

## Prophylactic Intravenous Immunoglobulin Injections to Mothers with Primary Cytomegalovirus Infection

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### ABSTRACT

**The aim of this trial study was to assess the preventive efficacy of immunoglobulin with a high titer of anti-CMV antibody for mother-to-fetus cytomegalovirus (CMV) transmission among pregnant women with primary/acute CMV infection. The primary CMV infection in mothers was diagnosed by a positive test for CMV IgM and/or low IgG avidity. Intact type immunoglobulin with a high titer of anti-CMV antibody was injected intravenously at a dosage of 2.5-5.0 g/day for consecutive 3 days to mothers with primary CMV infection. Four pregnant women were enrolled. One pregnancy ended in no congenital infection, while two pregnancies ended in congenital CMV infection. The other one pregnancy was terminated. The mother-to-fetus CMV transmission rate was found to be high as 66.7% (2/3). This preliminary result suggests that intravenous immunoglobulin injections are not effective for the prevention of mother-to-fetus CMV transmission in the present protocol.**

### INTRODUCTION

Cytomegalovirus (CMV) is the most common cause of intrauterine infection, occurring in 0.2-2.0% of live born infants [1]. When pregnant women have primary/acute CMV infection during the first trimester, approximately 25% of their fetuses will be infected [2]. Although 10-15% of infected fetuses show symptomatic congenital CMV infection at birth, the clinical manifestations including fetal growth restriction, low birth weight, central nervous system and multiple organ involvement may be so severe as to lead to a high perinatal mortality rate and major neurological sequelae in approximately 90% of the surviving infants [2-4]. In addition, 10-15% of infants with asymptomatic congenital infection will develop long-term sequelae, namely progressive sensorineural hearing difficulty and mental retardation [3, 4]. No guidelines concerning medical intervention for prenatally diagnosed congenital CMV infection is currently available. Therefore, aiming improvement of fetal/infantile prognosis many clinicians have tried a variety of fetal therapies for symptomatic congenital CMV infection including ganciclovir injection into fetal umbilical cord blood [5], hyper-immunoglobulin injection into peritoneal cavity of a fetus [6-8], hyper-immunoglobulin injection into maternal blood, amniotic fluid and umbilical cord blood [9, 10] and valaciclovir injection into maternal blood [11]. On the other hand, studies to prevent mother-to-fetus CMV transmission have reported a modality of intravenous injections of hyper-immunoglobulin [10, 12] to pregnant women with primary CMV infection. In these reports, immunoglobulin enriched for antibodies against CMV, such as Cytogam (CSL Behring) and Cyotect (Biotest AG), was used, whereas they are not available in many countries, including Japan. Therefore, we used conventional polyclonal-immunoglobulin with a high titer of anti-CMV antibody as substitutes for hyper-immunoglobulin. The aim of the present trial study was to assess the preventive efficacy of immunoglobulin with a high titer of anti-CMV antibody for mother-to-fetus CMV transmission among pregnant women with primary CMV infection.

### PATIENTS AND METHODS

This trial study was performed prospectively with informed consent from all of the patients. The institutional ethical boards of the Kobe University Hospital study approved this study. During the period between August 2009 and April 2013, pregnant women who had primary CMV infection were enrolled. The

primary CMV infection in mothers was diagnosed by a positive test for CMV IgM and/or low IgG avidity. Women with fetal abnormalities detected by ultrasound were excluded from the study.

After confirmation of CMV primary infection, intact type immunoglobulin with a highest titer of anti-CMV antibody which was available at the time of treatment, Kenketsu venilon-I (Teijin Pharma, Tokyo), was injected intravenously at a dosage of 2.5-5.0 g/day for consecutive 3 days to mothers with informed consent. A couple was counseled about a possible risk of indefinite infection and other adverse effects of immunoglobulin, and they selected daily dose of 2.5 g or 5.0 g. The amniocentesis followed by PCR analysis for CMV DNA was performed with informed consent, if a couple desired it.

The diagnosis of congenital CMV infection was determined by the presence of CMV DNA in the urine or blood of neonates. Live-birth neonates received the workup for congenital CMV infection. Ophthalmofunduscopy, cerebral ultrasound, physical and neurological examinations were performed. Head MRI and CT were used if necessary. Auditory brain-stem response was periodically tested to find sensorineural hearing difficulty as one of major sequelae. Neurological development of the infants was followed up.

Serological tests for CMV IgG (negative 0-230.99, borderline 231-239.99, positive  $\geq 240$ ) and IgM (negative 0-0.89, borderline 0.90-1.99, positive  $\geq 2.0$ ) were performed using EIA kits produced by Siemens Healthcare Diagnostics (Tokyo, Japan). CMV IgG avidity was measured in the Aisenkai Nichinan Hospital, and the index of 35% or less is defined as low IgG avidity as described previously [13]. A real-time PCR analysis was performed at a commercial laboratory (SRL, Tokyo, Japan). C7-HRP (CMV antigen test "TEIJIN" TFB, Tokyo, Japan) was used as CMV antigenemia test.

