Palma-Dias R, Wood EM, Shekleton P, Giles ML. Cytomegalovirus in pregnancy: to screen or not to screen. BMC Pregnancy Childbirth 2013;13:96.

7. Revello MG, Fabbri E, Furione M, Zavattoni M, Lilleri D, Tassis B, et al. Role of prenatal diagnosis and counseling in the management of 735 pregnancies complicated by primary human cytomegalovirus infection: a 20-year experience. J Clin Virol 2011;50:303-7.

8. Lazzarotto T, Guerra B, Gabrielli L, Lanari M, Landini MP. Update on the prevention, diagnosis and management of cytomegalovirus infection during pregnancy. Clin Microbiol Infect 2011;17:1285-93.

9. Manicklal S, Emery VC, Lazzarotto T, Boppana SB, Gupta RK. The "silent" global burden of congenital cytomegalovirus. Clin Microbiol Rev 2013;26:86-102.

10. Lazzarotto T, Guerra B, Lanari M, Gabrielli L, Landini MP. New advances in the diagnosis of congenital cytomegalovirus infection. J Clin Virol 2008;41:192-7.

11. Ebina Y, Minematsu T, Sonoyama A, Morioka I, Inoue N, Tairaku S, et al. The IgG avidity value for the prediction of congenital cytomegalovirus infection in a prospective cohort study.

J Perinat Med 2014;42:755-9.

12. Tanimura K, Tairaku S, Morioka I, Ozaki K, Nagamata S, Morizane M, et al. Universal screening with use of immunoglobulin g avidity for congenital cytomegalovirus infection. Clin Infect Dis 2017;65:1652-8.

13. Wang C, Zhang X, Bialek S, Cannon MJ. Attribution of congenital cytomegalovirus infection to primary versus non-primary maternal infection. Clin Infect Dis 2011;52:e11-13.

14. Kenneson A, Cannon MJ. Review and meta-analysis of the epidemiology of congenital cytomegalovirus (CMV) infection. Rev Med Virol 2007;17:253-76.

15. Soper DE. Congenital cytomegalovirus infection: an obstetrician's point of view. Clin Infect Dis 2013;57 Suppl 4:S171-3.

16. Fowler KB, Stagno S, Pass RF, Britt WJ, Boll TJ, Alford CA. The outcome of congenital cytomegalovirus infection in relation to maternal antibody status. N Engl J Med 1992;326:663-7.

17. Boppana SB, Fowler KB, Britt WJ, Stagno S, Pass RF.

Symptomatic congenital cytomegalovirus infection in infants born to mothers with preexistin gimmunity to cytomegalovirus. Pediatrics 1999;104:55-60.

18. Ross SA, Fowler KB, Ashrith G, Stagno S, Britt WJ, Pass RF, et al. Hearing loss in children with congenital cytomegalovirus infection born to mothers with preexisting immunity. J Pediatr 2006;148:332-6.

19. Puhakka L, Renko M, Helminen M, Peltola V, Heiskanen-Kosma T, Lappalainen M, et al. Primary versus non-primary maternal cytomegalovirus infection as a cause of symptomatic congenital infection - register-based study from Finland. Infect Dis (Lond) 2017;49:445-53.

20. Nigro G, Capretti I, Manganello AM, Best AM, Adler SP. Primary maternal cytomegalovirus infections during pregnancy: association of CMV hyperimmune globulin with gestational age at birth and birth weight. J Matern Fetal Neonatal Med 2015;28:168-71.

21. Lorenzoni F, Lunardi S, Liumbruno A, Ferri G, Madrigali V, Fiorentini E, et al. Neonatal screening for congenital cytomegalovirus infection in preterm and small for gestational age infants. J Matern Fetal Neonatal Med 2014;27:1589-93.

22. Cook CH, Zhang Y, McGuinness BJ, Lahm MC, Sedmak DD, Ferguson RM. Intra-abdominal bacterial infection reactivates latent pulmonary cytomegalovirus in immunocompetent mice. J Infect Dis 2002;185:1395-400.

23. Pereira L, Maidji E, McDonagh S, Genbacev O, Fisher S. Human cytomegalovirus transmission from the uterus to the placenta correlates with the presence of pathogenic bacteria and maternal immunity. J Virol 2003;77:13301-14.

24. Tanimura K, Tairaku S, Ebina Y, Morioka I, Nagamata S, Deguchi K, et al. Prediction of congenital cytomegalovirus infection in high-risk pregnant women. Clin Infect Dis 2017;64:159-65.

