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Masaharu Murata*, Keisuke Ishii, Takako Taguchi, Aki Mabuchi, Haruna Kawaguchi, Ryo Yamamoto, Shusaku Hayashi and Nobuaki Mitsuda

The prevalence and clinical features of twin-twin transfusion syndrome with onset during the third trimester

Abstract

Objective: To describe the incidence and clinical features of twin-twin transfusion syndrome (TTTS) with third trimester onset.

Methods: We performed a retrospective chart review of monochorionic diamniotic (MD) twin pregnancies delivered during a recent 4-year period. The inclusion criterion was women who received prenatal care at our center from the first trimester onward. Serial ultrasound examinations were performed at least every 2 weeks until delivery to evaluate fetal growth as well as to estimate amniotic fluid volume. The prevalence of TTTS onset after 28 weeks of gestation and clinical features, including neonatal outcomes and placental findings, were elucidated.

Results: Meeting our inclusion criterion were 143 MD twin pregnancies, including 15 TTTS cases (10%). Five cases (4%) developed TTTS during the third trimester and underwent a cesarean section immediately after the diagnosis. All of these women exhibited either abdominal distension or uterine contractions. Recipient twins tended to require more intensive cardiopulmonary treatment than donors, however, neither a recipient nor a donor twin suffered neonatal death or neurological impairment. Placental arterio-arterial anastomoses were detected in three out of five cases. Arteriovenous anastomoses were present in all cases, however, venovenous anastomoses were not found in any case.

Conclusions: TTTS is a relatively rare complication during the third trimester. It is imperative to be observant for the development of TTTS in MD twin pregnancies with any abdominal symptoms, even if they appear insignificant.

Keywords: Monochorionic diamniotic twin pregnancy; perinatal outcome; third trimester; twin-twin transfusion syndrome.

***Corresponding author: Masaharu Muratra,** MD, PhD, Department of Maternal Fetal Medicine, Osaka Prefectural Hospital Organization, Osaka Medical Center and Research Institute for Maternal and Child Health, 840 Murodo-cho, Izumi, Osaka 594-1101, Japan, Tel.: +81-752-56-1220, Fax: +81-725-57-3207, E-mail: masaharu@mch.pref.osaka.jp

Keisuke Ishii, Takako Taguchi, Aki Mabuchi, Haruna Kawaguchi, Ryo Yamamoto, Shusaku Hayashi and Nobuaki Mitsuda: Department of Maternal Fetal Medicine, Osaka Prefectural Hospital Organization, Osaka Medical Center and Research Institute for Maternal and Child Health, Osaka, Japan

Introduction

Twin-twin transfusion syndrome (TTTS) occurs at a frequency of 8%–10% in monochorionic twin pregnancies [1, 9, 15], and the majority of those cases are diagnosed during the second trimester [25]. In the last two decades, the overall prognosis of TTTS cases diagnosed before 26 weeks of gestation has dramatically improved by the introduction of fetoscopic laser photocoagulation (FLP) for placental communicating vessels [18–20]. Recently, several studies have shown that FLP for cases with TTTS after 26 weeks of gestation resulted in outcomes equal to, or superior to, those undergoing conventional treatment strategies [3, 14, 26]. However, there have been only a few studies focused on the clinical features of TTTS developing after 28 weeks of gestation [3, 10, 14, 26], and it is currently unclear which management option minimizes adverse outcomes.

The aim of this study was to describe the incidence and clinical features of TTTS onset during the third trimester. To clarify the natural course of the cases, we performed a single-center cohort study and limited it to the cases managed at our center throughout pregnancy.

Methods

This was a retrospective cohort study performed at a tertiary perinatal care center in Japan, providing fetal treatment, including around 30 fetoscopic laser therapies per year. We conducted a retrospec-

tive chart review of all monochorionic diamniotic (MD) pregnancies managed from the first trimester onward that delivered at our center between January 2009 and December 2012. The cases with single or double fetal demise before 14 weeks of gestation, fetuses with major anomalies, and twin reversed arterial perfusion sequence (TRAPs) were excluded.

The diagnosis of monochorionicity and the decision of an accurate gestational age were made at first trimester by ultrasound examination. Serial sonographic examinations were performed until delivery at least every 2 weeks to evaluate fetal growth and amniotic fluid volume. Doppler measurements of the umbilical artery and the ductus venous (DV) were performed at 18–19 weeks of gestation, and additional Doppler evaluations were done at the attending physician's discretion when impaired fetal growth or discordant amniotic fluid volume between twins were found. Inpatient management was initiated only for obstetrical indications. The diagnosis of TTTS was based on the sonographic criteria of polyhydramnios with amniotic fluid volume (AFP) of 8 cm or greater in the recipient fetus and oligohydramnios with AFP of 2 cm or less in the donor fetus. Staging of the disease was done according to the Quintero's staging system [17]. We examined the prevalence of TTTS during the second and third trimesters.

Concerning cases with TTTS onset during the third trimester, we reviewed maternal demographics as well as the clinical course of these pregnancies. The data of neonatal clinical course, including the need of intratracheal intubation, the administration of catecholamine, transfusion, and neurological findings at 1 month and 6 months of life were also documented. Furthermore, placental findings were reviewed to investigate the features of placental anastomoses evaluated by a color dye injection test.

Results

Initially included in the study were 152 MD twin pregnancies; subsequently, nine cases (three cases with TRAPs, two with spontaneous abortion before 14 weeks of gestation, and four with major anomalies) were excluded (Figure 1). A total of 143 sets of MD twin pregnancies met our criteria and were analyzed. Table 1 shows baseline characteristic of this population. TTTS occurred in 15 cases (10%), 10 cases (6%) diagnosed during the second trimester, and five cases (4%) during the third trimester. Among 10 TTTS cases during the second trimester, eight cases underwent FLP, one underwent a cesarean section to transition to neonatal treatment, and one was a pregnancy termination.

The five cases with TTTS during the third trimester are summarized in Table 2. Gestational age at TTTS diagnosis ranged from 31+2 to 35+5 weeks. Two cases were Quintero's stage I and the other three were stage III or IV. Two cases (cases 1 and 3) were inpatients due to threatened preterm labor (TPL) followed by the diagnosis of TTTS. In case 1, amniotic fluid discordance expanded to meet the TTTS criteria; this occurred 2 days after the ultrasonographic evaluation, which revealed isolated oligohydramnios at 31+0 weeks of gestation. In case 3, estimated amniotic volume of both twins was noted to be normal at 33+2 weeks of gestation, however, the patient complained of uterine contractions. Severe polyhydramnios and oligohydramnios with a pericardial effusion and ascites in the recipient fetus were demonstrated 3 days later.

The other three cases (cases 2, 4, and 5) were managed as outpatients without obstetrical complications. All three women presented at our outpatient department and complained of abdominal distension, which began a few days before the diagnosis of TTTS. In case 2, sonographic

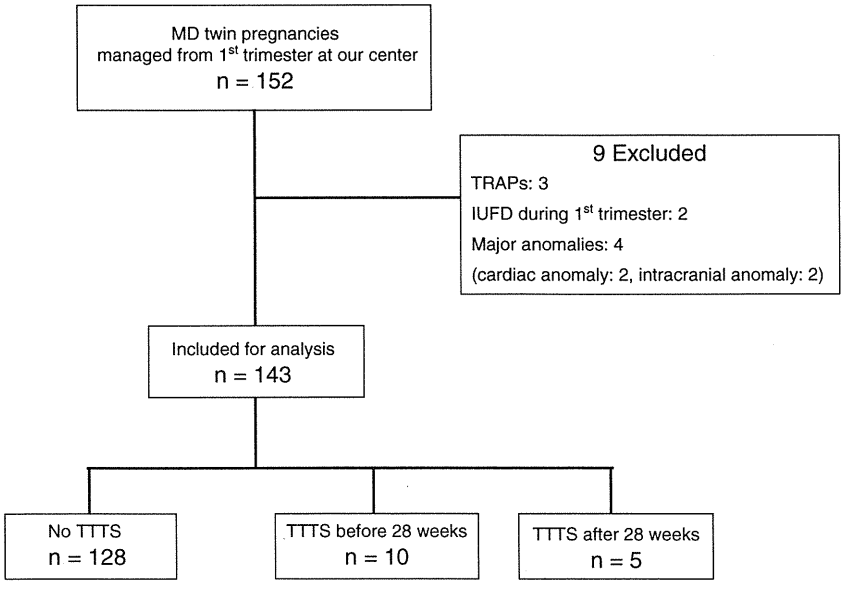

Figure 1 Diagram of study profile.