## RESULTS

Clinical findings of 4 women who received prophylactic immunoglobulin injections and the outcome are summarized in Table I.

Case 1 woman had antibody screening test and was found to have low CMV IgG avidity (22.7%) in the university hospital. CMV DNA in maternal blood was detected by PCR analysis. Intravenous immunoglobulin (IVIg) injections at a dosage of 2.5 g/day for 3 days were performed at 21 weeks of gestation (GW). Ultrasound examinations demonstrated no abnormalities of the fetus. CMV DNA in the amniotic fluid was not detected at 31 GW. A female baby weighing 2,650 g was delivered at 37 GW by elective cesarean section due to breech presentation. The baby had no congenital CMV infection and developed normally until 2 years 5 months old.

Case 2 woman was referred to the university hospital as she had antibody screening test and a positive test for CMV IgM in the former hospital. CMV IgG avidity (2.3%) in her blood was found to be extremely low. IVIg injections (2.5 g/day for 3 days) were performed at 17 GW. Ultrasound examinations demonstrated no abnormalities of the fetus. The pregnancy ended in preterm premature rupture of the membranes due to subchorionic hematoma and vaginal delivery of a stillbirth at 23 GW. The stillbirth had congenital CMV infection with the presence of CMV DNA in the cord blood serum. The consent to postmortem examinations was not obtained.

Case 3 woman was referred to the university hospital as she had liver dysfunction and a positive test for CMV IgM in the former hospital. CMV IgG avidity was found to be low as 12.6%. IVIg injections (5.0 g/day for 3 days) were performed at 13 GW. The amniocentesis and subsequent PCR analysis revealed the presence of CMV DNA in the amniotic fluid at 16 GW. A couple desired the continuation of the pregnancy and IVIg injections, so that IVIg injections with informed consent were performed additionally at 18, 22 and 26 GW. Thereafter, the couple declined further injections because of private reasons. Ultrasound examinations demonstrated no abnormalities of the fetus. A female baby weighing 2,758 g was delivered vaginally at 37 GW. The baby had congenital CMV infection with the presence of CMV DNA in the blood and the urine. She is 1 year and 5 months old and develops normally without any sequela.

Case 4 woman was referred to the university hospital as she had flu-like symptoms with fever and a positive test for CMV IgM in the former hospital. CMV IgG avidity was found to be low as 8.9%. Her blood was tested positive for CMV antigenemia at 18 GW. IVIg injections (5.0 g/day for 3 days) were performed at 18 GW. Ultrasound examinations demonstrated no abnormalities of the fetus. The pregnancy was terminated at 21 GW, and the consent to postmortem examinations was not obtained.

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**Table I.** Clinical findings of 4 women with prophylactic immunoglobulin injections and the outcome

Case no.	Maternal Gravida/Para Age (years old)	Maternal IgM/ IgG avidity (GW)	Maternal symptoms (GW)	GW of diagnosis*	Doses of maternal IVIg (GW)	Viral loads before / after injection** (GW)	Clinical findings at birth	IgM of neonatal blood	Viral loads in neonate** (days old)	Outcome
1	3/0 30	0 – (18)/ 22.7% (12)	Flu-like symptom (11)	18	7.5g (21)	MB 1.4 × 10 <sup>2</sup> (18)/ < 20 (22) AF ND/ <100 (31)	37 GW CS, 2.650g	0.1 –	Blood < 20 (0) Antigenemia – (3) Urine – (0)	No congenital infection
2	3/3 35	5.5+ (12)/ 2.3% (12)	Fever (5)	12	7.5g (17)	MB <20 (12)/ <20 (18)	23 GW VD, pPROM, still birth, 760g	1.0 ±	Serum 3.0 × 10 <sup>5</sup>	Congenital infection, stillbirth
3	0/0 29	9.3+ (13)/ 12.6% (13)	Fever, liver dysfunction (9)	13	15g (13) 15g (18) 15g (22) 15g (26)	MB <20 (13)/ <20 (34) AF ND/ 1.1 × 10 <sup>4</sup> (16) 1.8 × 10 <sup>5</sup> (24) 2.1 × 10 <sup>5</sup> (39)	39 GW VD, 2.758g	4.4+	Blood 1.7 × 10 <sup>2</sup> (0)/ – (1year old) Antigenemia – (0) Urine 5.3 × 10 <sup>7</sup> (0)/ 4.5 × 10 <sup>5</sup> (1year old)	Congenital infection, normal development at 1 year and 6 months old
4	2/1 24	4.6+ (12)/ 8.9% (18)	Fever, flu-like symptom (12)	18	15g (18)	MB < 20 (18)/ ND	21 GW TOP, 328g	ND	ND	Stillbirth

GW, weeks of gestation; IVIg, intravenous administration of cytomegalovirus hyper immunoglobulin; MB, maternal blood; AF, amniotic fluid; ND, not determined; CS, cesarean section; VD, vaginal delivery; pPROM, preterm premature rupture of the membranes; TOP, termination of pregnancy

\*Weeks of gestation when the maternal primary infection was diagnosed with laboratory data.

