Prospective Cohort Study of Congenital Cytomegalovirus Infection during Pregnancy with Fetal Growth Restriction: Serologic Analysis and Placental Pathology

Mitsuru Tsuge, MD, PhD[1], Akira I. Hida, MD, PhD[2], Toshio Minematsu, MD, PhD[3], Naotoshi Honda, MD, PhD[4], Yumi Oshiro, MD, PhD[5], Mikifumi Yokoyama, MD, PhD[4], and Yoichi Kondo, MD, PhD[6]

Objective To investigate prospectively the prevalence of congenital cytomegalovirus (CMV) infection and the pathologic features of the placenta in cases of fetal growth restriction (FGR).

Study design Forty-eight pregnant women who were diagnosed with FGR during pregnancy were enrolled for 15 months. Maternal CMV serologic tests, pathologic examinations of the placenta, and newborn urinary CMV-DNA polymerase chain reaction tests were performed in all the cases. The clinical characteristics and laboratory findings of the pregnant women and their newborns were collected. Biomarkers for inflammation, angiogenesis, and placental hormones were measured in the maternal serum at FGR diagnosis or in the neonatal urine at birth.

Results One of the 48 cases with FGR was a congenital CMV infection. CMV antigen was detected in the placenta of 7 cases with FGR. The change rate of the estimated fetal body weight was significantly lower in FGR cases with placental CMV detection. Placental villitis was observed more frequently in FGR cases with placental CMV detection. Human placental lactogen was significantly decreased in FGR cases with placental CMV detection. Increased C-reactive protein and serum amyloid A levels in the maternal serum were observed more frequently in FGR cases with placental CMV detection. Newborn urine β-2 microglobulin levels were significantly higher in FGR cases with placental CMV detection.

Conclusions Serologic tests for maternal CMV, the change rate of the estimated fetal body weight, analysis of several biomarkers, and placental pathologic examinations might be helpful in comprehensively predicting the possibility of congenital CMV infection. *(J Pediatr 2019;206:42-8).*

Human cytomegalovirus (CMV) is the leading cause of congenital viral infection, and it occurs in approximately 0.2%-3% of all live births in the US and Europe.[1] Congenital CMV infection causes prenatal neurologic damage, which is particularly severe when the primary maternal infection occurs during the first trimester.[2] A study screened urine from Japanese newborns for CMV-DNA and clarified that the prevalence of congenital CMV infection in Japan was 0.31%.[3] In contrast, a national survey in Japan reported only 140 cases of congenital CMV infections in 3 years, indicating that greater than 90% of congenital CMV-infected cases are overlooked.[4] The presence of CMV-IgM in the serum of pregnant women does not always indicate a recent infection because of the persistence of the IgM antibody,[5] and CMV-IgM in the serum of newborns is detected in only one-half of congenital CMV-infected newborns.[3] Newborn urinary CMV-DNA polymerase chain reaction (PCR) tests are not performed clinically in all suspicious cases.

Ultrasonographic findings are helpful in suspected intrauterine infections,[6] and frequent findings on congenital CMV infection include fetal growth restriction (FGR), cerebral ventriculomegaly, fetal ascites, intracranial calcifications, oligohydramnios, microcephaly, hyperechogenic bowel, hydrops fetalis, pleural effusion, and liver calcifications.[7-9] FGR is the most frequent finding during daily obstetric ultrasound examinations, and a recent study demonstrated that FGR was associated significantly with poor outcomes in congenital CMV infection.[10] Therefore, epidemiologically determining the extent of congenital CMV infection in pregnant women with FGR is important. Previous retrospective studies investigated congenital CMV infection in pregnant women with FGR[11,12] Thus, this study aimed to prospectively

AC	Abdominal circumference	FL	Femur length
BPD	Biparietal diameter	HPL	Human placental lactogen
CMV	Cytomegalovirus	IFN	Interferon
Cre	Creatinine	MCP-1	Monocyte chemoattractant protein-1
CRP	C-reactive protein	PCR	Polymerase chain reaction
EFBW	Estimated fetal body weight	SAA	Serum amyloid A
ELISA	Enzyme-linked immunosorbent assay	sFlt-1	Soluble fms-like tyrosine kinase-1
FGR	Fetal growth restriction	β2MG	β-2 microglobulin

From the [1]Department of Pediatrics, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama; [2]Department of Pathology, Kagoshima University Graduate School of Medicine and Dental Sciences, Kagoshima, Japan; [3]Research Center for Disease Control, Aisenkai Nichinan Hospital, Nichinan, Japan; [4]Department of Obstetrics and Gynecology; [5]Department of Pathology, Matsuyama Red Cross Hospital, Matsuyama, Japan; and [6]Department of Pediatrics, Matsuyama Red Cross Hospital, Matsuyama, Japan

Supported by research grants from the Japanese Society for Pediatric Infectious Diseases. The authors declare no conflicts of interests.

https://doi.org10.1016/j.jpeds.2018.10.003

evaluate the serologic CMV antibodies, the prevalence of congenital CMV infection, and the pathologic features of the placentas of pregnant women with FGR.