Table 1 Baseline characteristics.

No. of pregnancies, n	143
Maternal age, median (range)	31 (18–42)
Primipara, n (%)	78 (55)
Spontaneous conception, n (%)	120 (85)
Ovulation induction, n (%)	10 (7)
IVF/ICSI, n (%)	13 (9)
Delivery <22 weeks of gestation, n (%)	8 (6)
Both IUFD	5
Termination of pregnancy	2
Spontaneous abortion	1
IUFD, n (%)	9 (7)
Single IUFD, n	5
Both IUFD, n	4
TTTS, n (%)	15 (11)
Diagnosed <28 weeks of gestation	10
Diagnosed >28 weeks of gestation	5

IVF=*in vivo* fertilization, ICSI=intracytoplasmic sperm injection, IUFD=intrauterine fetal death, TTTS=twin-twin transfusion syndrome.

evaluation exhibited TTTS with reversal of DV flow in the recipient twin despite normal sonographic findings 9 days before the diagnosis of TTTS. In case 4, polyhydramnios, pleural effusion, and ascites in the recipient twin together with anhydramnios in the donor twin were found at an examination 7 days after the previous scan in which the amniotic fluid volume was noted to be normal. In case 5, amniotic fluid volume was found to be normal in both twins 14 days before the patient was diagnosed at 33+5 weeks with TTTS.

All cases underwent cesarean section immediately after diagnosis of TTTS without any fetal intervention, and subsequently, neonatal care was initiated by neonatologists. Neonatal and placental findings of the five cases are presented in Table 3. All five recipient neonates and two donor neonates required intratracheal intubation. Four out of five recipients required administration of catecholamine, whereas no donors required this therapy. Two recipient neonates required fresh frozen plasma transfusions to sustain hemodynamic status; one donor required red cell concentrate due to severe anemia. There was neither neonatal death nor abnormal neurological findings at the first month and 6 months after birth.

In regard to placental findings, arterioarterial anastomoses (AAA) were detected in three cases (cases 1, 2, and 5), and arteriovenous anastomoses were detected in all cases, however, venovenous anastomoses were not found in any case.

Discussion

The prevalence of TTTS onset after 28 weeks of gestation was 4% among the MD twin pregnancies uniformly managed at a single perinatal center from the first trimester onward. This prevalence is less than half that of previous reports including the typical onset of TTTS during the second trimester [1, 9, 15]. Thorson et al. reported five cases that were diagnosed after 28 weeks among 42 TTTS pregnancies (12%) [25]; however, that series consisted of patients referred from a number of hospitals at an unspecified stage of gestation.

It is well-known that placental angioarchitecture [4, 6, 8] and endocrinologic mechanisms [2, 7, 12] are involved in the pathogenesis of TTTS. It has been suggested that

Table 2 Five cases with twin-twin transfusion syndrome (TTTS) onset during the third trimester: pregnancy course.

Case	Characteristics			TTTS diagnosis	
	Age	Parity	GA (w)	Quintero stage	Comments
1	33	0	31+2	I	Inpatient due to TPL from 27 weeks of gestation Amniotic fluid discordance was detected at 31 weeks by chance
2	29	0	31+4	IIIRa[a]	Outpatient as uncomplicated MD twin until 30 weeks without amniotic fluid discordance Abdominal distension was started a couple of days before the date of TTTS diagnosis
3	30	1	33+5	IV	Inpatient due to TPL from 28 weeks Amniotic fluid discordance was detected at 33 weeks by chance
4	22	0	34+5	IV	Outpatient as uncomplicated MD twin until 33 weeks without amniotic fluid discordance Abdominal distension was started a couple of days before the date of TTTS diagnosis
5	33	0	35+5	I	Outpatient as selective IUGR type I until 34 weeks without amniotic fluid discordance Abdominal distension was started a couple of days before the date of TTTS diagnosis

[a]The suffix "R" indicates abnormal Doppler was found in recipient and the fetus suffix "a" means atypical. GA=gestational age, TPL=threatened preterm labor.

Table 3 Five cases with TTTS onset during the third trimester: neonatal and placental findings.

Case	Neonate									Placenta		
	GA (weeks)	Quintero stage	Birth weight (g)	Hb (g/dL)	Comments	Intratracheal intubation	Catecholamine	Transfusion	Neurological findings		AAA	AVA	VVA
					Fetus				at 1 month	at 6 months			
1	31+2	I	1602	20.0	Recipient	Day 0–1	Day 0–2	No	Normal	Normal	Yes	Yes	No
			1380	20.3	Donor	No	No	No	Normal	Normal			
2	31+4	IIIRa	1794	20.5	Recipient	Day 0–3	Day 0–2	FFP: day 0–2	Normal	Normal	Yes	Yes	No
			1158	10.0	Donor	Day 0–3	No	RCC: day 1–3	Normal	Normal			
3	33+5	IV	2376	14.0	Recipient	Day 0–7	No	FFP: day 3–4	Normal	Normal	No	Yes	No
			1774	17.6	Donor	No	No	No	Normal	Normal			
4	34+5	IV	2270	13.9	Recipient	Day 0–1	Day 0–4	No	Normal	Normal	No	Yes	No
			1901	16.0	Donor	Day 0–1	No	No	Normal	Normal			
5	35+5	I	2152	20.4	Recipient	Day 0–4	Day 1–5	No	Normal	Normal	Yes	Yes	No
			1526	20.4	Donor	No	No	No	Normal	Normal			

GA=gestational age, Hb=hemoglobin, RCC=red cell concentrate, AAA=arterio-arterial anastomoses, AVA=arteriovenous anastomoses, VVA=venovenous anastomoses, FFP=fresh frozen plasma.

thrombosis could cause hemodynamic changes between the twins leading to late onset TTTS [16, 24]. However, it remains to be fully clarified why TTTS develops during the third trimester after a previously uncomplicated prenatal course.

As twin pregnancies tend to be accompanied with threatened preterm labor, especially after the mid-trimester, uterine contractions might play some role in the development of TTTS. In animal studies, it has been demonstrated that non-labor uterine contractions cause significantly increased arterial and venous pressure in fetuses with oligohydramnios [21]. This could amplify blood transfusion from twins with relatively less amniotic fluid volume to co-twins. Moreover, it has been reported that uterine contractions could also affect fetal hemodynamic status in both normal and TTTS conditions [22, 23]. It appears to be imperative to explore the development of TTTS closely in MD twin pregnancies with clinical manifestations of TPL. However, the use of prophylactic tocolysis cannot be endorsed, considering that our five TTTS cases included two patients under medical treatment with intravenous tocolytic for TPL.

Several studies have shown that the presence of placental AAA plays a protective role against TTTS [5, 6]. In our series, AAA was detected in three out of five cases. Although our series is too small to verify the pathological significance of the presence of AAA, it is presumed that AAA may play a protective role against development of TTTS before the third trimester unless additional events, such as thrombosis and uterine contractions occur.

