The pulse Doppler gate was placed in the inlet of the DV.[11] The UA was identified in the amniotic fluid and the pulse Doppler gate was placed in the straightest portion. The insonation angle was always <60°. Pulsed Doppler signals were collected by a sample volume of 2.5–5 mm placed above the origin of the DV and the UA.

The reference ranges of each parameter between 12 and 14 GW were determined by the measurements values obtained from women who did not suffer PIH and delivered a normal infant after 37 GW. The mean values and standard deviations (SD) of CRL, BPD and FL at different gestational days were regressed using a simple linear equation. As references for DVPI and UAPI, the 5th, 10th, median, 90th and 95th percentiles in the different GW were calculated. To facilitate comparisons, the values of CRL, BPD and FL were converted to Z-scores according to the GW at the time of the scans. The Z-score was determined by the following formula: (XGD – MGD)/SDGD, where XGD is the measured value on a known gestational day, MGD is the mean value and SDGD is the SD obtained from the reference equations. The associations of these parameters with the obstetrical outcome were analyzed by Fisher's exact test and Kruskal–Wallis test. $P < 0.05$ was considered statistically significant. Cases resulting in termination of pregnancy (TOP) were excluded in analyses of SPD, PTL, IPD, PIH, SGA and LBW. Cases with fetal anomaly, fetal aneuploidy and IUFD were excluded in the analyses of SGA and LBW. Cases with IUFD before 22 GW were excluded in analyses of SPD, PTL, IPD and PIH. Cases with IUFD without sufficient information about the macroscopic findings and karyotype were excluded in analyses of fetal anomaly and fetal aneuploidy.

Results

Reference values between 12 GW and 14 GW

We determined the reference values of CRL, BPD, FL, UAPI and DVPI. The measurement values of CRL ($n = 339$), BPD ($n = 359$), FL ($n = 344$), UAPI ($n = 333$) and DVPI ($n = 277$) were obtained from women who did not suffer PIH and delivered a normal infant after 37 GW. Regression analysis demonstrated a significant positive correlation between the fetal biometry (CRL, BPD and FL) and gestational day (GD). The mean value and the SD of each biometry between 12 and 14 weeks of gestation were given by the following linear equations.

$$\text{CRL (mm)} = 1.501 \times \text{GD} - 69.780 \quad (R2 = 0.9788,\ P < 0.0001)$$

$$\text{SD-CRL (mm)} = 0.03390 \times \text{GD} + 3.095 \quad (R2 = 0.03541)$$

$$\text{BPD (mm)} = 0.5301 \times \text{GD} - 25.59 \quad (R2 = 0.9767,\ P < 0.0001)$$

$$\text{SD-BPD (mm)} = 0.04403 \times \text{GD} - 2.457 \quad (R2 = 0.4765)$$

$$\text{FL (mm)} = 0.4543 \times \text{GD} - 32.45 \quad (R2 = 0.963,\ P < 0.0001)$$

$$\text{SD-FL (mm)} = 0.05545 \times \text{GD} - 3.641 \quad (R2 = 0.2727)$$

The individual measurement values of UAPI and DVPI were plotted on the GD shown in Figure 2. We compared the data lumped for each GW by using the Kruskal–Wallis test and found that the values of UAPI and DVPI were significantly different depending on GW when they were measured. The 5th, 10th, median, 90th and 95th percentiles of the UAPI and DVPI in each GW are shown in Table 2. Using these reference ranges, the relationship of the biometry and pulse Doppler findings to the obstetrical outcomes was analyzed as follows.

Relationship between ultrasound findings and obstetrical outcomes

The results of the ultrasound findings and karyotypes of the poor-outcome group and good-outcome group are shown in Table 3.

Table 4 shows the effects of small CRL, BPD and FL (Z-score < –1 and –1.5) on the occurrence of SPD, PTL, IPD, PIH, fetal aneuploidy, fetal anomalies and delivery of SGA and LBW infants. No significant relationships were observed between small biometry and poor obstetrical outcomes. As shown in Table 5, an NT ≥ 3 mm was significantly associated with fetal aneuploidy (OR52.9, 95%CI 11.55–241.1). An increased UAPI (>90th and 95th percentiles) had no significant effect on the outcomes, and an increased DVPI (>90th and 95th percentiles) was a risk factor for SGA, LBW and fetal aneuploidy. Three of five fetuses with aneuploidy had a DVPI greater than the 90th percentile. Of three fetuses with aneuploidy and increased DVPI, two with trisomy 18 had an abnormal NT, but one with trisomy 21 did not have an abnormal NT (data not shown).

Discussion

It is considered that fetal growth in early pregnancy is minimally affected by pathological disorders. However, recent reports have suggested that fetal

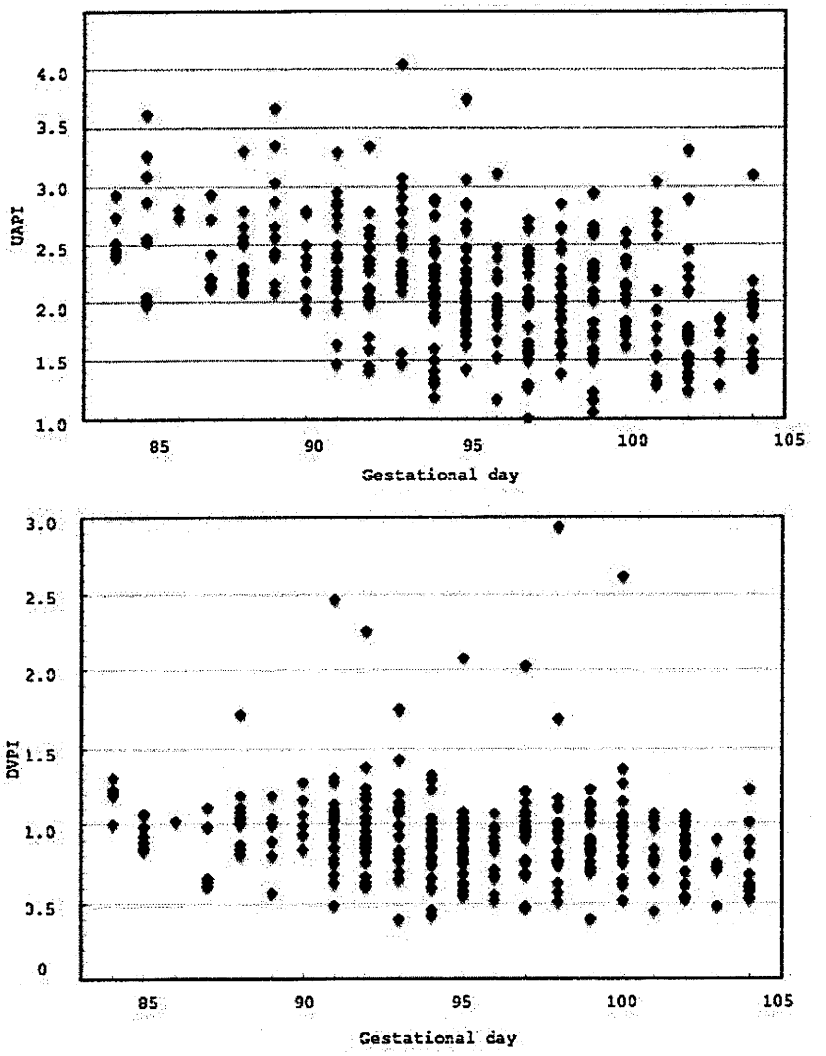

Figure 2 Relationship between gestation day and umbilical artery pulsatility index (UAPI) or ductus venosus pulsatility (DVPI). The measurement values of UAPI and DVPI of normal fetuses are plotted individually on each gestational day.

Table 2 Reference ranges of umbilical artery pulsatility index (UAPI) and ductus venosus pulsatility (DVPI)

	5th %tile	10th %tile	Median	90th %tile	95th %tile
UAPI					
12 GW	2.03	2.09	2.5	3.08	3.33
13 GW	1.41	1.53	2.19	2.84	2.96
14 GW	1.31	1.44	1.87	2.63	2.8
DVPI					
12 GW	0.64	0.80	1.00	1.21	1.28
13 GW	0.54	0.63	0.91	1.22	1.34
14 GW	0.53	0.59	0.85	1.21	1.23

%tile, percentile; GW, gestational weeks.