Methods

This study was performed in accordance with the appropriate clinical and experimental ethical guidelines, and the Ethics Committee of Matsuyama Red Cross Hospital approved the study protocol (No. 504). Informed consent was obtained from each pregnant woman for their participation in this study.

All Japanese singleton pregnant women diagnosed with FGR between January 2016 and March 2017 at Matsuyama Red Cross Hospital were recruited for the study. FGR was defined as an estimated fetal body weight (EFBW) below -1.5 SDs on at least 2 ultrasound biometrics performed by experienced obstetric physicians. EFBW was calculated from the biparietal diameter (BPD), abdominal circumference (AC), and femur length (FL) measurements using a local Japanese calculation formula.[13] The SD values of the EFBW, AC, BPD, and FL of each fetus were calculated from these measures depending on the Japanese standard fetus growth curves.[14] The rate of SD change, which is referred to as the delta SD (ΔSD), was calculated as the inclination value of the linear approximation curve from the SD values of each gestational period.

Experienced pediatric physicians evaluated the clinical symptoms of newborns at birth. Percentiles of birth weight, height, and head circumferences were calculated and charted against Japanese local reference values for gestational age and sex. Small for gestational age newborns were defined as babies with a birth weight of the <10th percentile, adjusted for the gestational age at delivery and the sex.[15] The Kaup index was calculated as the weight (kg) divided by the square of the length (m^2).

We collected maternal sera within 2 weeks after the FGR diagnosis, and newborn urine and blood were collected within 2 days after birth. Serum levels for CMV-IgG and CMV-IgM were measured quantitatively in a Japanese commercial laboratory (Bio Medical Laboratories, Tokyo, Japan) using enzyme-linked immunosorbent assay (ELISA) kits (Denka Seiken, Tokyo, Japan). Women with a positive CMV-IgG status were tested further for the CMV-IgG avidity index. The avidity test was performed using another commercial ELISA kit (Enzygnost anti-CMV; Siemens Healthcare Diagnostics, Tokyo, Japan), as reported previously.[16] Levels of C-reactive protein (CRP), serum amyloid A (SAA), human placental lactogen (HPL), creatinine (Cre), β-2 microglobulin (β2MG), and soluble interleukin-2 receptor were quantitatively measured at the Bio Medical Laboratories. The levels of soluble fms-like tyrosine kinase-1 (sFlt-1), placental growth factor, and monocyte chemoattractant protein-1 (MCP-1) were determined by ELISA (R&D Systems, Minneapolis, Minnesota) following the manufacturer's instructions.

DNA was extracted from 200 μL of the urine samples using a DNeasy blood and tissue kit (Qiagen, Valencia, California) according to the manufacturer's instructions. Detection of the CMV genome was accomplished by amplification of the CMV immediate early gene using the Taq PCR Master Mix Kit (Qiagen, Valencia, California), and the following primers: forward primer: 5′-GGT CAC TAG TGA CGC TTG TAT GAT GA-3′; and reverse primer: 5′-GAT AGT CGC GGG TAC AGG GGA CTC T-3′. Briefly, 25 μL of a reaction mixture contained 1 × Taq buffer, 0.2 μM of each primer, 0.5 unit Taq DNA polymerase, and 5 μL of extracted DNA. PCR was performed in a BioRad T100 Thermal Cycler (BioRad, Foster City, California). Cycle conditions were 1 cycle of 3 minutes at 94°C followed by 35 cycles of 45 seconds at 94°C, 45 seconds at 50°C, and 1 minute at 72°C, and final incubation for 10 minutes at 72°C. The PCR products were analyzed by gel electrophoresis. Positive and negative controls were included in all PCR runs. The lower detection limit for this conventional PCR method was 200 copies/μL.