Recently, several studies have been published concerning cases treated with FLP outside the customary period [3, 14, 26]. Middeldrop et al. demonstrated that FLP performed after 26 weeks of gestation prolonged gestational age and reduced neonatal morbidity compared to serial AR [14]. Baud et al. reported FLP performed between 26+1 and 30+3 weeks of gestation resulted in an improved survival rate with similar surgical feasibility compared to the customary period [3]. Conversely, the neonatal care of TTTS survivors is challenging because of hemodynamic aberrations in addition to prematurity [11]. In our series, recipient fetuses tended to require more intensive cardiopulmonary treatment than donor fetuses; however, neither neonatal death nor neurological impairment were seen in our series. Although serial amnioreduction is related to a low survival rate and high neuromorbidity rate [10, 13], the expectant management including strict monitoring, antenatal corticosteroid administration and amnioreduction would be a option, especially stage I TTTS in early third trimester. In contrast, for the TTTS

Q1: Please give the definition of AR in the sentence finishing "... compared to serial AR [14]."

cases with advanced staging or in late third trimester, the most reasonable option appears to be intensive fetal surveillance, to deliver promptly the patient when fetal status deteriorates, and to initiate intense neonatal treatment. In order to determine the optimal management of TTTS during the third trimester, a larger series of cases and long-term outcome must be evaluated.

In conclusion, TTTS occurs during the third trimester in 4% of MD twin gestations. Although the current study includes a case number too small to derive enough clinical considerations, it appears to be important to closely monitor women with MD twin pregnancies for the development of TTTS when they develop symptoms of threatened preterm labor even if their pregnancy has been uneventful until that point. Currently, therapeutic preterm delivery might be a primary option for third trimester TTTS.

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Magnetic resonance fetal right lung volumetry and its efficacy in predicting postnatal short-term outcomes of congenital left-sided diaphragmatic hernia

Nobuhiro Hidaka, Keisuke Ishii, Yoko Furutake, Ryo Yamamoto, Jun Sasahara and Nobuaki Mitsuda

Department of Maternal Fetal Medicine, Osaka Medical Center and Research Institute for Maternal and Child Health, Izumi, Japan

Abstract

Aim: We aimed to investigate whether the ratio of magnetic resonance imaging (MRI)-measured right lung volume (RLV) to ultrasonography-estimated bodyweight (RLV/BW) and observed-to-expected (o/e) RLV are of diagnostic value in predicting postnatal outcomes of left congenital diaphragmatic hernia (CDH).

Material and Methods: We included 32 CDH patients and 34 control subjects. Manually outlined fetal right lung areas on MRI were multiplied by the slice thickness and added to determine the entire volume. The association between RLV and RLV/BW with gestational age in the controls was examined using regression analysis. RLV/BW and o/e RLV were compared between surviving and non-surviving neonates with CDH.

Results: The expected fetal RLV was derived using the formula RLV (mm^3) = 1.717 × (gestational weeks)$^{2.82}$. In the controls, RLV/BW was nearly constant during the third trimester. The 27 survivors with CDH had a median RLV/BW of 10.7 and a median o/e RLV of 60.0, whereas the five non-surviving neonates had a median RLV/BW of 4.3 and a median o/e RLV of 22.6; the differences were statistically significant.

Conclusion: Assessment of fetal lungs by MRI volumetry is reliable for clinical use. RLV/BW and o/e RLV are potential predictors of postnatal outcomes of left CDH.

Key words: congenital diaphragmatic hernia, lung hypoplasia, magnetic resonance imaging, volumetry, prognosis.

Introduction

Congenital diaphragmatic hernia (CDH) occurs in 1:2500–3000 live births and is the most common congenital anomaly associated with pulmonary hypoplasia. Prenatal prediction of pulmonary hypoplasia and clinical outcomes is important for parental counseling, planning appropriate perinatal care, and determining the optimal center for delivery. Ultrasonography (US) remains the first-line imaging technique for the non-invasive assessment of fetal lungs, and various parameters measured by US have been proposed as predictors of postnatal outcome. Lung area to head circumference ratio (LHR) and lung–thorax transverse area ratio (LTR) measurements have been found to have clinical value in the prediction of poor postnatal outcome in CDH.[1–5] These parameters help assess postnatal outcome based on 2-D measurements and target the unaffected lung (right lung in left CDH). In healthy fetuses, the LHR increases with gestational age.[6] Therefore, in fetuses with CDH, it is important to correct for gestational-age-related changes in LHR. Jani *et al.*

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Reprint request to: Dr Nobuhiro Hidaka, Department of Maternal Fetal Medicine, Osaka Medical Center and Research Institute for Maternal and Child Health, 840 Murodocho, Izumi 594-1101, Japan. Email: nhidaka1120sylphy@yahoo.co.jp

demonstrated that this problem can be overcome by calculating the observed-to-expected (o/e) or the relative LHR and showed that this parameter was independent of gestational age and valuable for predicting the outcomes of neonates with CDH.[7] Regarding the other 2-D US parameter, LTR, Usui *et al.* performed a multicenter retrospective cohort study that included 114 isolated cases and reported that the LTR was not strongly influenced by gestational age and was a reliable prenatal predictive parameter in fetuses with isolated CDH.[1] Although the clinical application of these 2-D sonographic parameters has been proposed, unfavorable imaging conditions, such as polyhydramnios, oligohydramnios, and maternal obesity, can compromise the spatial resolution of ultrasonographic images. Under these conditions, accurate prediction of lung development using US is difficult, even when the procedure is performed by experienced technicians. In addition, the measurement itself is often operator dependent, thereby leading to poor reproducibility across different examiners and centers. Previously, Heling *et al.* investigated the correlation between 2-D lung biometry measurements and autopsy findings based on their own 29 cases and concluded that 2-D lung biometry is not a suitable method for antenatal detection of pulmonary hypoplasia.[8]

For some years, magnetic resonance imaging (MRI) fetal lung volume (FLV) measurement has been used to quantify pulmonary hypoplasia and to assess the probability of neonatal survival. However, the gestational age or fetal size at the time of MRI assessment can vary, and the evaluation method and the estimation of lung volume from MRI scans remain controversial. The parameter used most frequently to adjust the observed lung volume is o/e lung volume, that is, relative lung volume. O/e lung volume is calculated as a ratio of measured lung volume to appropriate mean for gestation. Several researchers have investigated the normative values of total FLV,[9–18] and MRI FLV has been found to be useful for antenatal prediction of survival in CDH in some of these studies.[9–13] The results of these studies show significantly impaired survival in children with an o/e FLV lower than 25–40%.[9–11] However, these studies have targeted total lung volume in CDH fetuses. As the established sonographic parameters (LHR or LTR) refer to the lung contralateral to the herniated side, accurate assessment of the hypoplastic lung on the herniated side is difficult in severe CDH cases. Therefore, the reliability of assessing total lung volume by MRI remains questionable. Moreover, previous studies defining the expected lung volume were mostly conducted using Western control subjects,[9–12,14–18] and although fetal lung size is expected to differ according to race, Japanese and Asian data in this regard are still lacking.

Here, we narrowed down the target to the fetal right lung and defined the expected right lung volume (RLV) based on Japanese controls. In addition to the o/e RLV, we defined RLV/BW (ratio of MRI-measured RLV to US-estimated fetal bodyweight) as another parameter for adjusting the observed lung volume. Using these parameters, we aimed to investigate whether the RLV/BW (mm^3/g) and o/e RLV are of diagnostic value in predicting postnatal short-term outcomes of Japanese fetuses with left CDH.

Methods

Patients

The records of all patients referred for obstetric MRI between January 2006 and August 2011 were reviewed, and all fetuses with a normal thorax were included in the control group. The clinical and imaging data for these control patients were retrospectively examined. The fetuses included in this group had no thoracic or lung abnormalities and demonstrated appropriate growth and biometric data for the respective gestational age at the time of the MRI, as confirmed by previous US images. The following exclusion criteria were applied:

1 Incomplete visualization of the right lung in MRI scans. Many of the examinations were performed for placental or fetal neurologic indications and the lungs were not included in the field of view.
2 Extrathoracic abnormality on US, such as gross hydrocephalus, that might have confounded the biometric measurements of fetal size.

