Feasibility and Reproducibility of Fetal Left Ventricular Twist Using Two-Dimensional Speckle-Tracking Analysis in a Japanese Population

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Summary
In fetal echocardiography, conventional parameters for assessing cardiac function are limited because of limited echocardiographic windows or the fetus’ position. We aimed to evaluate the feasibility and reproducibility of fetal left ventricular (LV) twist by two-dimensional, speckle-tracking echocardiography (2DSTE) in a Japanese population.

We included 55 normal fetuses at gestational ages between 21 and 36 weeks. Subjects with adverse maternal health issues were excluded. LV twist was calculated as the net difference between LV basal and apical rotation at end-systole estimated with 2DSTE.

We were able to analyze the 2DSTE images in 44 cases (80%). The mean (±SE) apical rotation, basal rotation, and LV twist were 7.88 ± 0.77, −3.68 ± 0.50, and 11.1 ± 0.75 degrees, respectively. We could not analyze 11 cases (20%) because of poor image quality due to fetal position in five cases (45.5%), failure to track the endocardium because of blurred images in five cases (45.5%), and failure to obtain images of the heart due to the presence of the placenta in front of the fetus in one case (9.1%). There were no significant differences in the demographic data between pregnant women in whom LV twist analysis was feasible and not feasible. The intra- and interobserver intraclass correlation coefficients were 0.67 and 0.64, respectively.

LV twist analysis by 2DSTE in the fetus was feasible in a substantial population and may provide new insight into cardiac function during the prenatal period. On the other hand, its reproducibility was moderate and needs to be improved.

Key words: Heart, Embryology, Fetus, Cardiac function, Echocardiography

Fetal echocardiography is a widely available imaging modality for assessing fetal cardiac function during the prenatal period. Currently, the cardiothoracic area ratio, which corresponds to the cardiothoracic ratio measured on a roentgenogram, and total cardiac dimension (TCD) are widely used to assess fetal cardiac function in a clinical setting because of limited echocardiographic windows in the fetus or the fetus’ position. However, these conventional 2-dimensional (2D) parameters provide only limited information on cardiac function and are often insufficient for determining the optimal therapeutic strategy before birth. The development of 2D, speckle-tracking echocardiography (2DSTE) provides angle-independent measurement of myocardial strain and strain rate that avoids tethering and myocardial translation effects.1-5) The assessment of left ventricular (LV) rotation, based on circumferential strain analysis in the apical and basal LV short-axis views, is available on 2DSTE. LV twist (or torsion), based on rotational and twist mechanics of the LV myocardium, is significantly associated with LV systolic and diastolic function,6,7) and LV twist characteristics may change during growth and maturation.8-10)

Recently, several studies have reported on 2DSTE’s feasibility for assessing cardiac function in the fetus, but the results were inconsistent.11-15) Li, et al.15) reported the feasibility of measuring fetal LV twist by 2DSTE, and this study was performed in a Western population. However, physical and racial differences can influence cardiac function and echocardiographic windows.16-19) Since fetal LV twist has not been reported in Asians, we aimed to evaluate the feasibility and reproducibility of fetal LV twist by 2DSTE in a Japanese population.
Methods

Study population: We initially recruited 76 consecutive fetuses (gestational fetal age, 21-36 weeks). All of their mothers were singleton pregnant and visited the Tokyo Medical And Dental University Hospital for a routine prenatal checkup between August 2010 and May 2012. Fetuses whose mothers had hypertension, systemic lupus erythematosus, hyperthyroidism, Kawasaki disease, Takayasu disease, and positive anti-Ro/SSA or anti-La/SSB antibodies were excluded. Subjects were also excluded if there was arrhythmia, fetal growth restriction, or suspected congenital heart disease (CHD). Finally, we included 55 study subjects, and fetal echocardiography was performed in these subjects. The study was approved by the ethics committee of the Tokyo Medical And Dental University Hospital, and we obtained informed consent from all women participating in this study.

Fetal echocardiography: We used the GE Vivid i ultrasound system with a 5-MHz probe (GE Healthcare, Milwaukee, WI, USA) for fetal echocardiography, which was performed by a pediatric cardiologist (Y.M.). After identifying the fetal LV, the fetal LV short-axis views were recorded both at the basal and apical levels (Figure 1). The LV short-axis view at the basal level was identified as images that included the mitral valve. Care was taken to avoid papillary muscles in the short-axis view at the apical level as much as possible. The frame rate was set as close as possible to 60-110 frames/second (range, 78-142 frame/second; mean, 102 frames/second), which is recommended for 2DSTE. Anatomic M-Mode images were also recorded to identify the fetal cardiac cycle. Fetal weight, gestational age, heart rate (HR), and frame rate/HR ratio (FR/HR) were recorded at the time of the examination. We measured the distance between the mother’s abdominal wall and the heart of the fetus, the size of the heart (TCD, cm), LV length (cm), LV end-systolic dimensions (mm), LV end-diastolic dimension (mm), and LV ejection fraction (%). Data were also collected on maternal age, maternal height and body weight, gestational age, and estimated fetal weight. Our echocardiography equipment and laboratory are maintained under the guidelines of the Japanese Society of Echocardiography.