Experienced pathologists performed the pathologic examination of the placentas. The pathologists were unaware of the clinical information. The placentas were fixed in 10% buffered formalin for 48 hours. The tissue specimens were obtained from 2 parts of the umbilical cord on the fetal and placental sides, a membrane roll, and 4 parts of placental parenchyma, including the decidua and chorionic plates. CMV immunostaining was performed automatically in a Benchmark XT staining module (Ventana Medical Systems, Tucson, Arizona). Briefly, antigen retrieval was performed by protease digestion and inhibition of peroxidase activity. The sections were incubated with a primary monoclonal antibody (Clones CCH2 + DDG9, M0854; Dako Corporation, Glostrup, Denmark) for 32 minutes at 37°C, washed, and processed for color development with a streptavidin-labeled biotinylated antibody method using the iVIEW DAB Universal Kit (Ventana Medical Systems). The severity of chorioamnionitis, funisitis, and villitis was graded as mild, moderate, or severe according to a previously described staging and grading system.[17]

Statistical analyses were performed using SPSS software v 17.0 (SPSS, Inc, Chicago, Illinois). Quantitative variables were described as the median and IQR and were compared using the nonparametric Mann-Whitney U test. Categorical variables were expressed using frequency measures, and comparisons between groups were performed with the Fisher exact test. Statistical significance was set at P values of less than .05. No adjustment was made for multiple comparisons.

Results

Among the 48 pregnant women with FGR, 36 (75.0%) were positive for serum CMV-IgG at FGR diagnosis, and 5 (10.4%) women were positive for serum CMV-IgM. Eight (16.7%) of the 36 women who were positive for CMV-IgG exhibited a serum CMV-IgG avidity of 40% or lower. Three of the 5 women who were positive for CMV-IgM exhibited a low CMV-IgG avidity (**Figure 1**). One of the 48 newborns (2.1%) was positive for serum CMV-IgM antibody and CMV-DNA in the urine. Therefore, this newborn was diagnosed with a fetal infection. This congenitally infected newborn presented with small for gestational age, jaundice, ventriculomegaly, and periventricular calcification. Maternal serologic test of this case with a fetal infection was positive for both CMV-IgG (21.2 IU/

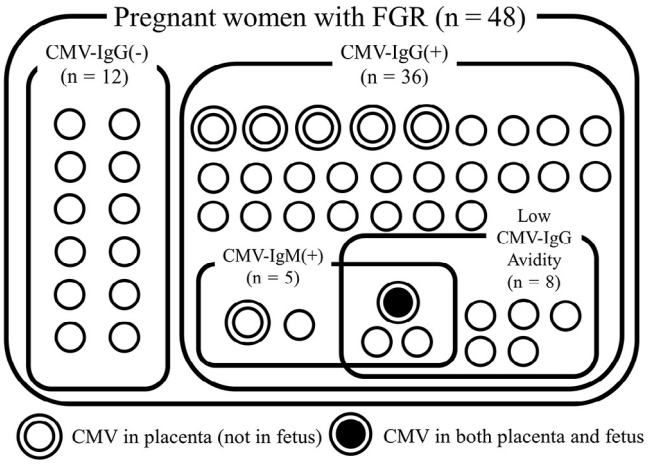

Figure 1. CMV serologic status of 48 pregnant women with FGR. The CMV serologic test was performed within 2 weeks after the FGR diagnosis.

mL) and CMV-IgM (1.28 IU/mL), and CMV-IgG avidity was low (35.4%).

CMV immunostaining detected viral antigens in 7 (14.6%) of the delivered placentae, including the woman with a fetal infection. All the viral antigens were localized inside the placental villi in all 4 sites of the placental tissue samples in the case of fetal infection. Viral antigens were not found in the placental villus in the other 6 cases with no fetal infection, but the antigens were localized in the intervillous space or around the placental villi and were only detected in 1 or 2 sites of the placental tissue samples (**Figure 2**).

We compared the 6 cases with the CMV antigen in the placenta, excluding 1 congenitally infected case (placental CMV [+] group), with the 41 cases without CMV antigen (placental CMV [−] group) to clarify the clinical characteristics of the FGR cases with placental CMV detection. There were no significant differences in the characteristics of the pregnant women, such as age, prepregnancy body mass index, parity, or obstetric histories, between the 2 groups. It should be noted that 4 cases (8.3%) of preeclampsia were confirmed during pregnancy as a pregnancy complication in our study. Three cases were included in the placental CMV (+) group, and 1 case was included in the placental CMV (−) group. There was a significantly higher prevalence of preeclampsia in the placental CMV (+) group than in the placental CMV (−) group ($P = .005$). Preeclampsia is also commonly associated with the cause of FGR; therefore, it must be considered that the presence of preeclampsia becomes a major confounder in our study. **Table I** (available at www.jpeds.com) shows the characteristics of the pregnant women.