In all cases, pregnancy dating was confirmed based on the last menstrual period and US measurements of the fetal crown–rump length obtained at 8–10 weeks of gestation. In all control fetuses, bodyweight was calculated using US either on the same day or within 3 days before or after fetal MRI. From control subjects, 21 fetuses were selected at random to evaluate the reliability of RLV measurement on MRI.

Next, the charts of all fetuses undergoing prenatal MRI examination for evaluation of fetal left-sided CDH at our institution between January 2006 and August 2012 were reviewed. Fetuses with other congenital anomalies or chromosomal abnormalities were excluded from the analysis because they often follow a

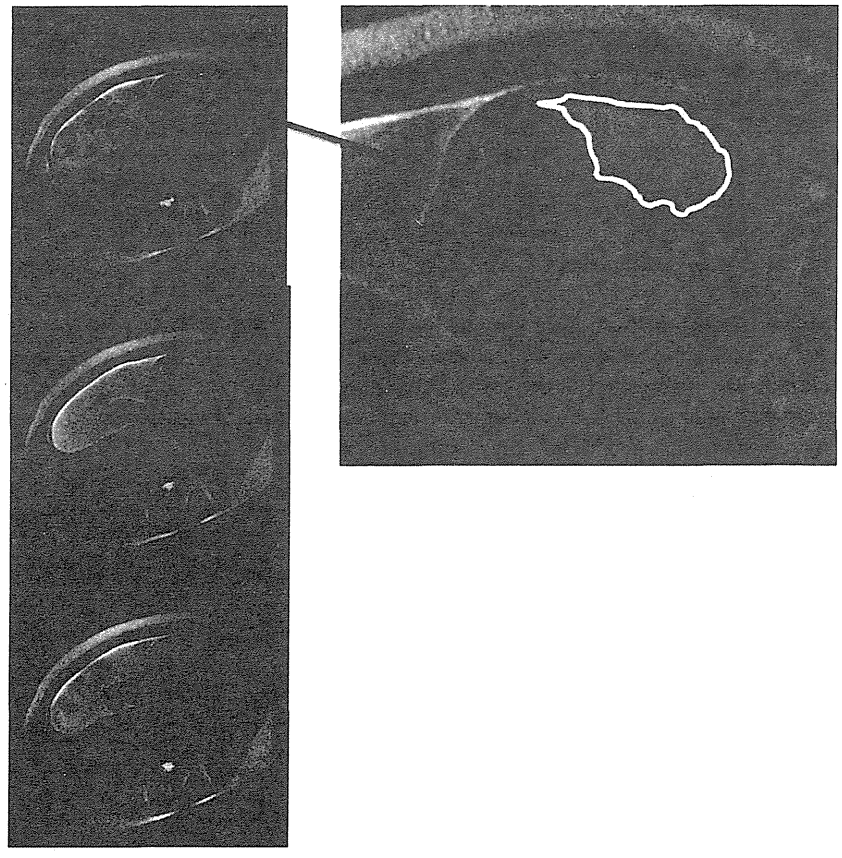

Figure 1 Illustration showing the manual segmentation of the right lung of a fetus with left-sided diaphragmatic hernia.

different course from that of the more common left-sided isolated defects. In all CDH fetuses, bodyweight was calculated by US performed on the same day as fetal MRI.

Fetal MRI technique and measurements

Fetal MRI examinations were performed on a 1.5-T SIGNA MRI system. Informed consent was obtained from all mothers for performing fetal MR imaging. Fetal imaging was performed through the uterus by using T2-weighted half-Fourier single-shot turbo spin echo with the following parameters: echo time, 100 msec; repetition time, 3100–4600 msec; matrix, 256 × 256; and slice thickness, 5 mm. The subjects were scanned in the supine or lateral decubitus position; no sedative was administered for reducing fetal movement. Sequences altered by fetal movements were repeated. The examination time was usually 20–30 min.

The fetal RLV was calculated as follows: in the transverse plane that allowed complete imaging of the right lung, planimetry was performed by manual tracing around the visible portions of lung parenchyma in all consecutive sections, excluding the pulmonary hila, and the areas measured were multiplied by the slice thickness and added (Fig. 1).

US measurements

Prenatal US was performed by experienced obstetricians by using an abdominal approach with a Voluson E6 or E8 US system. Freeze-frame capabilities were available, and on-screen calipers were used for all measurements. The US-estimated fetal bodyweight (BW) (g) was derived by using an established formula for Japanese fetuses. The formula was based on biparietal diameter (BPD) (cm), abdominal circumference (AC) (cm), and femur length (FL) (cm) and was as follows: $BW = (1.07 \times BPD^3) + (0.30 \times AC^2 \times FL)$.[19]

According to institutional policy, LHR and LTR were calculated weekly or every 2 weeks in cases of prenatal diagnosis of CDH. These parameters were measured using a cardiac four-chamber view of the fetal chest with the heart in diastole. The lung area was determined by multiplying the longest axis by the longest measurement perpendicular to it, and the LHR was

determined by dividing the lung area expressed in mm^2 by the head circumference expressed in mm. LTR was defined as the area of the contralateral lung divided by the area of the thorax. The area of the thorax bound by the inner border of the ribs on both sides, the posterior edge of the sternum, and the outer edge of the vertebra was measured by manual tracing.

Study design

This retrospective review of prospectively acquired data was performed to assess the reliability of MRI fetal right lung volumetry, chronological changes in RLV and RLV/BW during gestation, and the diagnostic value of RLV/BW and o/e RLV in predicting the postnatal short-term outcomes of CDH.

The reliability of RLV measurement in the control group was analyzed using the intraclass correlation coefficient. The measurements were obtained by two experienced obstetricians (N.H., Observer 1; Y.F., Observer 2). Observer 1 obtained two measurements at different time intervals. To determine intraobserver reliability, Observer 1 blindly measured fetal RLV twice, and to assess interobserver reliability, Observer 2 obtained one set of measurements. The two observers performed all measurements independently to eliminate bias. Normative RLV and RLV/BW were determined in the control group to calculate the expected RLV at each gestational age.

Next, Observer 1, who was blinded to the patient's identity, gestation, and outcome, performed RLV measurements from stored MRI images of fetuses with isolated left CDH, and RLV, RLV/BW, and o/e RLV were calculated. The postnatal clinical course in terms of survival at discharge was determined. CDH subjects were classified into two groups: the survivor group and the non-survivor group. The differences between RLV/BW, o/e RLV, and other sonographic parameters, such as LHR and LTR, for the two groups were examined for statistical significance.

Institutional therapeutic strategy for newborns with CDH

The therapeutic strategy included early surgery after preoperative stabilization and nitric oxide inhalation, as previously reported.[20] Briefly, high-frequency oscillatory ventilation was used routinely; exogenous surfactant was administered immediately after birth in cases of surfactant deficiency noted on the stable microbubble test. Nitric oxide was administered in cases where the preductal arterial saturation was less than 90%. Extracorporeal membrane oxygenation was not used. The blood flow through the ductus arteriosus was measured immediately after birth by using the color Doppler method. Prostaglandin E_1 was administered in cases where right-to-left shunting occurred for a greater duration at the ductus arteriosus than left-to-right shunting. Patient management practices were maintained constantly during the study period.

Statistical analysis

Data reported by the two observers were plotted and a line of equality was drawn to determine the degree of agreement. The consensus between and among observers was analyzed using the intraclass correlation coefficient for fetal right lung volumetry.[21] The intraclass correlation coefficient measures the strength of agreement of the variables, independent of the dimension of the variable considered; the following benchmarks were used for intraclass correlation coefficient characterization:[22] slight reliability (0–0.2), fair reliability (0.21–0.4), moderate reliability (0.41–0.6), substantial reliability (0.61–0.8), and almost perfect reliability (0.81–1.0). The degree of agreement was determined using the Bland–Altman test,[23] which allows calculation of the range in which 95% of the disagreement between and within observers is likely to occur.