Table 1. Seven newborns with congenital cytomegalovirus infection from mothers with non-primary cytomegalovirus infection

Case	Age (years old)	Gravidity / Parity	Pregnancy complications (Weeks of gestation at admission)	Weeks of gestation at flu-like symptoms	Weeks of gestation at IgG avidity measurements	CMV IgG	IgG avidity index (%)	CMV IgM (Index)	Weeks of gestation at birth	Birth weight (g)	Symptoms of congenital infection	Development/ Sequela (age)
1	31	3/3	Twin pregnancy TPD (33)	12	15	13,876	76.2	<0.8	35w2d	2,362	None	Normal (3 years old)
2	29	1/1	TPD (29)	30	32	13,000	66.2	<0.8	40w5d	2,956	None	Normal (3 years old)
3	33	4/2	Thyroid disease TPD (29)	None	28	12,000	78.8	<0.8	40w6d	3,320	None	Normal (3 years old)
4	34	1/1	Myotonic dystrophy Polyhydramnios TPD (23)	None	16	3,746	61.3	<0.8	31w6d	1,822	Abnormal ABR Ventriculomegaly	Myotonic dystrophy Cerebral palsy (1 year 6 months old)
5	35	1/1	SLE Fetal growth restriction (36)	None	17	37,722	47.7	<0.8	37w5d	2,144	Small for gestational age	Normal (1 year 6 months old)
6	30	1/1	Twin pregnancy TPD (25)	23	25	11,915	63.3	<0.8	26w5d	1,080	Low platelet counts Liver dysfunction	Cerebral palsy (1 year 6 months old)
7	40	1/1	HDP (32)	None	16	15,091	83.1	<0.8	33w0d	1,744	Low platelet counts	Normal (1 year 3 months old)

TPD, threatened premature delivery; SLE, systemic lupus erythematosus; HDP, hepertensive disorders during pregnancy; ABR, auditory brain-stem response

Table 2. Clinical characteristics of pregnant women with non-primary cytomegalovirus infection

	All n=1,287	Congenital infection n=7	No congenital infection n=1,280	*P* -value
Age, years old	33.1±5.5	33 (29-40)	34 (14-46)	0.8
Gravidity	1.4±1.5	0 (0-3)	1 (0-13)	0.2
Parity	0.6±0.8	0 (0-2)	0 (0-6)	0.5
BMI prior to pregnancy, kg/m2	21.6±3.8	22.9 (17.4-27.7)	20.7 (15.1-41.0)	0.3
Referral	34.6%	57.1%	34.5%	0.2
IVF-ET	13.3%	0.0%	13.4%	0.6
History of recurrent pregnancy loss	9.5%	14.3%	9.5%	0.5
Maternal fever or ful-like symptoms	16.9%	42.9%	16·7%	0.1
GW at the initial CMV IgG measurements	18.1±8.6	15 (8-32)	16 (5-36)	0.9
GW at delivery	37.3±2.9	35 (26-40)	38 (22-42)	0.1
Birth weight, g	2725.4 ± 628.1	2,253 (1,080-3,320)	2,820 (314-4,208)	0.04
Maternal complications				
Hypertensive disorders	4.0%	14.3%	3.9%	0.2
Thyroid diseases	4.9%	14.3%	4.8%	0.3
Diabetes mellitus/Gestational diabetes mellitus	6.7%	0%	6.7%	1.0
Medical diseases requiring immunosuppressive therapy	4.0%	14.3%	3.9%	0.2
Obstetric complications				
Threatened premature delivery	19.3%	71.4%	19.1%	0.004
Multiple pregnancy	5.4%	28.6%	5.3%	0.05
Fetal growth restriction	8.3%	14.3%	8.3%	0.5
Preterm delivery	25.0%	57.1%	24.8%	0.07
Light-for-date	11.0%	28.6%	10.9%	0.2

BMI., Body Mass Index. GW., Weeks of gestation. IVF., In vitro fertilization. ET., Embryo transfer
Data are expressed as the average ± standard deviation, median (range), or %.

Table 3. Clinical factors associated with congenital cytomegalovirus infection in pregnant women with non-primary cytomegalovirus infection

	Univariate analysis		Multivariable analysis	
	Odds ratio (95% CI)	*P* -value	Odds ratio (95% CI)	*P* -value
Maternal fever or flu-like symptoms	3.7 (0.8-16.9)	0.09		
Maternal complications				
Hypertensive disorders	4.1 (0.5-34.7)	0.2		
Thyroid diseases	3.3 (0.4-27.6)	0.3		
Medical diseases requiring immunosuppressive therapy	4.1 (0.5-34.7)	0.2		
Obstetric complications				
Threatened premature delivery	10.6 (2.0-55.0)	0.005	8.4 (1.5-48.1)	0.02
Multiple pregnancy	7.1 (1.4-37.4)	0.02	2.6 (0.4-15.0)	0.3
Fetal growth restriction	1.8 (0.2-15.5)	0.6		
Preterm delivery	4.0 (0.9-18.1)	0.07		
Light-for-date	3.3 (0.6-17.0)	0.2		

CI., confidence interval.