The ΔSD of the EFBW after FGR diagnosis was significantly lower in the placental CMV (+) group than in the placental CMV (−) group ($P = .005$). There was no significant difference in the ΔSDs of the BPD between the 2 groups, but the ΔSD of the AC and FL after FGR diagnosis were significantly lower in the placental CMV (+) group than in the placental CMV (−) group ($P = .021$ and $P = .015$, respectively). **Table II** shows the ultrasonographic findings.

There was a significantly higher prevalence of preterm delivery in the placental CMV (+) group than in the placental CMV (−) group ($P = .030$). There were no significant differences in other pregnancy outcomes between the 2 groups. There were also no significant differences in height, weight, or the head circumference of newborns between the 2 groups.

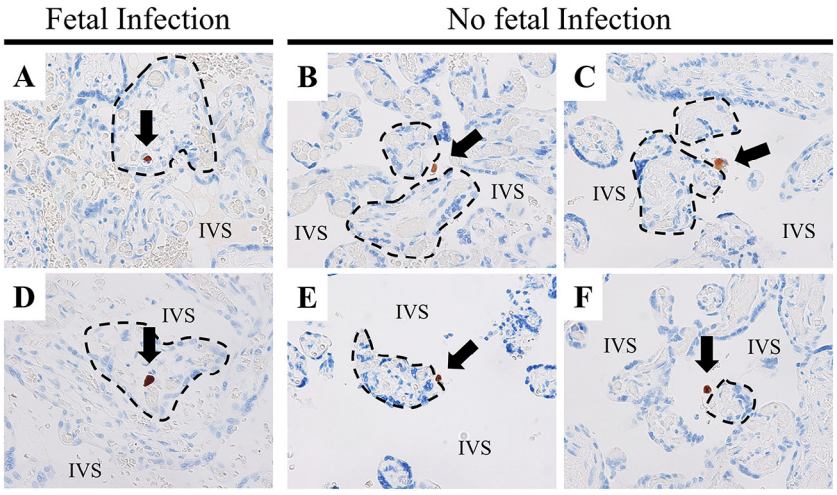

Figure 2. CMV immunohistochemical staining in the placenta. **A** and **B,** CMV is expressed inside the placental villous stroma (×200 magnification). **C, D, E,** and **F,** CMV is expressed in the intervillous space (×200 magnification). *IVS*, intervillous space. Arrows indicate a positive stain for the CMV antigen. Dotted lines indicate the surface of the placental villi. Representative images are shown for each immunohistochemical stain.

Table II. Ultrasound sonography/Doppler findings after FGR diagnosis

	Placental CMV (+) group (n = 6)	Placental CMV (–) group (n = 41)	*P* value
Oligohydramnios, n (%)	0 (0.0)	3 (7.3)	1.00
Changes of fetal growth			
ΔSD of EFBW (/mo)	−0.44 (−1.20-−0.31)	0.06 (−0.13-0.23)	**.005**
ΔSD of BPD (/mo)	0.34 (−0.36-0.80)	0.19 (−0.22-0.54)	.58
ΔSD of AC (/mo)	−0.26 (−0.33-−0.22)	−0.01 (−0.18-0.32)	**.021**
ΔSD of FL (/mo)	−0.40 (−1.29-0.40)	0.05 (−0.26-0.70)	**.015**
Resistance index			
UmA-RI > 90%tile, n (%)	3 (50.0)	7 (19.0)	.10
MCA-RI < 10%tile, n (%)	1 (16.7)	9 (24.3)	1.00
UmA-RI/MCA-RI >1, n (%)	2 (33.3)	8 (21.6)	.59

MCA-RI, middle cerebral artery resistance index; *UmA-RI*, umbilical artery resistance index.
Significant *P* values are in bold.
Data are expressed as median (IQR) or n (%).

However, the Kaup index was significantly lower in the placental CMV (+) group than in the placental CMV (−) group ($P = .047$). **Table III** (available at www.jpeds.com) shows the pregnancy outcomes and newborn characteristics.

Pathologic examinations revealed that the prevalence of grade 2 or higher chronic villitis was significantly higher in the placental CMV (+) group than in the placental CMV (−) group ($P = .013$). **Table IV** (available at www.jpeds.com) shows the pathologic findings of the placentas and umbilical cords.

The prevalence of the maternal CMV antibody (CMV-IgG and CMV-IgM) in the serum at the FGR diagnosis was not significantly different between the 2 groups, and there was no significant difference in the prevalence of FGR cases with low CMV-IgG avidity (≤40%) between the 2 groups. The prevalence of pregnant women with positive SAA (≥8.0 μg/mL) or CRP (≥1.0 mg/dL) at FGR diagnosis was significantly higher in the placental CMV (+) group than in the placental CMV (−) group ($P = .021$ and $P = .039$, respectively). The placental CMV (+) group exhibited significantly lower HPL levels and higher sFlt-1 levels compared with the placental CMV (−) group ($P = .013$ and $P = .043$, respectively). **Table V** shows the laboratory findings of the maternal serum at FGR diagnosis.