The RLV and RLV/BW were assessed throughout gestation in the control group. Regression analysis was performed to determine the relationship between RLV and gestational age, and the linear function, logarithm function, power math function, and exponential function were calculated to assess the impact of gestational age on fetal RLV in the controls. The regression formula showing the highest and satisfactory determination coefficient was defined. The distribution of the RLV/BW during pregnancy was determined.

In the CDH group, the differences in RLV/BW and o/e RLV were analyzed between the surviving and non-surviving neonates by using the Mann–Whitney *U*-test. For other sonographic parameters, univariate analysis was performed by Fisher's exact test or Mann–Whitney tests for categorical and continuous outcomes, respectively.

A *P*-value of less than 0.05 was considered statistically significant. Statistical analysis was performed using SPSS 21.0 for Windows.

Results

Thirty-four pregnant women met the inclusion criteria for the control group. The indications for MRI

Table 1 Reasons for the MRI examination in the 34 control pregnancies

• Placenta previa or suspected placenta accreta	15
• Vasa previa	1
• Fetal anomaly on US (including suspected cases)	
Megacisterna magna	1
Borderline ventriculomegaly	3
Arachnoid cyst	3
Lissencephaly	1
Hydronephrosis (Grade 2)	1
Esophageal atresia	1
Echogenic intrahepatic foci	1
Meningomyelocele	1
Ovarian cyst	3
Neck cyst	1
Enlarged thymus	1
Polyhydramnios	1

MRI, magnetic resonance imaging; US, ultrasonography.

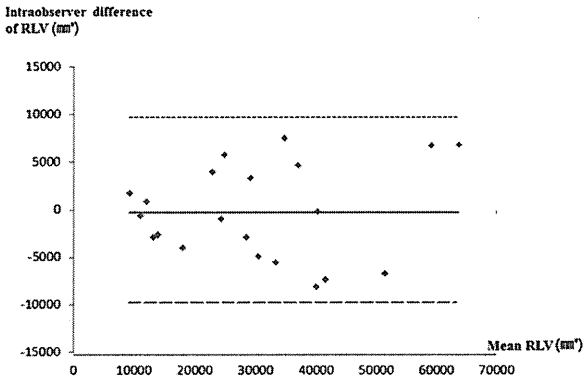

Figure 2 Bland–Altman plot showing the range in which 95% of the intraobserver disagreement for fetal right lung volume (RLV) is likely to occur. RLV is expressed in mm^3. —, mean; -----, minimum detectable change (MDC)95; ––, +MDC95.

examination in the 34 pregnancies were placenta previa or suspected placenta accreta in 15 cases, vasa previa in one case, and anomalies suspected by US in 18 cases (Table 1). The mean gestational age at the time of MRI examination was 32.3 weeks (range, 26–37 weeks). Fetal RLV on MRI was satisfactorily obtained in all cases.

Figure 2 shows the Bland and Altman plots of the differences in fetal RLV measurements obtained by Observer 1, expressed as proportions against the mean. Figure 3 shows plots of the differences in fetal RLV measurements between observers against the line of equality. All the points seem to lie randomly around this line, indicating the absence of bias. The variables

Table 2 The ICC values and their 95%CI

	ICC (95%CI)
Intraobserver	0.952 (0.888–0.980)
Interobserver	0.896 (0.756–0.957)

CI, confidence interval; ICC, intraclass correlation coefficient.

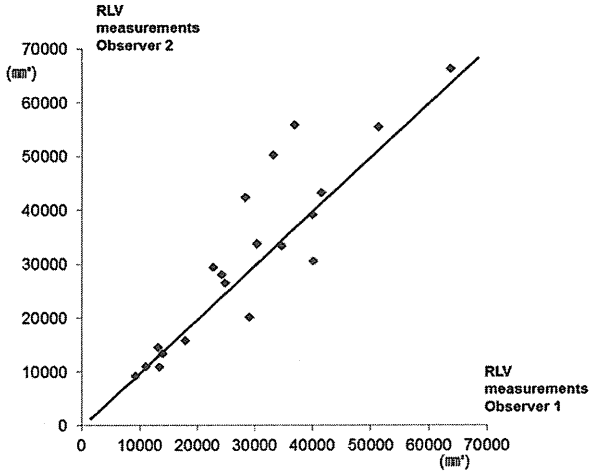

Figure 3 Fetal right lung volume measurements obtained by observers and the line of equality.

are close to the line of equality as well, indicating good agreement and seemingly minor differences between observers. Table 2 summarizes the intraclass correlation coefficient values and their 95% confidence interval (CI). The intraobserver reliability was almost perfect at 0.952 (95%CI, 0.888–0.980), and the interobserver reliability was 0.896 (95%CI, 0.756–0.957), also reflecting almost perfect reliability.

Figure 4 shows the RLV and RLV/BW normative distribution plotted against gestational age in weeks in the control group. With respect to the RLV, the highest determination coefficient, which was observed in the power match function, was 0.546, showing satisfactory prediction accuracy. The regression analysis yielded the following standard formula: RLV (mm^3) = $1.717 \times (\text{gestational age in weeks})^{2.82}$ ($P < 0.0001$). The best fit for RLV/BW was represented by the regression line RLV/BW (mm^3/g) = $-0.10 \times$ (gestational age in weeks) + 20.23. Based on this, RLV/BW decreased slightly with gestational age during the third trimester, but was nearly constant. Table 3 shows the expected RLV at each gestational age (26–37 weeks) calculated from the standard formula shown above.

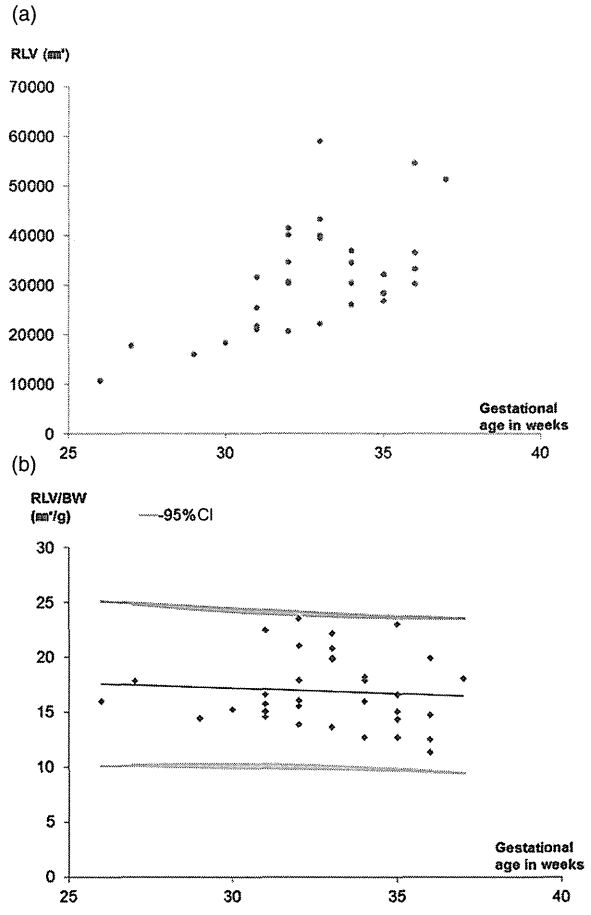

Figure 4 Scatter plots showing the changes in (a) fetal right lung volume (RLV) (mm^3) and (b) RLV/ultrasound-estimated bodyweight ratio (mm^3/g) during the gestational period in 34 control fetuses.

Table 3 Expected fetal RLV calculated from our standard formula: RLV = 1.717 × (gestational age in weeks)$^{2.82}$

Gestational age (weeks)	Fetal RLV (mm^3)
26	15 185
27	17 262
28	19 532
29	22 005
30	24 690
31	27 600
32	30 743
33	34 130
34	37 773
35	41 682
36	45 868
37	50 342

RLV, right lung volume.