Table 3 Results of ultrasound findings and karyotypes of the poor-outcome group and good-outcome group

		Good-outcome group (n = 406)			Poor-outcome group (n = 110)		
		Mean or median	SD or IQR	n	Mean or median	SD or IQR	n
CRL z score		0.04†	0.95‡	381	−0.12†	1.03‡	103
BPD z score		0.04†	1.00‡	403	0.11†	1.05‡	109
FL z score		−0.05†	1.05‡	388	−0.18†	1.10‡	103
UAPI							
	12w	2.50§	2.18–2.79¶	63	2.46§	2.20–2.71¶	20
	13w	2.20§	1.92–2.48¶	182	2.22§	1.84–2.47¶	49
	14w	1.86§	1.56–2.21¶	129	1.76§	1.51–2.25¶	30
DVPI							
	12w	1.00§	0.86–1.09¶	48	0.94§	0.90–1.50¶	14
	13w	0.91§	0.76–1.04¶	152	0.97§	0.84–1.17¶	40
	14w	0.84§	0.73–1.01¶	107	0.71§	0.71–1.06¶	25
NT ≥ 3mm (%)				12/397 (3.0%)			11/108 (10.2%)
Fetal karyotyping							
	Normal			10			9
	Abnormal			10			3
Neonatal karyotyping				0			6
	Normal			0			2
	Abnormal			0			0
							2

†Mean; ‡SD; §median; ¶IQR. BPD, biparietal diameter; CRL, crown-rump length; DV, ductus venosus; DVPI, ductus venosus pulsatility; FL, femur length; IQR, interquartile range; NT, nuchal translucency; PI, pulsatility index; SD, standard deviation; UA, umbilical artery.

growth restriction may be present as early as in the first trimester and is associated with poor obstetrical outcomes.[2-4] In this study, reduced CRL, BPD and FL between 12 and 14 GW had no significant association with delivery of SGA and LBW infants or other poor obstetrical outcomes. It may be impossible to detect statistical differences because this study population of our research was smaller than those of earlier reports.

A DVPI greater than the 90th percentile in the first trimester was significantly associated with delivery of SGA and LBW infants. The DV is a venous shunt between the intra-abdominal umbilical vein and the inferior vena cava. An increase in the DV shunting rate is a general adaptation mechanism in the presence of placental insufficiency to ensure an adequate supply of oxygen and glucose to vitally important organs, such as the brain and heart, but it also results in a reduction of hepatic blood supply.[12–15] An increased DVPI has commonly been found in IUGR and SGA cases in the second or third trimesters. It has been reported that an increased DVPI was observed several days prior to delivery in IUGR fetuses[16–19] and that Doppler examination of DV was useful to predict fetal compromise, morbidity and mortality and to determine optimal timing of intervention.[16–18,20,21] Our results suggest the possibility that a change in DV flow may occur as early as in the first trimester in some growth-restricted fetuses. There are, however, few reports on the relationship between abnormal DV flow in the first trimester and the risk of SGA or LBW infants. Oh *et al.* reported that there were no differences in the ratio of IUGR and birth weight between fetuses with abnormal DV flow and a control group.[22] Oh *et al.* defined abnormal DV flow as absent or reverse flow during atrial contraction,[22] which have often been used as a DV parameter in studies on associations with aneuploidy and cardiac defects,[5–10] while we look at the DVPI value, which can evaluate the DV flow velocity quantitatively. The degree of abnormal DV flow observed in growth-restricted fetuses may be so mild that the association can not be proved using absent or reverse flow during atrial contraction as a parameter. A DVPI increase up to a CRL of 63 mm (12 weeks and 6 days) and the cause thereof is considered as the absence of trophoblastic migration in early pregnancy. In the trophoblastic migration, placental vascular resistance decreases and, as a result, the DVPI decreases.[23] Inadequate trophoblastic migration may be the cause of the increased DVPI observed in growth-restricted fetuses between 12 and 14 GW.

Table 4 Association between perinatal outcome and biometry

Poor outcome	*n*§	*n*¶	Positive† negative‡ CRL Z score < −1.0		OR	95%CI			*P-value*
SPD	23	447	5/75	18/395	1.50	0.54	–	4.16	0.3909
PTL	28	442	5/75	23/372	1.16	0.42	–	3.14	0.7899
IPD	13	457	2/75	11/395	0.96	0.21	–	4.41	1.0000
SGA	40	412	8/72	32/380	1.36	0.60	–	3.09	0.4961
LBW	47	405	9/72	38/380	1.29	0.59	–	2.79	0.5285
PIH	16	454	2/75	14/395	0.75	0.17	–	3.35	1.0000
Aneuploidy	8	467	2/77	6/398	1.74	0.34	–	8.80	0.6216
Anomaly	12	462	2/77	10/397	1.03	0.22	–	4.81	1.0000
			CRL Z score < −1.5						
SPD	23	447	2/23	21/447	1.93	0.42	–	8.79	0.3121
PTL	28	442	0/23	28/447	0.31	0.02	–	5.29	0.3854
IPD	13	457	2/23	11/447	3.78	0.79	–	18.13	0.1287
SGA	40	412	4/31	36/431	2.58	0.82	–	8.09	0.1043
LBW	47	405	3/21	44/431	1.47	0.42	–	5.18	0.4702
PIH	16	454	2/23	14/447	2.95	0.63	–	13.81	0.1813
Aneuploidy	8	467	0/23	8/452	1.11	0.06	–	19.88	1.0000
Anomaly	12	462	1/23	11/451	1.82	0.22	–	14.73	0.4534
			BPD Z score < −1.0						
SPD	24	476	2/77	22/423	0.49	0.11	–	2.11	0.5595
PTL	32	468	5/77	27/423	1.02	0.38	–	2.73	1.0000
IPD	13	487	2/77	11/423	1.00	0.22	–	4.60	1.0000
SGA	44	437	8/76	36/405	1.21	0.54	–	2.71	0.6646
LBW	48	433	7/76	41/405	0.90	0.39	–	2.09	1.0000
PIH	17	483	4/77	13/423	1.73	0.55	–	5.45	0.3132
Aneuploidy	8	497	1/78	7/427	0.78	0.09	–	6.43	1.0000
Anomaly	13	491	0/78	13/426	0.20	0.01	–	3.32	0.2354
			BPD Z score < −1.5						
SPD	24	476	0/31	24/469	0.29	0.02	–	4.86	0.3874
PTL	32	468	3/31	29/469	1.63	0.47	–	5.67	0.4384
IPD	13	487	1/31	12/469	1.27	0.16	–	10.10	0.5694
SGA	44	437	2/30	42/451	0.70	0.16	–	3.02	1.0000
LBW	48	433	3/30	45/451	1.00	0.29	–	3.44	1.0000
PIH	17	483	3/31	14/469	3.48	0.94	–	12.83	0.0812
Aneuploidy	8	497	1/32	7/473	2.15	0.26	–	18.02	0.4099
Anomaly	13	491	0/32	13/472	0.52	0.03	–	9.01	1.0000
			FL Z score < −1.0						
SPD	23	478	4/84	19/417	1.05	0.35	–	3.16	1.0000
PTL	29	430	7/84	22/375	1.46	0.60	–	3.54	0.4546
IPD	12	447	3/84	9/375	1.51	0.40	–	5.69	0.4666
SGA	43	401	9/82	34/362	1.19	0.55	–	2.59	0.6796
LBW	46	398	9/82	37/362	1.08	0.50	–	2.34	0.8415
PIH	17	442	4/84	13/375	1.39	0.44	–	4.38	0.5289
Aneuploidy	7	456	2/85	5/378	1.80	0.34	–	9.43	0.6173
Anomaly	10	452	1/85	9/377	0.49	0.06	–	3.90	0.6971
			FL Z score < −1.5						
SPD	23	478	2/40	21/461	1.10	0.25	–	4.88	0.7045
PTL	29	430	2/40	27/419	0.76	0.17	–	3.34	1.0000
IPD	12	447	2/40	10/419	2.15	0.45	–	10.19	0.2814
SGA	43	401	5/39	38/405	1.42	0.52	–	3.85	0.5670
LBW	46	398	5/39	41/405	1.31	0.48	–	3.52	0.5820
PIH	17	442	2/40	15/419	1.42	0.31	–	6.44	0.6521
Aneuploidy	7	456	1/41	6/422	1.73	0.20	–	14.77	0.4798
Anomaly	10	452	1/41	9/421	1.14	0.14	–	9.27	0.6090

P-values were determined by Fisher's exact test. †Number of the poor outcomes with the positive finding/number of total study population with the positive findings; ‡number of the poor outcomes with the negative finding/number of total study population with the negative findings; §number of cases with the poor outcome with data; ¶number of cases without the poor outcomes with data. BPD, biparietal diameter; CI, confidence interval; CRL, crown-rump length; FL, femur length; IPD, iatrogenic preterm delivery; LBW, low birth weight; OR, odds ratio; PIH, pregnancy-induced hypertension; PTL, preterm labor; SGA, small for gestational age; SPD, spontaneous preterm delivery.