Newborn blood tests revealed no significant differences in platelet count, the total bilirubin, aspartate aminotransaminase, total IgM, or CMV-IgM levels between the 2 groups. Newborn urinary β2MG/Cre and urinary MCP-1/Cre levels were significantly higher in the placental CMV (+) group than in the placental CMV (−) group ($P = .009$ and $P = .036$, respectively). **Table VI** (available at www.jpeds.com) shows the laboratory findings of the newborns at birth.

Discussion

FGR is indicative of congenital CMV infection during pregnancy, but placental pathologic examinations or urinary CMV-DNA tests are not routinely performed in all suspicious cases. Our study revealed that CMV infected the fetus in 2.1% of pregnant women with FGR during pregnancy, which is similar to the findings of previous retrospective studies on the prevalence of congenital CMV infection in FGR cases.[11,12]

The CMV antibody prevalence in women of childbearing age in Japan was 95% in the 1970s, and it has since declined to <75%,[18,19] which is similar to our result. We found that 10.4% of pregnant women with FGR were positive for CMV-IgM antibody. The presence of CMV-IgM in serum does not always indicate a recent infection because of the persistence of the IgM antibody.[5] The CMV IgG avidity test is a reliable tool for distinguishing primary infection from nonprimary infection.[20,21] CMV-IgG avidity was low in 3 of the 5 women positive for CMV-IgM in our study, which included a woman with congenital CMV infection. This result suggests that CMV-IgG avidity may be useful for diagnosing the possible congenital CMV infection.

Pathologic examinations revealed that CMV antigens were detected in the placenta in 14.6% of the pregnant women with FGR in our study. A previous study reported that CMV DNA was found in 39% of placentas with a high-avidity IgG in the maternal serum.[22] In our study, biopsies were performed from 4 small tissues from each placenta; therefore, some false-negative results because of sampling errors in the case of focal placental infection might exist. Moreover, the absence of CMV antigens does not necessarily indicate the absence of CMV antigen in the entire placenta. In addition, the PCR technique is usually a more sensitive method in comparison with the CMV immunohistochemical reaction; therefore, we might be able to detect the CMV-DNA in the placenta using PCR or in situ DNA hybridization instead of the immunohistochemical

Table V. Laboratory findings in maternal serum at the diagnosis of FGR

	Placental CMV (+) group (n = 6)	Placental CMV (–) group (n = 41)	*P* value
CMV-IgG titer positive (>2.0), n (%)	6 (100.0)	29 (70.7)	.32
CMV-IgM titer positive (>0.8), n (%)	1 (16.7)	3 (7.3)	.43
Low CMV-IgG Avidity (<40%), n (%)	0 (0.0)	7 (17.1)	.57
CRP (>1.0 mg/dL), n (%)	2 (33.3)	1 (2.4)	**.039**
Serum amyloid A (>8 μg/mL), n (%)	4 (66.7)	7 (17.1)	**.021**
HPL (μg/mL)	3.4 (2.7-4.0)	5.7 (4.8-8.4)	**.013**
sFlt-1 (pg/mL)	17 503 (7294-21 999)	4891 (2561-11 189)	**.043**
Placental growth factor (pg/mL)	181.1 (61.6-368.4)	177.8 (119.1-336.6)	.56
Soluble interleukin-2 receptor (U/mL)	540.0 (431.8-545.0)	523.0 (436.7-613.4)	.87

Significant *P* values are in bold.
Data are expressed as median (IQR) or n (%).

method. Notably, we detected CMV antigens inside the placental villi in a congenitally infected woman, but these antigens were detected in the intervillous space or around the placental villi in the all other 6 cases. The CMV antigen may exist in the placenta during CMV reinfection or reactivation in these 6 women based on their high-avidity IgG in the maternal serum. CMV antigen in the maternal bloodstream may have prevented the entry of the virus into the placental villi at the maternal-fetal interface and prevented fetal infection, as previously reported.[23]

Our study demonstrated that the ΔSD of the EFBW after FGR diagnosis was significantly lower in the FGR cases with placental CMV detection, which indicates that FGR progressively worsened. Some fetuses develop along the normal growth curve despite the FGR diagnosis, which may result from a variety of reasons, such as a temporary deterioration of the placental-fetal circulation, change in fetal body position, physicians' interexaminer errors, or human errors. Our results revealed the significance of the changing EFBW rate after the FGR diagnosis, which may help predict the placental CMV detection. The ΔSD of the AC and FL was significantly lower in the FGR cases with placental CMV detection in our study, whereas that of the BPD was not significantly different. The Kaup index, which is a nutritional index for infants, was significantly lower at birth in the FGR cases with placental CMV detection. These results indicate that the trunks of the fetuses became progressively thinner because of the nutritional disturbance. We presumed that the asymmetrical FGR occurred because of a brain-sparing effect caused by the uterine-placental circulation insufficiency in the FGR cases with placental CMV detection.