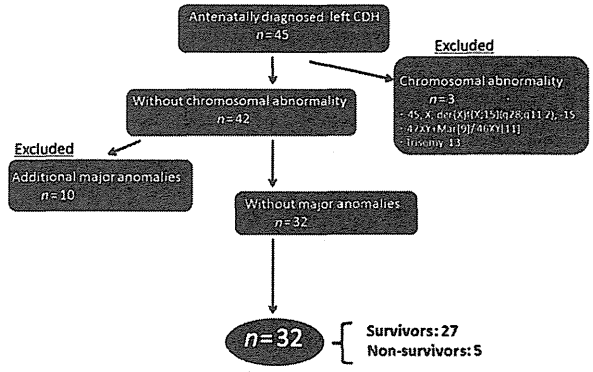

Figure 5 Enrollment of isolated left congenital diaphragmatic hernia (CDH) patients.

Table 4 Patient demographics of CDH cases

Demographics	Values
Patients (*n*)	32
Fetal sex, male	17 (53.1%)
Gestational age at diagnosis (weeks)	28 (19–36)
Liver herniation (*n*)	11 (34.4%)
Herniation of more than half of the stomach into the right chest (*n*)	7 (21.9%)
Polyhydramnios (*n*)	7 (21.9%)
Gestational age at MRI exam (weeks)	31 (26–36)
RLV/BW (mm^3/g)	9.6 (1.8–18.4)
o/e RLV (%)	47.7 (11.5–115.4)
Gestational age at delivery (weeks)	37 (33–40)
Birthweight (g)	2623 (1834–3324)
Survival at discharge	27 (84.4%)

CDH, congenital diaphragmatic hernia; BW, bodyweight; o/e RLV, observed-to-expected right lung volume.

From January 2006 to August 2012, 45 fetuses with antenatally diagnosed left CDH were evaluated at our unit. Of these 45 fetuses, three had a chromosomal abnormality and 10 had major additional anomalies and were excluded from this study. Thirty-two patients met the criteria for the diagnosis of isolated left CDH on gestational MRI evaluation (Fig. 5). The patient demographics are listed in Table 4. The median gestational age at diagnosis was 28 weeks (range, 19–36 weeks), and the median gestational age at the time of the MRI was 31 weeks (range, 26–36 weeks). The overall survival rate at discharge was 84.4% (27 of 32).

Table 5 shows the prognostic value of RLV/BW, o/e RLV, and other sonographic parameters for predicting survival at discharge in patients with left CDH. The 27 surviving neonates with CDH had a median RLV/BW of 10.7 (range, 2.8–18.4) and a median o/e RLV of 60.0

Table 5 Perinatal factors and risk of mortality in 32 CDH fetuses

Variable	Non-survivors ($n = 5$)	Survivors ($n = 27$)	*P*-value
RLV/BW, mm^3/g	4.3 (1.8–4.5)	10.7 (2.8–18.4)	<0.01*
o/e RLV, %	22.6 (11.5–31.6)	60.0 (16.3–115.4)	<0.01*
Male sex, *n* (%)	1 (20%)	16 (59%)	NS
Gestational age at diagnosis, weeks	27 (23–31)	28 (19–37)	NS
Polyhydramnios, *n* (%)	0 (0%)	7 (26%)	NS
Intrathoracic position of liver, *n* (%)	4 (80%)	7 (26%)	0.04*
Herniation of more than half of the stomach into the right chest, *n* (%)	4 (80%)	3 (11%)	<0.01*
LTR before delivery	0.07 (0.02–0.06)	0.12 (0.04–0.25)	0.02*
LHR before delivery	1.34 (0.37–1.77)	1.93 (0.56–3.56)	0.04*
Gestational age at delivery, weeks	37 (37–38)	37 (33–40)	NS
Birthweight, g	2670 (2372–3308)	2622 (1834–3324)	NS

*Statistically significant. BW, bodyweight; LHR, lung area to head circumference ratio; LTR, lung–thorax transverse area ratio; NS, not significant; o/e RLV, observed-to-expected right lung volume.

(range, 16.3–115.4), whereas the non-surviving five neonates had a median RLV/BW of 4.3 (range, 1.8–4.5) and a median o/e RLV of 22.6 (range, 11.5–31.6); the differences between the two groups were statistically significant. LHR and LTR measured immediately before delivery were significantly lower in the non-survivor than in the survivor group.

Figure 6 shows the comparison of RLV/BW and o/e RLV between the survivor and non-survivor groups. The survival rate of patients with RLV/BW > 5 mm^3/g was 100%, whereas that of patients with RLV/BW < 5 mm^3/g was 17% (1/6). The survival rate of patients with o/e RLV > 35% was 100%, whereas that of patients with o/e RLV < 35% was 17% (1/6). Table 6 shows the predictive value of RLV/BW and o/e RLV for postnatal death, with cut-off values of 5 mm^3/g and 35% for RLV/BW and o/e RLV, respectively.

Discussion

Reliability is the ability to repeat, reproduce or consistently obtain the same measurements under identical conditions. Reliability analysis enables evaluation of the properties of measurement scales and their component items. The reliability analysis procedure calculates a number of commonly used measures by using a reliability scale and provides information about the relation between individual items in the scale. Intraobserver reliability indicates the ability to consistently obtain similar measurements by the same observer, whereas interobserver reliability indicates the ability to obtain similar measurements by different observers under the same circumstances. Consensus between and among observers is commonly reached by using the intraclass correlation coefficient and interclass correlation coefficient. Only a few previous studies on normal fetal lung volumetry performed using MRI have analyzed the reliability of measurements. Rypens *et al.* reported an interobserver correlation coefficient of 0.96.[14] Ward *et al.* showed that the interobserver correlation coefficient was 0.76 and intraobserver correlation coefficient was 0.83 in transverse plane measurements.[24] In this study, we have additionally shown the reliability of RLV measurements by using the manual tracing method on MRI scans.

Over the last decade, multiple MRI studies have reported normal FLV according to gestational age.[9–18] However, these values were obtained using mostly Western controls. Relative FLV can be calculated by applying several formulas that have been derived from the nomograms of healthy fetal lungs and are designed to determine the expected lung volume.[12–15,17,18] Fetal lung size is expected to differ according to race, and Japanese and Asian data in this regard are still lacking. Indeed, several researchers have reported race-based differences in gestational age-specific birthweights. Wang *et al.*[25] showed that the gestational age-specific mean birthweights of Japanese babies were lower than those of white American babies. The FLV reported in a previous study performed using the Japanese population[13] were smaller than those obtained by US and MRI in other fetal lung volumetry studies. These findings suggest the presence of race-based differences in lung volumes. Furthermore, the treatment of neonates with CDH varies in different countries. In patients with apparently isolated CDH, the overall survival rate was reported to be approximately 60–70% in several recent

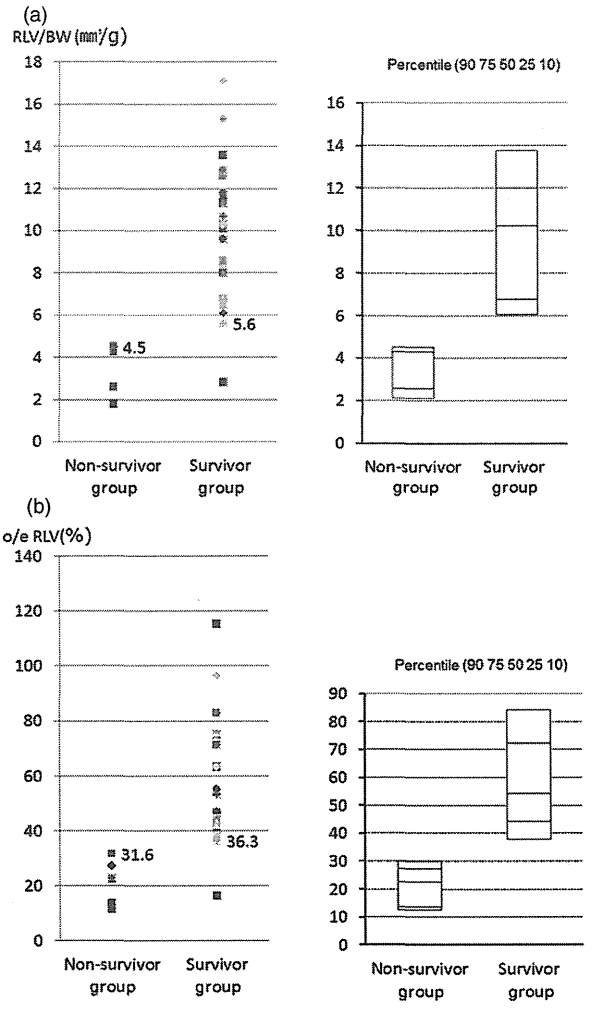

Figure 6 Comparison of right lung volume (RLV)/bodyweight (BW) and observed-to-expected (o/e) RLV between the non-survivor group and the survivor group. (a) The highest value of RLV/BW in the non-surviving group is 4.5, whereas the second lowest value in the surviving group is 5.6. (b) The highest value of o/e RLV in the non-surviving group is 31.6, whereas the second lowest value in the surviving group is 36.3.