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Table 5 Association between perinatal outcome and Doppler study or nuchal translucency (NT)

			Positive† negative‡					
	n§	*n*¶	UAPI > 90th %tile	OR	95%CI			*P-value*
SPD	23	442	0/42	23/423	0.20	0.01	–	3.36	0.2509
PTL	29	436	3/42	26/423	1.18	0.34	–	4.06	0.7378
IPD	12	453	0/42	12/423	0.39	0.02	–	6.66	0.6128
SGA	40	407	4/40	36/407	1.15	0.39	–	3.40	0.7716
LBW	46	402	3/40	43/408	0.69	0.20	–	2.33	0.7849
PIH	16	449	1/42	15/423	0.66	0.09	–	5.15	1.0000
Aneuploidy	6	463	1/43	5/426	2.01	0.23	–	17.57	0.4402
Anomaly	13	455	2/42	11/426	1.89	0.40	–	8.81	0.3284
			UAPI > 95th %tile					
SPD	23	442	0/24	23/441	0.36	0.02	–	6.17	0.6223
PTL	29	436	3/24	26/441	2.28	0.64	–	8.15	0.1822
IPD	12	453	0/24	12/441	0.70	0.04	–	12.20	1.0000
SGA	40	407	4/23	36/424	2.27	0.73	–	7.03	0.1398
LBW	46	402	3/23	43/425	1.33	0.38	–	4.67	0.7200
PIH	16	449	0/24	16/441	0.53	0.03	–	9.04	1.0000
Aneuploidy	6	463	1/25	5/444	3.66	0.41	–	32.57	0.2814
Anomaly	13	455	1/24	12/444	1.57	0.19	–	12.57	0.5002
			DVPI > 90th %tile					
SPD	21	360	3/44	18/337	1.30	0.37	–	4.60	0.7221
PTL	29	352	5/44	24/337	1.67	0.60	–	4.64	0.3586
IPD	10	371	1/44	9/337	0.85	0.10	–	6.86	1.0000
SGA	30	337	7/41	23/326	2.71	1.08	–	6.79	0.0613
LBW	36	331	8/41	28/326	2.58	1.09	–	6.12	0.0450
PIH	11	370	2/44	9/337	1.74	0.36	–	8.31	0.3692
Aneuploidy	5	379	3/46	2/338	11.72	1.90	–	72.17	0.0136
Anomaly	10	374	2/46	8/338	1.88	0.39	–	9.12	0.3413
			DVPI > 95th %tile					
SPD	21	360	1/29	20/352	0.59	0.08	–	4.59	1.0000
PTL	29	352	4/29	25/352	2.09	0.68	–	6.49	0.2604
IPD	10	371	1/29	9/352	1.36	0.17	–	11.14	0.5514
SGA	30	337	7/26	23/341	5.09	1.94	–	13.37	0.0026
LBW	36	331	7/26	29/341	3.96	1.54	–	10.21	0.0080
PIH	11	370	2/29	9/352	2.82	0.58	–	13.73	0.2007
Aneuploidy	5	379	2/30	3/354	8.36	1.34	–	52.13	0.0509
Anomaly	10	374	2/30	8/354	3.09	0.63	–	15.26	0.1795
			NT ≥ 3 mm					
SPD	24	470	0/16	24/478	0.56	0.03	–	9.66	1.0000
PTL	31	463	0/16	31/478	0.43	0.03	–	7.35	0.6139
IPD	13	481	1/16	12/478	2.59	0.32	–	21.23	0.3517
SGA	43	432	0/12	43/463	0.39	0.02	–	6.65	0.6130
LBW	48	427	0/12	48/463	0.34	0.02	–	5.88	0.6205
PIH	17	477	0/16	17/478	0.80	0.05	–	13.88	1.0000
Aneuploidy	8	491	5/20	3/479	52.89	11.55	–	242.10	<0.0001
Anomaly	13	485	2/19	11/479	5.01	1.03	–	24.37	0.0837

P-values were determined by Fisher's exact test. †Number of the poor outcomes with the positive finding/number of total study population with the positive findings; ‡number of the poor outcomes with the negative finding/number of total study population with the negative findings; §number of cases with the poor outcome with data; ¶number of cases without the poor outcomes with data. %tile, percentile; CI, confidence interval; DV, ductus venosus; IPD, iatrogenic preterm delivery; LBW, low birth weight; OR, odds ratio; PI, pulsatility index; PIH, pregnancy-induced hypertension; PTL, preterm labor; SGA, small for gestational age; SPD, spontaneous preterm delivery; UA, umbilical artery, UAPI, umbilical artery pulsatility index.

In this study, abnormal NT and high DVPI were significantly associated with fetal aneuploidy and many studies have already suggested an association between aneuploidy and abnormal DV flow.[1,5–10] In two reports, in which the DVPI was used as the parameter, the 95th percentile was used as the cutoff value.[24,25] Of our study patients, there was one fetus whom we had not been able to diagnose as having trisomy 21

prenatally. Abnormal NT and morphological abnormalities could not be detected by our ultrasound examination during the whole pregnant period, and an increased DVPI during the first trimester was the only abnormal sign. It has already been reported that absent or reverse DV flow during atrial contraction was a significant predictor of aneuploidy, even in fetuses with normal NT.[6,22] However, it is controversial as to whether isolated abnormal DV flow should be an indication for invasive testing of fetal karyotypes.

These results suggest that abnormal DV flow should be a useful predictor of delivery, not only of infants with aneuploidy but also of SGA and LBW infants. Further studies are needed for determining which method is better, quantitative analysis such as the DVPI or a qualitative one (absent or reverse flow of DV).

The present study has two limitations. The first limitation is the accuracy of gestational age. The gestational age was adjusted by CRL measurement when estimated to be more than 7 days earlier than the date of the last menstrual period. Even in a normal fetus, the CRL has a variation of several days. In a fetus whose growth is already restricted early in the pregnancy, the gestational age may be shifted towards earlier than the actual age. The scientific validity of our study would have improved if the study population was restricted to women who had a regular 28-day menstrual cycle or to those who conceived through assisted reproductive technology. The second limitation was that we determined the normal references of UAPI and DVPI by lumping the measurement values to complete weeks disregarding days. These limitations may have affected our study but probably to a negligible degree because variation with gestational age is small (Table 2).

In conclusion, increased pulsation in the ductus venosus blood flow velocity is associated with increased risk of aneuploidy, but also fetal growth restriction.

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ORIGINAL ARTICLE

Establishment of reference ranges for ductus venosus waveform indices in the Japanese population

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Abstract
Objective To establish reference ranges for ductus venosus waveform indices in the Japanese population.
Methods In this retrospective cross-sectional study, 791 singleton fetuses of healthy Japanese couples were examined from January 2004 to January 2008. Reference ranges for ductus venosus waveform indices were constructed from cross-sectional data obtained at between 18 and 41 weeks of gestation.
Results With a success rate of 84%, a total of 667 measurements in 791 women were eligible for analysis. The median pulsatility index (PI) of fetal ductus venosus decreased from 0.54 at 18 weeks of gestation to 0.30 at 41 weeks of gestation. The median end-diastolic velocity/peak systolic velocity (a/S) of the ductus venosus increased from 0.56 at 18 weeks of gestation to 0.76 at 41 weeks of gestation.
Conclusions In this study, we established reference ranges for the PI and a/S of the ductus venosus in the Japanese population, which differed slightly from other published reference data. The results will be useful for further studies to determine the validity of the clinical importance of the ductus venosus for at-risk fetuses.

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Keywords Doppler velocimetry · Ductus venosus · Fetus · Reference range · Ultrasound

Introduction

Of all the precordial veins, the ductus venosus is the most commonly studied vessel. It yields the best and most reliable information on myocardial hemodynamics and functions of the fetal heart, with good reproducibility [1].

The ductus venosus is a precordial vein in the fetus that reflects the physiological status of the right ventricle. It appears to be an important regulator in the distribution of oxygen-rich blood between the liver and the heart. In the case of hypoxia, the fraction of ductus venosus shunting from the umbilical vein increases in order to maintain the oxygen supply to the brain and myocardium. Thus, the ductus venosus is important as a distributor of well-oxygenated blood to the fetal brain and myocardium [2–4].

Kiserud et al. [5] first reported Doppler ultrasound investigation of the ductus venosus as a diagnostic tool. Recent reports have indicated a relationship between the degree of acidemia and values of waveform indices of the ductus venosus due to chronic placental insufficiency associated with severe intrauterine growth restriction (IUGR), and have indicated a role in the diagnosis of fetal congestive cardiocirculatory diseases [6–8]. A relationship between perinatal outcome in early-onset IUGR and abnormalities of velocimetry in the ductus venosus has also been reported. In particular, the current standard management of IUGR involves Doppler examination of the ductus venosus [9–13].

Although many reference ranges have been established for the ductus venosus [1, 8, 14–21], most of the data reported were derived from Caucasian population-based

studies, with only a few reports concerning the Japanese population [17]. The aim of this study is to establish reference ranges and intra-interobserver variations for ductus venosus waveform indices of fetuses in the Japanese population, in order to facilitate the application of these indices in the prenatal fetal assessment.

Patients and methods

This is a retrospective cross-sectional study. Seven hundred ninety-one (791) singleton fetuses of healthy Japanese couples between 18 and 40 weeks of gestation were involved. They were recruited from patients visiting our routine antenatal outpatient department from January 2004 to January 2008. The study protocol was approved by the ethics committee of the institution, and informed consent was obtained for participation in this study. The criteria for participation were as follows: the gestational age was confirmed by fetal biometry (crown-rump length or biparietal diameter) between 9 and 11 weeks, and the fetuses were anatomically normal on prenatal and neonatal examinations at birth. In addition, all fetuses were term infants with an Apgar score of not less than 8 points at 1 and 5 min, their birth weights were between the 10th and 90th percentiles of the Japanese standard birth weight curve, and participants who had complications or took medications that might affect fetal circulation were excluded.

Pulsed-wave Doppler ultrasonographic examinations of fetal ductus venosus were performed transabdominally using a Voluson 730 Expert ultrasound device (GE Yokogawa Medical Systems, Tokyo, Japan) equipped with a 3.5-MHz curved-array transducer.