\*\* Numbers shown are the results of real time PCR analyses. Units of amniotic fluid/urine /serum and blood samples are gene copy number/ ml and gene copy number/ 10<sup>6</sup> white blood cells, respectively.

### DISCUSSION

This trial study of prophylactic IVIg injections enrolled 4 pregnant women with primary/acute CMV infection. Three of the four women had low IgG avidity and high IgM levels. The diagnosis of primary CMV infection in pregnant women based on CMV IgM is difficult, because the presence of CMV IgM may represent either recent primary infection or reactivation. CMV IgG avidity tests have been used to distinguish recent from distant infection. In addition, Ebina et al. suggested that a cut-off value of <40% IgG avidity index had a specificity of 96.1% and a sensitivity of 64.3% sensitivity for prediction of congenital infection[14]. For these reasons, a positive test for CMV IgM and/or low IgG avidity were used as diagnostic tools for detecting primary CMV infection in this study. The other woman, case 1 had low IgG avidity level and CMV DNA but not a positive test for IgM in her blood. Only one pregnancy (case 1) ended in no congenital infection, while two pregnancies (case 2 and case 3) ended in congenital CMV infection. Examinations of CMV infection for the stillbirth could not be performed in case 4. Therefore, excluding one pregnancy, mother-to-fetus CMV transmission rate was found to be high as 66.7% (2/3) among pregnant women who received prophylactic IVIg injections in the present study. This preliminary result suggests that IVIg injections are not effective for the prevention of mother-to-fetus CMV transmission. The plausible reasons for the ineffectiveness might involve delay of injections and insufficient doses of IVIg. Interval periods between appearance of maternal symptoms and IVIg injections ranged from 4 to 12 weeks in the present study. Case 3 experienced general symptoms of CMV infection at 9 GW and IVIg injections were able to start at 13 GW. However, CMV already existed in the amniotic fluid at 16 GW. The case 3 received relatively a high dose (a total of 60g) of IVIg within 26 weeks. Her baby had asymptomatic congenital CMV infection but normally developed without any sequela. It is suggested that the prevention of mother-to-fetus CMV transmission with use of IVIg is not easy, but the high amount of IVIg with repeated injections may reduce the severity of congenital CMV infection symptoms and the sequelae.

Nigro et al. reported that 37 pregnant women with primary CMV infection received prophylactic IVIg and 6 (16%) pregnancies ended in asymptomatic congenital CMV infection [10]. Buxmann et al. also reported that 39 pregnant women with primary CMV infection received prophylactic IVIg and 9 (23%) pregnancies ended in congenital CMV infection consisting of 8 asymptomatic infection and 1 termination of pregnancy [12]. In

these two studies, IVIg was injected repeatedly. The Cochrane review of randomized controlled trials mentioned that there was no sufficient evidence to recommend use of any particular strategy to prevent mother-to-fetus transmission of CMV [15]. Pass et al. investigated the prevention of maternal CMV infection by administering a vaccine consisting of recombinant CMV envelope glycoprotein B to non-pregnant women [16]. The results of this trial suggest that CMV glycoprotein B vaccine has the potential to decrease incident cases of maternal and congenital CMV infection, however further phase 3 and 4 clinical trials are required. Pending the availability of an active vaccine, IVIg is thought to be one of the most promising candidates as prophylactic agent, because IVIg has been used safely in the previous studies [17-21]. The effects of IVIg may be related to not only the ability to inhibit the replication of CMV, but also the ability to inhibit the activity of immune cells producing harmful cytokines such as tumor necrosis factor  $\alpha$  [22,23].

Number of subjects in the present study is very small, but the preliminary result suggests that IVIg injections in the present protocol are not effective for the prevention of mother-to-fetus CMV transmission. However, in our study, conventional polyclonal-immunoglobulin with a high titer of anti-CMV antibody was substituted for hyper-immunoglobulin. The titers of anti-CMV antibody contained in immunoglobulin used in our study may be much lower than that of hyper-immunoglobulin used in western countries, so we may have to use much higher doses of immunoglobulin. Therefore, we have discontinued the IVIg study of the present form. A new prophylactic study with use of a high dose IVIg has started.

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