studies, with the remaining babies usually dying in the neonatal period because of pulmonary hypoplasia and/or pulmonary hypertension.[26–29] The survival rate in our study was 84.4%, which is comparatively higher than that reported in previous studies. Based on a Japanese multicenter retrospective cohort, Usui *et al.* reported that the mortality at 90 days was 79%.[2] An assessment of the specific prognosis of Japanese patients and the indications for fetal surgery is a clinical necessity.

Table 6 Predictability for postnatal death using RLV/BW and o/e RLV

Parameter	RLV/BW (cut-off, 5 mm^3/g)	o/e RLV (cut-off, 35%)
Sensitivity	5/5 (100%)	5/5 (100%)
Specificity	26/27 (96%)	26/27 (96%)
PPV	5/6 (83%)	5/6 (83%)
NPV	26/26 (100%)	26/26 (100%)
LR+	25	25

BW, bodyweight; LR, likelihood ratio; NPV, negative predictive value; PPV, positive predictive value; o/e RLV, observed-to-expected right lung volume.

In patients with CDH, the degree of pulmonary hypoplasia is an important determinant of mortality. Therefore, prenatal evaluation of the fetal lung is important to accurately predict the prognosis and make the appropriate predelivery choices regarding ventilatory support, surgery, and parent counseling. Although 2-D US is the first-line technique of choice for quantitative evaluation of lung hypoplasia, accurate prediction of lung development and maturity by using US remains somewhat challenging. In contrast, MRI is becoming increasingly popular and can provide not only anatomical information but also the functional details of the malformed fetus. This has led to an increased number of studies discussing the potential of MRI for assessment of the fetal lung. Most investigations have focused on bilateral assessment of the lung; however, the accurate detection and tracing of the lung on the herniated-side lung remains challenging, especially in the case of severe CDH. Here, we assessed the lung contralateral to the herniated side and defined the expected RLV. We used the o/e RLV and RLV/BW as parameters to adjust the observed lung volume. In our cohort, all patients with RLV/BW < 2.8 mm^3/g died, whereas all patients with RLV/BW > 4.5 mm^3/g survived. Furthermore, all patients with o/e RLV < 16.3% died, whereas all patients with o/e RLV > 31.6% survived. The results of this study thus confirm the feasibility of using volumetric measurements based on MRI scans to quantitate pulmonary hypoplasia in fetuses with abnormal lungs.

Our study has some limitations. First, our control group consisted of fetuses who had normal lungs and had been taken from a population referred for prenatal MRI because of various extrathoracic abnormalities

and therefore may not have been representative of truly healthy fetuses. We tried to minimize this effect by excluding fetuses with thoracic abnormalities on US and those with ultrasonographic abnormalities that were likely to confound biometric measurements. Consequently, all controls showed a good respiratory outcome after birth. Second, in the CDH group, we could not prepare a receiver–operator curve to determine the appropriate cut-off value because the nonsurvivor group consisted of only five patients. The survival rate of patients who had isolated left CDH and were treated at our institution was approximately 85%; therefore, we need to continue to obtain a bigger sample size. Third, fetal bodyweight was sonographically estimated using an established Japanese-specific formula. This formula includes fetal abdominal circumference as a measurement, and the abdominal circumference in fetuses with CDH tends to be small, depending on the herniated organs. Therefore, the bodyweight of some CDH fetuses might be underestimated.

In conclusion, our data suggest that RLV/BW and o/e RLV are reliable parameters for estimating the neonatal outcomes of left CDH. MRI is not a replacement for US as a first-line screening technique. However, fetal MRI is a feasible technique for assessing malformed lungs, particularly when sonographic imaging conditions are negatively affected (e.g., in oligohydramnios, maternal obesity, and unfavorable fetal positions), and RLV/BW and o/e RLV provide information useful for predicting neonatal survival. The prognostic and therapeutic importance of our results needs to be assessed in further clinical studies.

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Disclosure

The authors have no financial interest to declare in relation to the content of this article.

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Significance of maternal screening for toxoplasmosis, rubella, cytomegalovirus and herpes simplex virus infection in cases of fetal growth restriction

Ryo Yamamoto, Keisuke Ishii, Mayumi Shimada, Syusaku Hayashi, Nobuhiro Hidaka, Masahiro Nakayama and Nobuaki Mitsuda

Department of Maternal Fetal Medicine, Osaka Medical Center and Research Institute for Maternal and Child Health, Osaka, Japan

Abstract

Aim: The objective of this study was to evaluate the significance of maternal toxoplasmosis, rubella, cytomegalovirus (CMV) and herpes simplex virus (TORCH) screening in cases of fetal growth restriction (FGR).

Material and Methods: The medical records of women carrying fetuses with FGR who underwent TORCH screening over a 10-year period were retrospectively reviewed for maternal and congenital TORCH infection. Women carrying fetuses with FGR routinely underwent serologic TORCH tests and systematic ultrasound evaluation for congenital abnormalities. If a congenital CMV infection was suspected, amniotic fluid, placenta or neonatal urine was used for CMV DNA detection by polymerase chain reaction.

Results: In 319 patients, no cases of maternal or congenital infection with toxoplasma, rubella, or herpes simplex virus were found. Conversely, six cases (1.8%) were diagnosed with congenital CMV infection, two of which had no structural abnormalities other than FGR.

Conclusions: A complete maternal TORCH screening for cases of FGR appears to be unnecessary. Although a maternal CMV test can be considered, the incidence of congenital CMV infection was found to be low in FGR cases.

Key words: congenital infection, cytomegalovirus, fetal growth restriction, maternal TORCH test.

Introduction

Because there may be a wide variety of factors that result in fetal growth restriction (FGR), its pathophysiology is an important research topic. The incidence of microorganism-induced FGR is estimated to be approximately 5% of all cases.[1] Among various microorganisms, the combination of toxoplasmosis, rubella, cytomegalovirus (CMV) and herpes simplex virus (HSV), known as TORCH, was regarded as one of the major causes of FGR due to congenital infection.[2] *Toxoplasma gondii*, rubella, CMV, and HSV have the potential to cause a congenital infection; furthermore, each may cause FGR as well as a variety of other clinical features. When an *in utero* infection is suspected based on maternal serologic testing or abnormal ultrasonographic findings, including FGR, antenatal diagnostic tests of the fetus should be considered.[3] Although routine TORCH screening for all pregnant women has not been recommended,[4,5] the significance of maternal TORCH screening for FGR has not been fully elucidated. The low yield of work-up for TORCH infection among infants with FGR has already been described.[6]

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Reprint request to: Dr Ryo Yamamoto, Department of Maternal Fetal Medicine, Osaka Medical Center and Research Institute for Maternal and Child Health, 840 Murodo Izumi, Osaka 594-1101, Japan. Email: yamaryo@mch.pref.osaka.jp

The aim of this study was to evaluate the significance of maternal TORCH screening in cases of FGR by means of reviewing the frequency of positive maternal serologic TORCH tests and congenital infections.