Using color Doppler, blood flow signals of the ductus venosus were depicted either in a mid-sagittal longitudinal plane of the fetal trunk (Fig. 1a) or in an oblique transverse plane of the fetal upper abdomen (Fig. 1b). The sample volume was positioned directly at the entrance of the ductus venosus from the umbilical vein, where color Doppler indicated the highest velocities. Low-pass filters were set at 50–100 Hz to detect all velocities appropriately. To avoid detecting signals from the adjacent hepatic and umbilical veins, the size of the sample volume was adjusted such that it only covered the entire vessel lumen. The insonation angle between the ultrasound beam and direction of blood flow was kept as low as possible and always at $\leq 50°$. All Doppler recordings used for measurements were obtained in the absence of fetal breathing movements, fetal gross body movements, and uterine contractions. Fetal heart rate was regular, within the range of 120–160 beats/min. Measurements were frequently repeated to ensure that the mechanical and thermal indices for soft tissue were maintained at or below 1.1. Only one measurement from each participant was included in the statistical analysis. Cases in which a satisfactory waveform could not be recorded were not included in the study.

The normal velocity waveform of the ductus venosus exhibits continuous, triphasic forward flow throughout the cardiac cycle. The peak forward flow velocities during ventricular systole (S); the peak forward flow velocities during early ventricular diastole (D), which corresponds to passive ventricular filling; and the lowest forward velocity or peak reversed velocity in late ventricular diastole during atrial contraction (a) (Fig. 2) were the quantities determined for ductus venosus waveform analysis. The pulsatility index (PI) was defined as (S − a)/time-averaged maximum velocity. a/S was defined as end-diastolic velocity/peak systolic velocity. PI and a/S were independent of the angle of insonation and were measured as mean values of at least three consecutive uniform waveforms. Mono- or biphasic flow patterns with comparably high late ventricular diastole were included in the analysis as they are considered a normal variant [22].

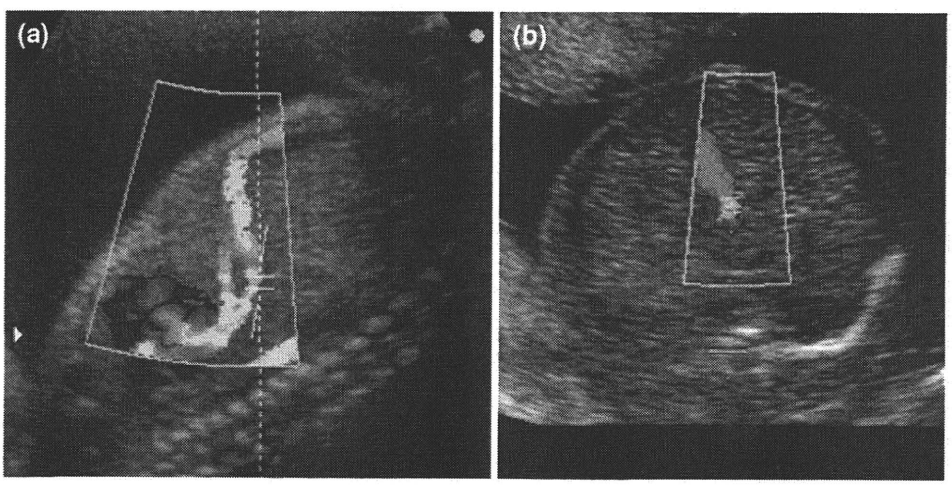

Fig. 1 Visualization of the ductus venosus **a** in a mid-sagittal longitudinal plane and **b** in an oblique transverse plane of the fetal abdomen. There is a marked difference in blood flow velocities between the umbilical vein and the ductus venosus. The higher flow velocity in the ductus venosus causes aliasing, which appears as an area of color reversal

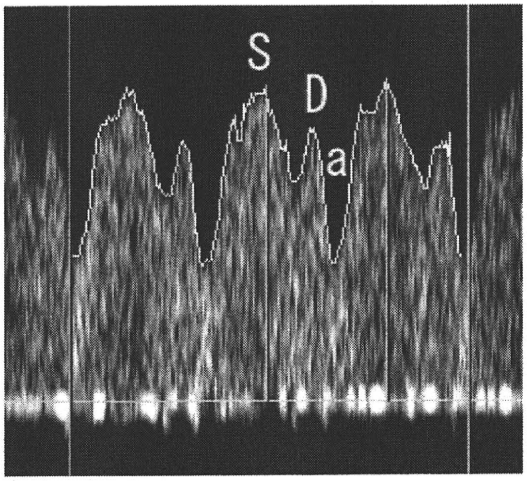

Fig. 2 Typical flow velocity waveforms of the ductus venosus in a normal fetus at 30 weeks of gestation: the peak forward flow velocities during ventricular systole (*S*); the peak forward flow velocities during early ventricular diastole (*D*), which corresponds to passive ventricular filling; and the lowest forward velocity or peak reversed velocity in late ventricular diastole during atrial contraction (*a*)

For the assessment of intraobserver variation, 52 participants were examined by one operator, and for assessment of interobserver variation, 17 participants were examined by two operators. These participants were chosen arbitrarily from all study participants and repeat Doppler measurements of the ductus venosus were performed.

Statistical analyses

The power of the study was sufficient as per previous cross-sectional studies [1, 8, 14–20]. The reference ranges for the respective gestational week were determined to construct smooth growth curves for the entire gestation period. These ranges were estimated by the methods described by Royston and Wright [23]. The assumption of normal distribution was checked for outcome variables, and Box-Cox transformation was used to achieve normal distribution. Least-squares regression analysis was performed on the transformed data to estimate both the mean and SD curves as polynomial functions of gestational age in transformed units. The 5th and 10th percentiles were calculated by subtracting 1.64 and 1.28 SD, respectively, from the mean in transformed units. The 90th and 95th percentiles were calculated by adding the respective SD multiples to the mean transformed units. The limits of the calculated reference ranges were subjected to anti-Box–Cox transformation.

Intra- and interobserver variations were calculated by the methods described by Bland and Altman [24, 25]. The mean difference was determined between these two measurements with a 95% confidence interval (CI) and limits of agreement (1.96 SD of the mean difference). Statistical analysis was performed with SPSS software (12.0.1 J for Windows; SPSS Japan, Tokyo, Japan). A P value of 0.05 was considered statistically significant.

Table 1 Background of 791 participants

Characteristic	Value
Median maternal age	32.1 years (range 19.2–45.8)
Median gestational age at delivery	39 weeks + 5 days (range 37 weeks + 0 days to 41 weeks + 6 days)
Median birth weight	3,042 g (range 2,316–3,910)
Sex of neonates	Male $n = 419$, female $n = 372$

Results

A total of 791 patients met the inclusion criteria. The background of the participants was as follows (Table 1): the median maternal age was 32.1 years (range 19.2–45.8), the median gestational age at delivery was 39 weeks + 5 days (range 37 + 0 to 41 + 6), the median birth weight was 3,042 g (range 2,316–3,910), and the fetal sex distribution was 419 males (53.0%) and 372 females (47.0%).

Satisfactory, clear, and uniform waveforms were obtained in 84% of cases (667 of 791). Fetal activity, breathing movements, and unfavorable fetal position lowered the success rate to 84%. The median PI of the ductus venosus decreased throughout the observation period, from 0.54 at 18 weeks to 0.30 at 41 weeks (Fig. 3; Table 2). The median a/S of the ductus venosus increased from 0.56 at 18 weeks to 0.76 at 41 weeks (Fig. 4; Table 3). Reference ranges for the PI and a/S of the ductus venosus with respect to each gestational age are shown in Tables 2 and 3, and regression curves of the 5th, 10th, 50th, 90th, and 95th percentiles for the same are graphically illustrated in Figs. 3 and 4. Formulae for the PI and a/S of the ductus venosus with gestational age are as follows: $\mathrm{PI} = (-0.009809 \times \mathrm{GA} + 0.7855 + K \times 0.1413)^{1.25}$ and $\mathrm{a/S} = (-0.00014 \times \mathrm{GA}^2 + 0.01512 \times \mathrm{GA} + 0.4392 + K \times 0.8787)^{10/7}$, where GA indicates gestational age (weeks) and the 5th, 10th, 50th, 90th, and 95th percentiles were calculated by $K = -1.64, -1.28, 0, 1.28$, and 1.64, respectively.

Inter- and intraobserver variations are presented as the limits of agreement (Table 4). Figures 5, 6, 7, and 8 give detailed information about inter- and intraobserver variations.