Our study revealed that chronic villitis was more frequently observed in the placentas with CMV detection. CMV infection in the placenta causes chronic villitis, and the CMV antigen was detected in some placentas with villitis of an unknown etiology.[24,25] CMV antigens initiate placental inflammation in a Toll-like receptor-2-dependent manner, without a direct infection, as an innate defense response.[26,27] We hypothesize that the CMV antigen derived from the maternal bloodstream to the placenta via maternal CMV infection initiates placental inflammation and produces FGR as a complication of chronic villitis, even without fetal infection.

Placental inflammation caused by chronic villitis increases some inflammatory markers in the serum of pregnant women.[28] Our study demonstrated that increased CRP or SAA levels in the serum was frequently observed in the FGR cases with placental CMV detection. Other inflammatory diseases, such as a bacterial infection or connective tissue disease, may have caused this increase, but the CRP or SAA levels during pregnancy may be biomarkers to predict placental inflammation caused by the CMV antigen.

The newborn urinary β2MG level at birth was significantly higher in the FGR cases with placental CMV detection. β2MG is a component of human leukocyte antigen class I molecules, and it is produced by lymphoid tissues, increased in blood, and excreted in urine. Activated lymphocytes produce β2MG, and inflammatory cytokines, such as interferon (IFN)-γ, accelerate its production. Previous reports demonstrated that β2MG exhibited a diagnostic efficacy for symptomatic CMV congenital infections,[29-31] and CMV infection triggered an innate immune response that was predominated by IFN-γ induction in the maternal decidua during a viral-tissue contact.[32] We hypothesize that CMV antigen in the placenta induced the production of various cytokines, such as IFN-γ, which promoted β2MG production in the fetus. We also found that the MCP-1 level in the urine was increased in the FGR cases with placental CMV detection. A previous study also reported elevated MCP-1 in the amniotic fluid with congenital CMV infection.[33] CMV-infected placentas exhibited significantly elevated MCP-1 levels compared with uninfected placentas, and CMV infection significantly elevated MCP-1 expression in ex vivo placental explants.[34] β2MG and MCP-1 in the newborn urine may be biomarkers to predict the presence of CMV antigen in the placenta of pregnant women with FGR.

The measurement of placenta-derived hormones in serum during pregnancy is one method to examine placental functions. Syncytiotrophoblasts in the placental villi secrete HPL, and its concentration in the serum increases gradually as the gestational age progresses and decreases and returns to the normal levels after delivery. HPL does not directly affect the fetus, but it acts on pregnant mothers and promotes fetal development via glycolipid metabolism.[35,36] Our study demonstrated that the level of serum HPL at FGR diagnosis was significantly lower in the placental CMV (+) group, which indicates that HPL production was reduced in the placental villi and suggests that the destruction of the placental villi caused by chronic villitis damaged its functions.

We also demonstrated that sFlt-1, which is an antiangiogenic factor, was significantly higher in the maternal serum in the FGR cases with placental CMV detection, which is consistent with a previous study.[37] However, the prevalence of preeclampsia in our study could influence this result as a major confounder because sFlt-1 is similar to a predictive biomarker for preeclampsia . Therefore, further studies using larger samples without preeclampsia should be performed.

Our study had several limitations. First, the study had a small sample size and only 7 patients had positive placental pathology findings. Second, the enrolled pregnant women had varying maternal and clinical backgrounds, pregnancy complications, and causes of FGR. In particular, the presence of preeclampsia should be considered a major confounding factor in our study. Third, some false-negative results might have been obtained owing to sampling errors or the sensitivity of immunohistochemical staining. The presence of false discovery rate is possible because no adjustment was made for multiple comparisons. Future studies will require careful case ascertainment and healthy and disease control matching to minimize the impact of confounding variables, such as maternal backgrounds, pregnancy complications, and pharmacologic interventions, in a sufficiently large cohort of patients.