Methods

All pregnant women with FGR who underwent a TORCH test between January 2001 and December 2010 at Osaka Medical Center and Research Institute for Maternal and Child Health were eligible for the study. Estimated fetal bodyweight (EFBW) was calculated by means of the Japanese local calculating formula.[7] FGR was defined by an estimated bodyweight below −1.5 standard deviation (SD) via at least two ultrasound biometrics conducted by experienced physicians. An EFBW below −1.5 SD, which is equal to a 6.7 percentile, is typically used as a criterion of FGR in Japan.[7]

Medical charts in our computerized database were retrospectively reviewed for maternal baseline characteristics, perinatal outcome, and presence or absence of maternal and congenital TORCH infection. When ultrasonographic abnormalities possibly associated with intrauterine TORCH infections were detected prenatally, they were also noted. At our institution, women carrying a fetus with FGR routinely underwent serologic TORCH tests and systematic ultrasound investigations for congenital abnormalities. The maternal tests for TORCH screening were *Toxoplasma gondii* antibody using the passive hemagglutination, rubella antibodies (immunoglobulin [Ig] G and IgM) determined by the enzyme immune assay (EIA) technique, and CMV antibodies (IgG and IgM) determined by EIA technique. The tests for HSV antibodies were conducted when appropriate; however, they were not always necessary. If maternal toxoplasma antibody titer was >160 and if the rubella IgM index or the CMV IgM index was above 1.2, the case was defined as positive. When a congenital CMV infection was suspected, the amniotic fluid, placenta, or neonatal urine was used for CMV DNA detection by polymerase chain reaction (PCR).

If a non-reassuring fetal status (NRFS) was suspected during the observational period, serial intensive evaluation of fetal status by cardiotocogram (CTG) and ultrasonography was performed. When fetuses were diagnosed with NRFS by CTG or a biophysical profile (BPP), elective delivery was performed. All neonates received systematic physical assessments by neonatologists within the first several days after birth and at 1 month of age.

Results

During the study period, 363 women carried a fetus diagnosed with FGR; all women underwent TORCH screening and 319 women, including 26 cases with twin pregnancies, fit the inclusion criteria. Forty-four cases for which perinatal information was unavailable were excluded.

Maternal baseline characteristics are presented in Table 1. Obstetrical complications included 31 cases of pregnancy-induced hypertension (9.7%); eight cases of placental abruption (2.5%); four cases with placenta previa (1.2%); three cases of twin–twin transfusion syndrome (0.94%), and one case of a placental tumor (0.31%). In seven cases, the parents opted for termination of pregnancy because of severe FGR or multiple fetal abnormalities (Table 2). Major congenital anomalies without chromosomal abnormalities occurred in 28 cases, including congenital heart disease in 12 cases, multiple malformations in six, renal agenesis in two, duodenal atresia in two, and one case each of meconium peritonitis, gastroschisis, holoprosencephaly, polycystic kidney, sacrococcygeal teratoma and congenital myopathy. In regard to chromosomal abnormalities, there were eight cases with trisomy 21 and another eight cases with trisomy 18.

Table 1 Maternal baseline characteristics

Age (years), median (range)	30 (18–44)
Nulliparity, *n* (%)	190 (59)
Twin pregnancy, *n* (%)	26 (8.1)
Conception	
Spontaneous, *n* (%)	298 (93)
Ovulation induction, *n* (%)	13 (4.0)
IVF-ET, *n* (%)	10 (3.0)
Gestational age at FGR diagnosis (weeks), median (range)	27 (18–38)

FGR, fetal growth restriction; IVF-ET, *in vitro* fertilization – embryo transfer.

Table 2 Pregnancy outcomes

Gestational age at delivery (weeks), median (range)	37 (23–41)
Male, *n* (%)	121 (37)
Birthweight (g), median (range)	1989 (326-3,200)
Cesarean section, *n* (%)	162 (50)
TOP, *n* (%)	7 (2.1)
IUFD, *n* (%)	19 (5.9)
Major congenital anomalies, *n* (%)	28 (8.7)
Chromosomal abnormality, *n* (%)	19 (5.9)

IUFD, intrauterine fetal death; TOP, termination of pregnancy.

There were no positive maternal serologic screening tests for either *Toxoplasma gondii* or rubella virus. Although 17 women were seropositive for HSV, no visible pathology was noted on the vulva or elsewhere. No congenital infections of toxoplasmosis, rubella, or HSV were found. In six cases that tested positive for maternal CMV, there were three cases with congenital infection and three other cases were defined as negative by CMV PCR of the neonatal urine or placenta (Table 3). Conversely, among six cases (1.8%) diagnosed with congenital CMV infection, three cases were serologically negative in the maternal test. In 246 isolated FGR cases without ultrasonographic abnormalities, congenital anomalies, or chromosomal abnormalities, there were two cases (0.8%) with congenital CMV infection.

Ultrasonographic abnormalities other than FGR were found in 31 cases; of these, four cases of congenital CMV infection were confirmed. Ultrasound findings and pregnancy outcome in six cases of confirmed congenital CMV infection are summarized in Table 4. In cases 1, 2, and 4, CMV DNA was detected in a fetal blood sample, amniotic fluid, or placenta. In all three liveborn neonates, a congenital CMV infection was confirmed by PCR of urine. In two cases without specific ultrasonographic abnormalities (Cases 3 and 5), cerebral palsy (CP) was noted in one case and the other was found to have normal neurological development. Case 3 underwent a cesarean delivery because of nonreassuring fetal status at 26 weeks of gestation. A 628-g neonate with Apgar scores of 5 and 6 at 1 and 5 min was delivered and diagnosed with CP.

Table 3 Prevalence of positive maternal TORCH tests and congenital TORCH infection in FGR

	Positive maternal serologic screening test	Congenital infection
Toxoplasmosis, *n* (%)	0 (0)	0 (0)
Rubella, *n* (%)	0 (0)	0 (0)
CMV, *n* (%)	6 (1.8)†	6 (1.8)
HSV, *n* (%)	17 (6.0)	0 (0)

†Three cases were congenitally infected. CMV, cytomegalovirus; FGR, fetal growth restriction; HSV, herpes simplex virus; TORCH, toxoplasmosis, rubella, cytomegalovirus and herpes simplex virus.

Discussion

This study suggests that it is not always necessary to conduct a complete maternal TORCH screening when FGR is found. We found no cases of maternal or congenital infections by any organisms other than CMV.

There were 47 cases (14.7%) with either congenital anomalies or chromosomal abnormalities in the study group. This higher rate appears to be due to the fact that many FGR cases with ultrasonographic abnormalities are referred to our tertiary care center. The reason why our cases included more female than male infants was unclear.

The incidence of primary maternal toxoplasmosis varies from 0.1% to 0.8%[8] and the prevalence of congenital toxoplasmosis ranges from 1 per 1000 live births to 1 per 10 000 live births.[9,10] Typical fetal ultrasonographic features of congenital toxoplasmosis are ventriculomegaly, intracranial calcifications, liver calcifications, increased thickness and high echogenicity of the placenta, and ascites.[11] However, there was no evidence of association between fetal toxoplasmosis and FGR in a recent study.[12] This is consistent with our study, suggesting that maternal toxoplasmosis screening may not be indicated for women carrying a fetus with FGR.

Table 4 Pregnancy outcomes in cases of congenital cytomegalovirus infections

Case	Ultrasound findings	Maternal IgM	Outcome
1	Oligohydramnios, ascites ventriculomegaly	Negative	TOP
2	Ascites, hepatosplenomegaly, liver and brain calcifications	Positive	IUFD
3	None	Positive	Live birth CP
4	Ascites, hepatomegaly, ventriculomegaly	Negative	TOP
5	None	Positive	Live birth
6	Oligohydramnios	Negative	Live birth

CP, cerebral palsy; IgM, immunoglobulin M; IUFD, intrauterine fetal death; TOP, termination of pregnancy.