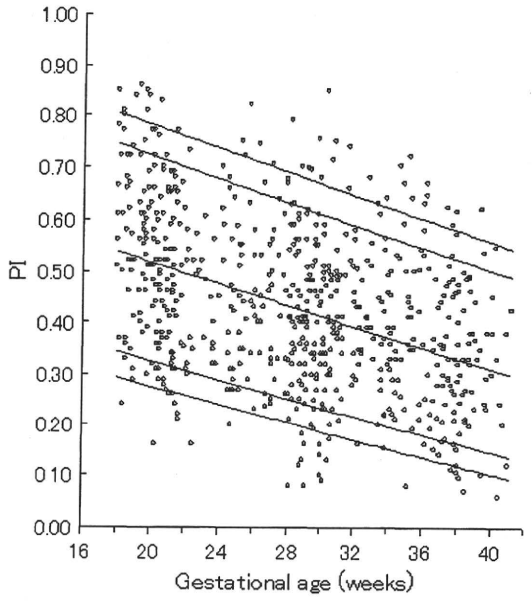

Fig. 3 Reference ranges for the pulsatility index (PI) of the ductus venosus based on 667 observations. The 5th, 10th, 50th, 90th, and 95th percentiles are shown

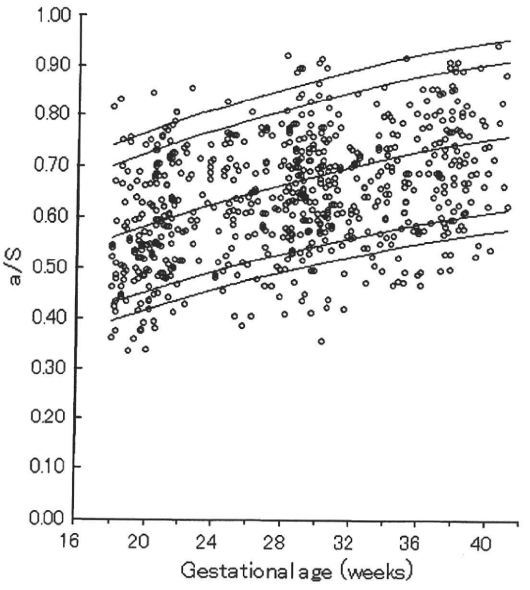

Fig. 4 Reference ranges for the end-diastolic velocity/peak systolic velocity (a/S) of the ductus venosus based on 667 observations. The 5th, 10th, 50th, 90th, and 95th percentiles are shown

Table 2 Reference ranges for the pulsatility index (PI) of the ductus venosus based on 667 observations

Gestational age (weeks)	Percentile				
	5th	10th	50th	90th	95th
18	0.30	0.35	0.54	0.74	0.80
19	0.29	0.34	0.53	0.73	0.79
20	0.28	0.33	0.52	0.72	0.78
21	0.27	0.32	0.51	0.71	0.77
22	0.26	0.31	0.49	0.70	0.76
23	0.25	0.30	0.48	0.69	0.75
24	0.24	0.29	0.47	0.68	0.74
25	0.23	0.28	0.46	0.66	0.72
26	0.22	0.27	0.45	0.65	0.71
27	0.21	0.26	0.44	0.64	0.70
28	0.20	0.25	0.43	0.63	0.69
29	0.19	0.24	0.42	0.62	0.68
30	0.19	0.23	0.41	0.61	0.67
31	0.18	0.22	0.40	0.60	0.66
32	0.17	0.21	0.39	0.59	0.64
33	0.16	0.20	0.38	0.58	0.63
34	0.15	0.20	0.37	0.56	0.62
35	0.14	0.19	0.36	0.55	0.61
36	0.13	0.18	0.35	0.54	0.60
37	0.13	0.17	0.34	0.53	0.59
38	0.12	0.16	0.33	0.52	0.58
39	0.11	0.15	0.32	0.51	0.57
40	0.10	0.14	0.31	0.50	0.56
41	0.09	0.14	0.30	0.49	0.54

Table 3 Reference ranges for the end-diastolic velocity/peak systolic velocity (a/S) of the ductus venosus based on 667 observations

Gestational age (weeks)	Percentile				
	5th	10th	50th	90th	95th
18	0.39	0.43	0.56	0.70	0.74
19	0.41	0.44	0.57	0.71	0.75
20	0.42	0.45	0.58	0.72	0.77
21	0.43	0.46	0.59	0.74	0.78
22	0.44	0.47	0.61	0.75	0.79
23	0.45	0.48	0.62	0.76	0.80
24	0.46	0.49	0.63	0.77	0.81
25	0.47	0.50	0.64	0.78	0.82
26	0.47	0.51	0.65	0.79	0.84
27	0.48	0.52	0.66	0.80	0.85
28	0.49	0.53	0.67	0.81	0.86
29	0.50	0.54	0.68	0.82	0.87
30	0.51	0.55	0.68	0.83	0.88
31	0.52	0.55	0.69	0.84	0.88
32	0.52	0.56	0.70	0.85	0.89
33	0.53	0.57	0.71	0.86	0.90
34	0.54	0.58	0.72	0.87	0.91
35	0.54	0.58	0.72	0.87	0.92
36	0.55	0.59	0.73	0.88	0.92
37	0.56	0.59	0.74	0.89	0.93
38	0.56	0.60	0.74	0.89	0.94
39	0.57	0.61	0.75	0.90	0.94
40	0.57	0.61	0.75	0.90	0.95
41	0.58	0.61	0.76	0.91	0.95

Table 4 Intra- and interobserver variation of ductus venosus flow waveform indices

	Paired differences			*df*	K-S test *P*	S-W test *P*	Limits of agreement	
	Mean (95% CI)	SD	SE				Upper (95% CI)	Lower (95% CI)
Intraobserver variation								
PI	0.00 (−0.02, 0.01)	0.06	0.01	52	>0.200	0.719	0.12 (0.09, 0.15)	−0.12 (−0.15, −0.09)
a/S	0.00 (−0.01, 0.02)	0.06	0.01	52	>0.200	0.719	0.10 (0.08, 0.12)	−0.10 (−0.12, −0.08)
Interobserver variation								
PI	0.02 (−0.01, 0.05)	0.06	0.03	17	>0.200	0.991	0.15 (0.09, 0.20)	−0.11 (−0.16, −0.05)
a/S	−0.01 (−0.03, 0.01)	0.04	0.01	17	>0.200	0.950	0.07 (0.04, 0.11)	−0.10 (−0.13, −0.06)

SD Standard deviation of the mean, *SE* standard error of the mean, *df* degrees of freedom, *K-S test* Kolmogorov-Smirnov test, *S-W test* Shapiro-Wilks test, *PI* pulsatility index, *a/S* end-diastolic velocity/peak systolic velocity

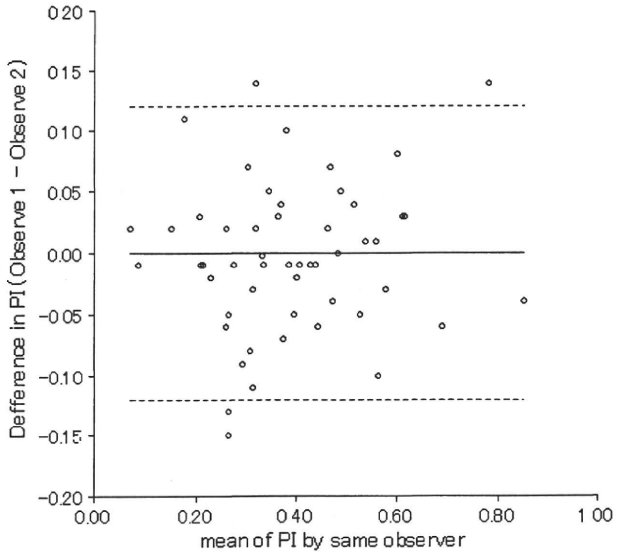

Fig. 5 Plot of difference against mean for the measurements of pulsatility index (PI) of the ductus venosus by the same observer, with the mean difference (*solid line*) and 95% limits of agreement (*dashed lines*) indicated

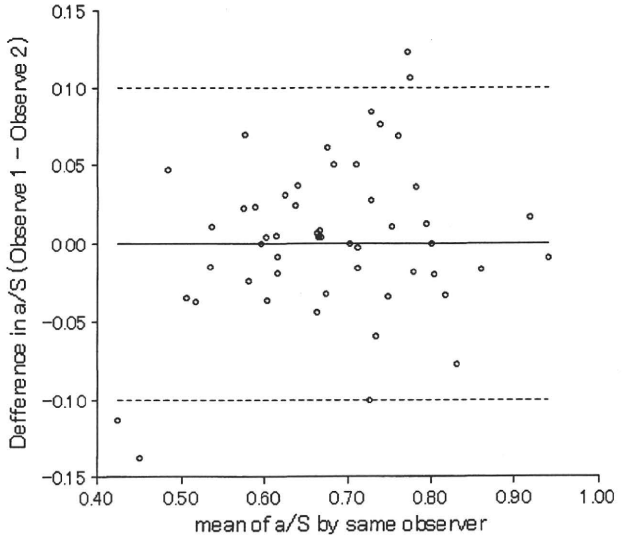

Fig. 6 Plot of difference against mean for measurements of the end-diastolic velocity/peak systolic velocity (a/S) of the ductus venosus by the same observer, with the mean difference (*solid line*) and 95% limits of agreement (*dashed lines*) indicated