The inflammatory biomarkers or placental hormones in the maternal serum at the FGR diagnosis may be helpful in establishing a diagnosis of placental chronic villitis. It is important to predict comprehensively the possibility of congenital

CMV infection using maternal CMV serologic tests, the change rate of the EFBW, several biomarkers, and placental pathologic examinations. If CMV is causally associated with adverse pregnancy outcomes in the absence of fetal infection, this data would significantly add to the urgent need to develop an effective CMV vaccine. ■

We thank all the obstetricians at the Matsuyama Red Cross Hospital for enrolling the pregnant women with FGR, all the pediatricians for treating the newborns in the neonatal intensive care unit, and all the technicians in the Pathology department for their assistance with the tissue samples.

Submitted for publication May 8, 2018; last revision received Aug 30, 2018; accepted Oct 2, 2018

Reprint requests: Akira I. Hida, MD, PhD, 8-35-1 Sakuragaoka, Kagoshima 890-8544, Japan. E-mail: aihida@m.kufm.kagoshima-u.ac.jp

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50 Years Ago in *The Journal of Pediatrics*

Normal Inner Canthal and Outer Orbital Dimensions

Laestadius ND, Aase JM, Smith DW. J Pediatr 1969:74;465-8.

In view of inadequate data for the clinicians to determine hypo-hypertelorism in children, Laestadius et al acquired data for the normal ranges of inner canthal and outer orbital distances in 472 Caucasians, using ruled calipers. The results were published as mean (SD) for various ages ranging from premature newborns to adults.

The last 50 years have witnessed publication of more than 50 studies presenting and analyzing inner-outer intercanthal distances in different population subsets. Also, various terminologies and concepts have emerged with regards to hypertelorism. The term "orbital hypertelorism" was proposed by Tessier[1] in 1972 as the true form with the lateralization of entire bony orbit. He differentiated it from telecanthus or pseudohypertelorism, where there is no change in the position of the lateral wall of orbit; and ocular hypertelorism, which could be present in either condition. In 2001, Everekliogl et al[2] coined the term interpupillary index as a new variable to evaluate the presence of ocular hypo-hypertelorism; the concept of canthal index was also introduced. The normative values of inner canthal, orbital distances, and interpupillary distance have also been applied in calculating other anthropometric values. In 2008, Etezad-Razavi et al[3] developed a regression equation using inner-outer intercanthal distance to calculate interpupillary distance. Later, researchers found another application of inner canthal distance as a reliable predictor of width of maxillary incisor teeth. Despite this work on ocular anthropometry, no single cut-off value or a diagnostic index has emerged for hypo-hypertelorism. Though these normative values and variables do help oculoplastic surgeons in planning and execution of complex corrective surgical procedures; for a clinician in general practice, the definition of diagnosis still relies on subjective perception of "abnormal increase" in interorbital distance. Failure to arrive at a consensus can in part, but not justifiably so, be attributed to variance in human race across the globe.

Payal Gupta, DNB
Department of Ophthalmology
All India Institute of Medical Sciences
Bhopal, India

Piyush Gupta, MD, FAMS
Department of Pediatrics
University College of Medical Sciences
Delhi, India

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Table I. Maternal characteristics participating in the study

	Placental CMV (+) group (n = 6)	Placental CMV (–) group (n = 41)	*P* value
Maternal age (y)	38 (30-41)	31 (29-37)	.24
Prepregnancy BMI (kg/m^2)	21.8 (19.5-26.9)	19.8 (18.4-22.2)	.32
Parous at FGR diagnosis, n (%)	4 (66.7)	20 (48.8)	.67
Previous miscarriage or stillbirth, n (%)	1 (16.7)	9 (22.0)	1.00
Previous pregnancy with FGR n (%)	0/4 (0.0)	6/20 (30.0)	.56
Gestational age at FGR diagnosis (wk)	29 (25-32)	32 (28-36)	.10
SD score of EFBW at FGR diagnosis (SD)	–1.8 (–2.1–-1.6)	–1.6 (–1.8–-1.5)	.53
Pregnancy complication			
Preeclampsia, n (%)	3 (50.0)	1 (2.4)	**.005**
Gestational diabetes mellitus, n (%)	0 (0.0)	5 (12.2)	1.00
Threatened premature delivery, n (%)	1 (16.7)	19 (46.3)	.22
Pregnancy anemia, n (%)	5 (83.3)	23 (56.1)	.38

BMI, body mass index.
Significant *P* values are in bold.
Data are expressed as median (IQR) or n (%).