Discussion

The present findings reveal that the PI of the ductus venosus decreases and the a/S of the ductus venosus increases with advancing gestation during the second and third trimesters of pregnancy. This conclusion is in agreement with those of previous studies. However, our reference ranges of PI were lower and those of a/S were higher compared to data obtained by Hecher et al. [8], Bahlmann et al. [18], Baschat et al. [19], Axt-Fliedner et al. [20], and Kessler et al. [21]. For example, Kessler et al. [21] found higher values of PI and lower values of a/S throughout the whole observation period. However, their 50th percentile value of PI was merely 0.06–0.13 (21–39 weeks) higher, and their 50th percentile value of a/S was merely 0.06–0.13 (21–39 weeks) lower compared with our data with advancing gestation. Their data were in good agreement with our data, especially at mid-gestation. These differences could have been due to variations in the study size, study population, and statistical methods. In general, the selection of the study population is extremely important when establishing reference ranges. On the one hand, Kessler et al. [21] included participants with complications that affect fetal circulation, while our approach might produce supernormal reference ranges. However, many patients were referred to our hospital for management of maternal complications or a targeted ultrasound examination for fetal anomalies or suspected IUGR, which was the reason for the exclusion of all conditions that might be associated with pathology. Reasonableness and necessity are controversial for ethnic reference ranges for fetal Doppler waveforms. However, the apparent difference from previous studies was ethnicity. Most of the reported

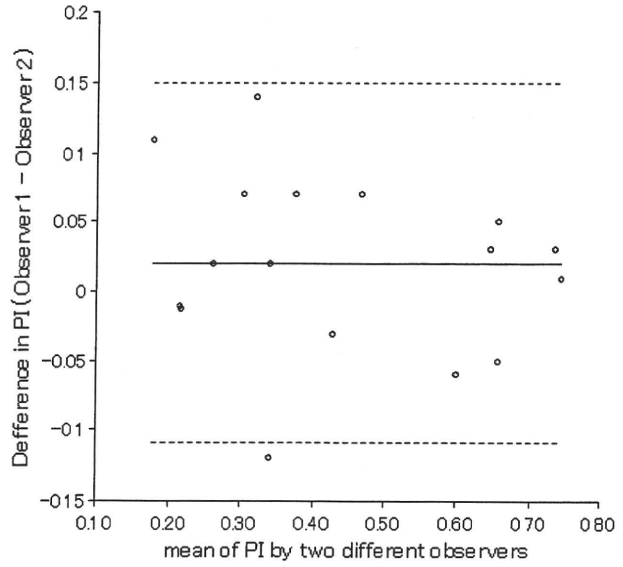

Fig. 7 Plot of difference against mean for measurements of pulsatility index (PI) of the ductus venosus by two different observers, with the mean difference (*solid line*) and 95% limits of agreement (*dashed lines*) indicated

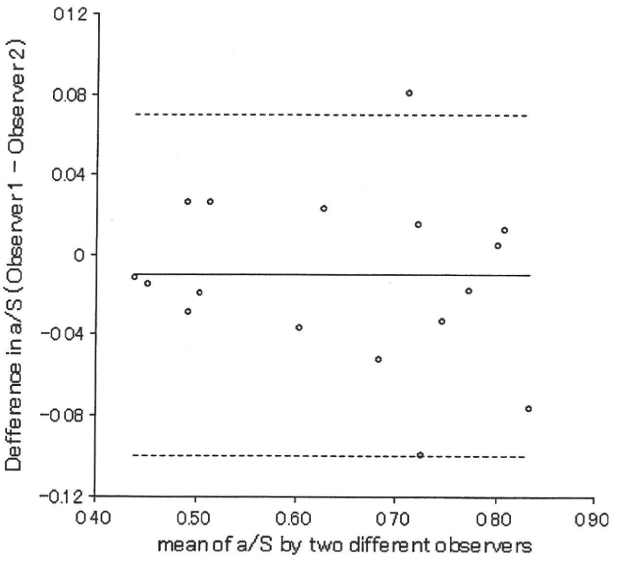

Fig. 8 Plot of difference against mean for measurements of the end-diastolic velocity/peak systolic velocity (a/S) of the ductus venosus by two different observers, with the mean difference (*solid line*) and 95% limits of agreement (*dashed lines*) indicated

data concerning reference ranges of ductus venosus waveforms have been derived from Caucasian population-based studies [1, 8, 14–16, 18–21]. There is a possibility that these reference ranges may differ in part according to ethnicity. Previously, Nakata et al. [17] reported reference ranges for flow velocities of the ductus venosus during the prenatal course in the Japanese population. However, the validity of the reference ranges is diminished by the small number of cases and the absence of information on the variation of Doppler ultrasound measurements and on the measuring parameters used.

The present inter- and intraobserver variation expressed by the 95% CI of the difference is in accordance with that reported by Kessler et al. [21] and is small enough to lead to the assumptions that any variation seen in the present dataset is mainly due to biological variation rather than methodological variation. However, further clinical research is necessary to assess whether inter- and intraobserver variations are within the clinically acceptable ranges.

Reference ranges of the ductus venosus in the fetus have been needed for many years in Japan. In this study, we established reference ranges for the PI and a/S of ductus venosus waveform indices of fetuses in the Japanese population. Further clinical research is necessary to determine the validity of the clinical importance of the ductus venosus in at-risk fetuses.

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Fulminant type 1 diabetes mellitus acutely emerged during pregnancy

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Abstract

A pregnant woman at 32 weeks of gestation was emergently admitted to our hospital with symptoms of nausea, vomiting, and uterine contraction. Cardiotocogram demonstrated a loss of variability and late deceleration in fetal heart rate pattern. Emergency cesarean section was performed, and a male infant weighing 1750 g was born with Apgar scores of 1 at 1 min, and 3 at 5 min after delivery. After cesarean section, the patient developed an acetone breath odor, and blood examination demonstrated remarkable acidemia and an extremely high level of blood glucose. The patient was diagnosed with ketoacidosis with acute onset of fulminant type 1 diabetes mellitus. Intensive care was applied due to the severe diabetes mellitus conditions. The patient's general condition ameliorated during the postoperative period, although there was a possibility of neurological complications in the infant.

Key words: cesarean section, critical care obstetrics, diabetes mellitus.

Introduction

Fulminant type 1 diabetes mellitus, which is included in type 1B diabetes, accounts for approximately 20% of cases of acute-onset type 1 diabetes, and is recognized as a novel subtype of type 1 diabetes.[1,2] The major clinical characteristics of fulminant type 1 diabetes mellitus are markedly abrupt onset of disease, very short (<1 week) duration of diabetic symptoms, and severe acidosis at the time of diagnosis. A recent 5-year nationwide survey performed in Japan reported 18 cases of fulminant type 1 diabetes mellitus that emerged during pregnancy, and that the infants were rescued in only six cases (approximately one per year).[3] We report the clinical course of a pregnant woman with typical fulminant type 1 diabetes mellitus who had an extremely acute onset of symptoms and whose baby was rescued.

Case Report

A pregnant woman at 32 weeks of gestation was emergently admitted to our hospital with symptoms of nausea, vomiting, and uterine contraction. Slight symptoms, such as thirst and polyuria, began roughly 1 day before her emergency hospitalization. The patient received routine prenatal care and examinations from an obstetrician in another prefecture. She was visiting her parents' home near our hospital when her symptoms occurred suddenly. Serial routine examinations had not revealed any urinary sugar, and a blood sugar examination performed at 28 weeks of gestation showed a normal value. The patient had no family history of diabetes mellitus, nor any past history of the disease.

On admission, the patient was alert and no sign of coma was observed. Cardiotocogram demonstrated a

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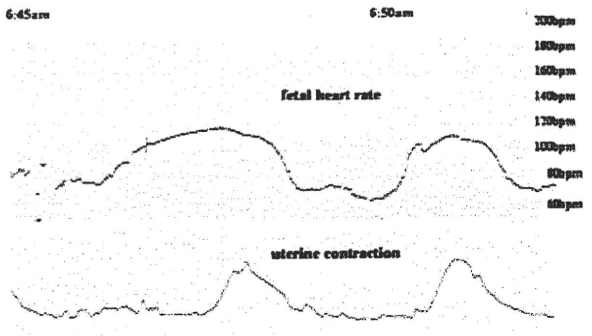

Figure 1 Cardiotocogram of the patient, examined just after emergency admission. Loss of variability and late deceleration were noted.

loss of variability and late deceleration in the fetal heart rate pattern (Fig. 1). Emergency cesarean section was performed, and a male infant weighing 1750 g was born with Apgar scores of 1 at 1 min after delivery, and 3 at 5 min. After the cesarean section, the patient developed an acetone breath odor, and blood examination demonstrated marked acidemia and an extremely high blood glucose level.

Results of laboratory examinations performed just after the surgery were as follows:

- Complete blood count: white blood cells 22 920/μL; red blood cells 461 × 10^4/μL; hemoglobin 14.0 g/dL; hematocrit 42.6%, platelet 28.3 × 10^4/μL.
- Biochemical examination: blood sugar 557 mg/dL; total protein 8.5 g/dL; albumin 4.0 g/dL; blood urea nitrogen 22 mg/dL; creatinine 0.77 mg/dL; aspartate aminotransferase 39 IU/L; alanine aminotransferase 46 IU/L; lactate dehydrogenase 269 IU/L; Na 129 mEq/L; K 5.8 mEq/L; Cl 101 mEq/L; serum amylase 173 IU/L.
- Arterial blood gas analyses: pH 7.058; pCO2 13.8 mmHg; pO2 131.3 mmHg; HCO_3^- 3.8 mmol/L; base excess −24.5 mmol/L; SaO2; 97.9%.
- Urinalysis: urinary sugar 4+; urinary acetone body 4+.

The patient was diagnosed with ketoacidosis and fulminant type 1 diabetes mellitus. The type of acidosis was determined to be metabolic because of low HCO_3^- and pCO2 values.

Results of laboratory examinations related to diabetes mellitus, performed during hospitalization, were as follows:

- Hemoglobin A1c 5.4%; urinary C peptide excretion 3.8 μg/day.
- Islet-related autoantibodies: antiglutamic acid decarboxylase (anti-GAD) antibodies negative; anti-islet cell (IC) antibodies negative; anti-islet antigen 2 (anti-IA2) antibodies negative.
- Serum exocrine pancreatic enzyme level: amylase 173 IU/L; lipase 269 IU/L.

Results of viral examinations performed at the third day after surgery were as follows:

- IgG to herpes simplex virus >128; IgG to cytomegalovirus >128; IgM to cytomegalovirus 0.46; IgG to Ebstein-Barr virus >160; IgM to Ebstein-Barr virus; <10; IgA to Ebstein-Barr virus <10; antibodies to poliovirus negative; antibodies to coxsachie virus negative; IgG to rubella virus 102; IgM to rubella virus 0.32; antibodies to measles ×8; antibodies to mumps ×16.

The genotype of human leukocyte antigens (HLA) of the patient was DRB1*0901/*1405 and DQB1*0303/*0503.

To treat the diabetes mellitus, rapidly acting recombinant human insulin was administered. First, 10 units of rapidly acting recombinant human insulin were injected intradermally several times, and thereafter 49.5 mL of physiological saline, including 50 units of rapidly acting recombinant human insulin, were continuously injected intravenously at a rate of 2 mL/h.

Blood glucose level decreased to 390 mg/dL on the night of surgery. Continuous insulin infusion was performed, and the glucose level decreased to within 250 mg/dL the next day. The sliding scale for using recombinant insulin was applied, and the blood glucose level improved close to the normal range. The general condition of the patient gradually ameliorated, and she was transferred to internal medicine on the 13th day after cesarean section. As for the neonatal condition, the results of umbilical arterial blood gas analyses were as follows: pH 6.660; pCO2 84.3 mmHg; pO2 13.5 mmHg; and BE −28.9 mmol/L; which indicated a severe acidemic and hypoxic condition in the infant. The infant was placed in the neonatal intensive care unit. The blood glucose level just after delivery was 294 mg/dL, and metabolic acidosis was adjusted by the infusion of sodium bicarbonate. Respiratory care was performed using high frequency oscillation with intubation. Surfactant was intratracheally administered for respiratory distress. The general condition of the infant gradually ameliorated, and artificial respiratory care ceased on the sixth day after birth. The infant was discharged on the 50th day after birth, with

the possibility of neurological complications due to hypoxic encephalopathy, as findings of periventricular leukomalasia was observed by ultrasonography and magnetic resonance image.

Discussion

Type 1 diabetes mellitus is characterized by insulin deficiency resulting from the destruction of pancreatic β-cells.[4,5] Type 1A diabetes mellitus involves direct damage to the pancreatic β-cells by islet-related autoantibodies,[6] and type 1B diabetes is considered to be idiopathic.[1] Of these, fulminant type 1 diabetes mellitus demonstrates the most severe clinical course.

The clinical characteristics of fulminant type 1 diabetes mellitus are as follows:[1,2] (i) markedly abrupt onset of disease; (ii) very short (<1 week) duration of diabetic symptoms (e.g. polyuria, thirst, and body weight loss); (iii) acidosis at the time of diagnosis; (iv) negative status of islet-related autoantibodies, such as islet cell antibodies, GAD ab, insulin autoantibodies, or IA-2ab; (v) virtually no C peptide secretion (<10 μg/day in the urine); and (vi) elevated serum pancreatic enzyme levels. The present patient fulfilled almost all of these criteria, therefore, she was diagnosed with typical fulminant type 1 diabetes mellitus.

The onset of the present case was markedly acute (i.e. no urinary sugar was detected on routinely performed prenatal examinations, and a blood sugar examination was normal at 28 weeks of gestation). Shimizu *et al.* compared the clinical characteristics between a patient group with pregnancy associated fulminant type 1 diabetes mellitus and non-pregnancy associated fulminant type 1 diabetes mellitus, and concluded that the clinical symptoms of pregnancy associated fulminant type 1 diabetes mellitus were more severe than non-pregnancy associated fulminant type 1 diabetes mellitus.[3,7] Recent reports have pointed to the possibility of viral infection, such as mumps and influenza, inducing fulminant type 1 diabetes mellitus.[8,9] Although several different viral examinations were performed, viral infection was not obvious in the present patient.

Serious systemic maternal diseases are related to onset of preterm uterine contraction.[10] In the present case, marked uterine contraction was observed on admission. Ketoacidosis during pregnancy decreases uteroplacental blood flow as the result of maternal hypovolemia and/or maternal acidemia itself.[11] Decreased uterine blood flow causes myometrial hypoxia, which provokes local inflammatory reaction and prostaglandin production. Such mechanisms are considered to have caused the manifestation of marked uterine contraction in the present case. Commonly used uterine relaxants, such as beta2 stimulant, will worsen diabetic hyperglycemia; therefore, careful management of uterine contraction must be applied in such cases.

In the present case, diabetic ketoacidosis was extremely marked at the onset of symptoms of fulminant type 1 diabetes mellitus. Diabetic ketoacidosis is a medical emergency during pregnancy because of maternal risks, such as manifestation of diabetic coma, as well as high rates of fetal mortality. Shimizu *et al.* reported that fetal demise occurred in 12 of 18 patients (67%) who developed fulminant type 1 diabetes mellitus during pregnancy, and cases demonstrating fetal demise showed more severe acidosis than patients with liveborn infants.[3] Montoro *et al.* reported that fetal loss was 35% in 20 patients with diabetic ketoacidosis during pregnancy,[12] although a lower percentage (9% of 11 cases) of fetal demise was reported by Cullen *et al.*[13] Montoro *et al.* demonstrated that new unrecognized onset diabetes accounted for 57% of fetal deaths compared with 21% in mothers with recognized disease.[12] Therefore, it is suggested that the abrupt onset of diabetes, as well as the severity of maternal diabetic ketoacidosis, might affect the high fetal mortality rate in pregnant women who develop fulminant type 1 diabetes mellitus.

The immunogenetic background of fulminant type 1 diabetes mellitus has been well analyzed (i.e. some susceptible human leukocyte antigens have been reported concerning the disease, especially pregnancy associated fulminant type 1 diabetes). Shimizu *et al.* demonstrated that the frequency of haplotype HLA-DRB1*0901-DQB1*0303 in pregnancy associated fulminant type 1 diabetes patients was 41.2%, which was significantly higher compared with non-pregnancy associated fulminant type 1 diabetes patients (17.5%), and controls (14.7%).[3] The HLA-class II genotype of our patient was DRB1*0901/*1405 and DQB1*0303/*0503, which strongly indicates that she had an immunogenetic predisposition to pregnancy associated fulminant type 1 diabetes.

As causes of the high fetal mortality rate in these pregnancies, various factors, such as maternal dehydration, which diminishes uteroplacental blood flow, and maternal acidosis leading to fetal acidosis, are considered relevant, although the precise mechanisms remain unclear.[11,14] Emergency cesarean section was performed in the present case to rescue the infant. Shimizu *et al.* reported that it may be possible for fetal lives to be

rescued if cesarean section, together with treatment for diabetic ketoacidosis is performed immediately after the development of diabetic ketoacidosis, as the duration of hyperglycemic symptoms tended to be shorter in cases of liveborn infants than in cases demonstrating fetal demise.[3]

As described above, a nationwide survey performed over 5 years in Japan indicated that there were 18 patients with fulminant type 1 diabetes mellitus emerging in the prenatal period, and the infants were rescued in only six of these cases (roughly one per year).[7] We encountered a pregnant woman with typical fulminant type 1 diabetes mellitus, whose infant was rescued by emergency cesarean section, although there was a possibility that the infant would have some neurological complications due to the acute onset of severe hypoxia in the fetus just before delivery. As slight symptoms, such as thirst and polyuria, commenced roughly 1 day before serious aggravation of the symptoms in the present case, it is possible that examinations for diabetes mellitus at the manifestation of slight symptoms could predict the onset of the fulminant type 1 diabetes mellitus. In this context, it is crucial that women with fulminant type 1 diabetes mellitus emerging during pregnancy be recorded and the clinical course of these patients be analyzed. Moreover, all physicians as well as obstetricians should recognize and treat fulminant type 1 diabetes mellitus as soon as possible if a pregnant women abruptly develops hyperglycemic symptoms.