Table III. Pregnancy outcomes and newborn characteristics

	Placental CMV (+) group (n = 6)	Placental CMV (–) group (n = 41)	*P* value
Pregnancy outcomes			
Emergency cesarean delivery, n (%)	2 (33.3)	6 (14.6)	.27
Meconium-stained amniotic fluid, n (%)	2 (33.3)	9 (22.0)	.61
Premature rupture of the membrane, n (%)	1 (16.7)	5 (12.2)	1.00
Loop or coiling of the umbilical cord, n (%)	3 (50.0)	13 (31.7)	.40
Nonreassuring FHR pattern, n (%)	1 (16.7)	14 (34.1)	.65
Preterm delivery, n (%)	4 (66.7)	8 (19.5)	**.030**
5-min Apgar score <7, n (%)	0 (0.0)	1 (2.4)	1.00
Umbilical artery pH	7.28 (7.24-7.32)	7.29 (7.25-7.31)	.74
Umbilical artery base excess	–2.4 (–4.4–-2.0)	–3.3 (–4.8–-1.5)	.70
Newborn characteristics			
Sex (male), n (%)	2 (33.3)	12 (29.3)	1.00
Birth weight percentile	5.8 (2.0-26.3)	7.9 (4.3-17.5)	.82
Height percentile	5.3 (1.7-32.1)	9.1 (2.9-16.5)	.85
Head circumstances percentile	20.0 (5.8-26.1)	15.0 (4.3-26.3)	.87
Small for gestational age*, n (%)	3 (50.0)	10 (24.4)	.33
Kaup index† (kg/m^2)	10.0 (9.0-11.4)	11.4 (10.7-12.2)	**.047**
Nonproportional microcephaly‡, n (%)	0 (0.0)	0 (0.0)	1.00

FHR, fetal heart rate.
Significant *P* values are in bold.
Data are expressed as median (IQR) or n (%).
*Small for gestational age was defined as a birthweight less than 10th percentile.
†Kaup index was calculated as weight (kg) divided by the squaring of length (m).
‡Nonproportional microcephaly: microcephaly (head circumstances <-3SD) in the setting of normal weight and height (>-1.5SD).

Table IV. Pathologic findings of placenta and umbilical cord

	Placental CMV (+) group (n = 6)	Placental CMV (–) group (n = 41)	*P* value
Placental weight (g)	366 (294-485)	480 (418-523)	.17
Placental thickness (cm)	2.0 (1.9-2.4)	2.0 (1.8-2.2)	.74
Placental breadth (cm)	17.3 (14.6-18.6)	16.5 (15.8-17.5)	.73
Fetal to placenta weight ratio	4.6 (3.6-5.6)	5.1 (4.4-5.5)	.50
Umbilical cord length (cm)	45.0 (39.3-53.0)	48.0 (42.0-54.0)	.66
Umbilical cord breadth (cm)	1.2 (1.0-1.4)	1.0 (1.0-1.3)	.74
Abnormal cord insertion (marginal or velamentous), n (%)	2 (33.3)	5 (12.2)	.21
Single umbilical artery and vein, n (%)	0 (0.0)	1 (2.4)	1.00
Maternal floor infarction, n (%)	3 (50.0)	7 (17.1)	.10
Chorioamnionitis, n (%)	2 (33.3)	12 (29.3)	1.00
Funisitis, n (%)	0 (0.0)	3 (7.3)	1.00
Chronic villitis, n (%)	5 (83.3)	11 (26.8)	**.013**

Significant *P* values are in bold.
Data are expressed as median (IQR) or n (%).

Table VI. Laboratory findings of newborn at birth

	Placental CMV (+) group (n = 6)	Placental CMV (–) group (n = 41)	*P* value
Blood tests			
Platelet count ($\times 10^4/\mu L$)	22.9 (19.4-24.4)	29.1 (25.2-35.1)	.050
Total bilirubin (g/dL)	3.1 (2.6-5.3)	5.3 (4.0-6.8)	.26
Aspartate aminotransferase (IU/L)	34.5 (32.3-37.5)	47.0 (38.0-69.0)	.053
CRP (mg/dL)	0.10 (0.10-0.12)	0.11 (0.10-0.30)	.46
IgM (mg/dL)	11.0 (9.0-14.8)	11.6 (9.5-14.4)	.77
CMV-IgG titer positive, n (%)	6 (100.0)	30 (73.2)	.31
CMV-IgM titer positive, n (%)	0 (0.0)	0 (0.0)	1.00
Urine test			
β2MG/Cre (μg/g)	17 285 (6256-20 338)	1507 (896-3725)	**.009**
MCP-1/Cre (ng/g)	867 (304-1690)	52 (0-224)	**.036**
Automated auditory brainstem response test			
Abnormalities, n (%)	0 (0.0)	0 (0.0)	1.00

Significant *P* values are in bold.
Data are expressed as median (IQR) or n (%).