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Fetal Atrioventricular Block and Postpartum Augmentative QT Prolongation in a Patient with Long-QT Syndrome With KCNQ1 Mutation

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2:1 AV Block in KCNQ1. **The case of a 32-year-old pregnant woman, who had had several syncopal episodes during swimming and running at 9 and 10 years of age and whose fetus had 2:1 AV block, is presented. The mother and baby had the same heterozygous single nucleotide substitution in KCNQ1 at T587M. After 27 weeks of gestation, the fetal 2:1 AV block disappeared, and 1:1 AV conduction resumed, with a fetal heart rate of 110–120 beats/min. The maternal electrocardiogram revealed a normal QTc interval (433 ms) without ST-T abnormalities at gestational week 23, but the QTc was 490 and 531 ms at 1 and 2 months postpartum, with biphasic T waves in leads V2 and V3. This case is the first report of fetal 2:1 AV block with KCNQ1 mutation (T587M) and unmasked maternal QT prolongation in the postpartum period.** *(J Cardiovasc Electrophysiol, Vol. 21, pp. 1170-1173, October 2010)*

atrioventricular block, KCNQ1, long-QT syndrome, neonate, pregnancy

Introduction

The congenital long-QT syndrome (LQTS) is a potentially lethal cardiac disease associated with ventricular tachyarrhythmias, especially torsade de pointes, due to abnormally prolonged repolarization.[1,2] Bradyarrhythmias during the fetal or perinatal stages may be associated with 2:1 atrioventricular (AV) block and carry a worse prognosis.[3,4] Previous reports of LQTS with 2:1 AV block have been related to homozygous mutations in HERG[5,6] or SCN5A.[7]

On the other hand, women with LQTS have a reduced risk for cardiac events during pregnancy, but an increased risk during the postpartum period, especially women with HERG and KCNQ1[8]; however, the change in the QT interval in the peripartum period is not yet well elucidated.

A case of maternal postpartum augmentative QT prolongation, whose fetus had 2:1 AV block associated with KCNQ1 mutation, is presented.

Case Report

A 32-year-old pregnant woman was referred to our clinic because her fetus had 2:1 AV block. Fetal echocardiography showed normal growth and cardiac structure, but the superior vena cava (SVC)/ascending aorta (AA) Doppler tracing showed 2:1 flow velocities, representing 2:1 AV block (Fig. 1A). The patient had had several syncopal episodes during swimming and running at 9 and 10 years of age, but she did not have a medical check-up. There was no family history of sudden death. Her chest X-ray, blood chemistry data, and cardiac echocardiography were normal. Autoantibody screening was negative for antinuclear antibodies anti-SS-A, anti-SS-B, anti-Sm, and anti-RNP. An electrocardiogram (ECG) revealed normal sinus rhythm at 63 beats/min, and the QTc interval was 433 ms without ST-T abnormalities at gestational week 23 (Fig. 2A). After gestational week 27, the fetal 2:1 AV block disappeared, and the fetal heart rate increased to 110–120 beats/min with 1:1 AV conduction. She had childbirth by a Caesarean operation week 38 of gestation (Apgar score: 7/9). At the age of 1 week, the neonate's QTc interval prolonged to 521 ms on ECG (Fig. 1B), but in the neonate's cardiac echocardiography there was no abnormality and the ejection fraction of the left ventricle was 67%. No arrhythmia including 2:1 AV block nor sinus bradycardia was seen in the infant period. The baby was given carteolol at a dose of 0.2 mg/kg/day from the age of 3 months. The mother's ECG showed QTc interval prolongation to 490 ms 1 month postpartum (Fig. 2B) and to 531 ms 2 months postpartum; in particular, biphasic T waves appeared in leads V2 and V3 (Fig. 2C). The mother was prescribed propranolol at a dose of 30 mg/day.

DNA Isolation and Mutation Analysis

Genomic DNA was isolated from leukocyte nuclei obtained from the mother and baby by conventional methods.[9] Direct PCR was performed for all exons$^{1-15}$ of KCNQ1 isoform 1.[10] The primers were constructed, and all PCR products were sequenced in both directions using the same primers as used for the first round PCR.[10] DNA sequencing was performed by an ABI-Prism 310 DNA sequencer (Perkin-Elmer/Applied Biosystems, Foster City, CA, USA) using BigDye terminator premix reagent. The mutations previously reported in HERG, SCN5A, KCNE1, and KCNE2 were also screened in the same way.

Results of Mutation Analysis

In the mother and neonate, the DNA sequencing identified the same heterozygous single nucleotide substitution in KCNQ1 at position 1760 (C to T), resulting in an amino acid substitution

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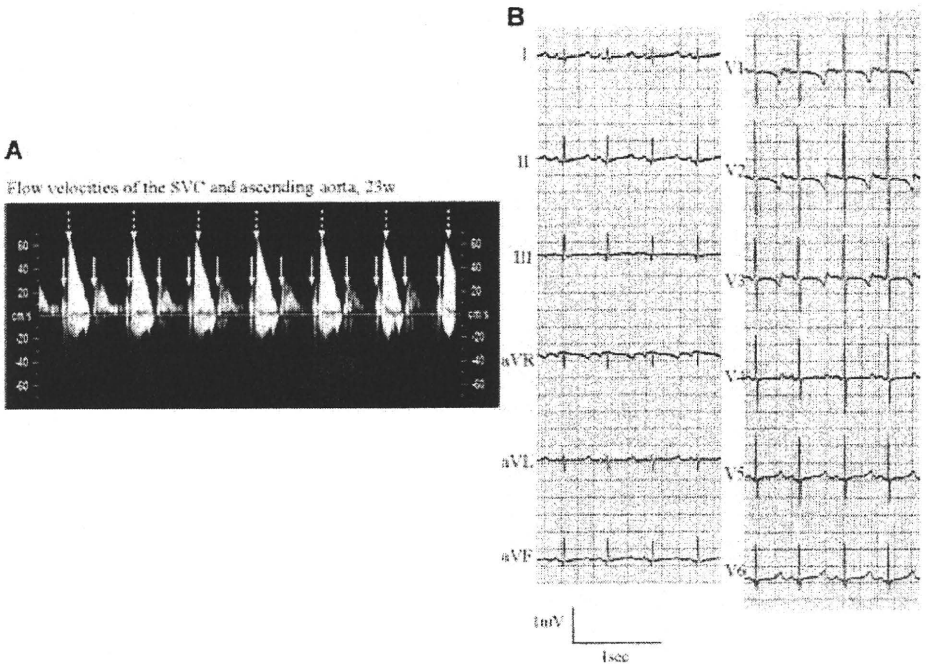

Figure 1. *(A) Flow velocities of the SVC and AA at gestational week 23, suggesting 2:1 AV conduction block. Solid arrows indicate reverse flow of the SVC, and dotted arrows indicate forward flow of the AA. (B) A 12-lead surface ECG of the newborn child at the age of 1 week. The corrected QT interval (QTc) at a sinus rate of 114/min is 521 ms.*

of methionine for threonine at codon 587 (T587M), which has been previously reported (Fig. 3A).[11] This heterozygous mutation was located in the C-terminal domain of KCNQ1 (Fig. 3B). The mutated alleles were also confirmed by restriction analysis with *PfuI*. The same nucleotide substitution was not observed in greater than 100 normal individuals.[11] Direct sequencing of other primer sets for KCNQ1, HERG, SCN5A, KCNE1, and KCNE2 revealed no mutations.

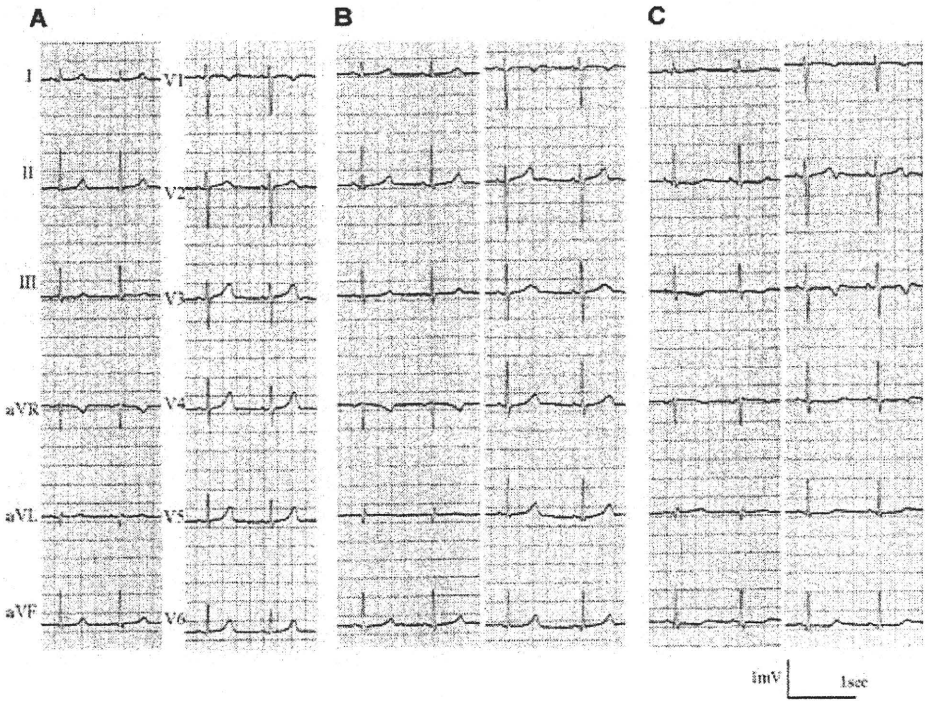

Figure 2. *A 12-lead surface ECG of the mother. The QTc interval is in the normal range (433 ms) without ST-T changes at gestational week 23 (A). The QTc interval is prolonged to 490 ms at 1 month postpartum (B) and to 531 ms at 2 months postpartum, with a biphasic T wave abnormality in leads V2 and V3 (C).*