Twist analysis: The echocardiographic images obtained were transferred from the ultrasound system to a computer workstation for offline 2DSTE analysis using EchoPAC software (Version 133). We defined LV end-diastole as the time point when the LV was most visibly expanded at the basal level (Figure 1). LV twist was measured according to methods used in previous studies. Specifically, the LV endocardial borders were manually traced in the basal and apical short-axis images at end-systole. The software automatically generated epicardial border tracing and a region of interest, which was adjusted to fit the LV wall thickness, when necessary. When the tracking algorithm was applied, segmental and average rotational displacement values throughout a cardiac cycle were automatically provided in degrees. Twist was determined by calculating the degree of rotation of the apex relative to the base by subtracting the rotation at the basal level from the rotation at the apical level. Viewed from the apex, counterclockwise rotation was defined as positive and clockwise rotation as negative. In fetal echocardiography, electrocardiography, which is essential to identify the cardiac cycle, cannot be recorded. Thus, LV endo-diastole was defined as the time point when the LV was most visibly expanded in 2D images simultaneously displayed with a 2DSTE graph. The time (ms) from the end-diastole to end-systole was measured using the timings of maximum and minimum LV diameters in M-mode separately (Figure 1). The timing of end-systole was defined on 2DSTE graph by using the time measured by M-mode. Apical rotation, basal rotation, and twist were plotted on a graph. LV twist was averaged over three cardiac cycles.

Reproducibility of twist analysis: To examine intra- and interobserver reproducibility of apical and basal rotation and LV twist, intraclass correlation coefficients (ICC) were calculated, and Bland-Altman analysis was performed. Ten randomly selected examinations were analyzed twice by a blinded first investigator (Y.M.) at a two-week interval to determine intraobserver reproducibility, as well as once by a blinded second investigator (Y.Y.) to determine interobserver reproducibility. The same images recorded by a single operator were used to assess intra- and interobserver reproducibility in each subject because of difficulty in repeated fetal echocardiography. For fixed bias, we calculated the 95% confidence interval (CI) of the mean difference among measured values (95% limits of agreement). For proportional bias, we calculated Pearson’s correlation coefficient.

Statistical analysis: All statistical analyses were performed with SPSS software (SPSS Inc., Chicago, IL, USA) version 25. Clinical and echocardiographic parameters are shown as mean ± standard deviation, and apical rotation, basal rotation, and twist are shown as mean ± standard error. Normality of apical and basal rotations and twist were confirmed by the Kolmogorov-Smirnov test. Comparison between the two groups was performed by an unpaired t-test. For all analyses, a probability value < 0.05 was considered statistically significant.

Results

General characteristics and the feasibility of LV twist analysis: We examined the data from 55 fetuses and were able to analyze the images in 44 cases (80%) (Figure 2). We could not analyze 11 cases (20%) because of poor image quality due to fetal position in five cases (45.5%), failure to track the endocardium because of blurred images in five cases (45.5%), and failure to obtain images of the heart due to the presence of the placenta in front of the fetus in one case (9.1%). There were no significant differences in maternal age, height, body weight, body mass index, and gestational age between pregnant women in whom LV twist analysis was feasible and not feasible (Table I).

Measurements of LV twist: Fetal demographics, the results of conventional fetal echocardiography, and results of LV twist analysis are summarized in Table II. 2DSTE clearly demonstrated LV rotational motion in the basal and apical short-axis views in cases in which clear images were obtained. (Figure 3). In all of the study fetuses, the
apical LV showed counterclockwise rotation, and the basal LV showed clockwise rotation. Based on 2DSTE analysis in the apical and basal LV short-axis views, an LV twist curve was constructed for each fetus (Figure 4).

Reproducibility of LV twist: The intraobserver ICC for twist was 0.67 (95% CI, 0.03 to 0.91), and the interobserver ICC was 0.64 (95% CI, 0.05 to 0.86) (Table III), suggesting moderate LV twist reproducibility. Figure 5 shows Bland-Altman plots of agreement for the intra- and interobserver measurements of apical and basal rotation, and LV twist. Although a small fixed bias was observed for both the intraobserver measurements (0.13 degrees) and interobserver measurements (−1.71 degrees), the 95% limits of agreement (±1.96 SD) were above and below a “0 value” (Table III, Figure 5), suggesting that the fixed bias was within an acceptable range. No significant proportional bias was detected for either the intra- or interobserver measurements.

Discussion

In the current study, we demonstrated that fetal LV twist analysis using 2DSTE was feasible in 80% of our study subjects. The fetus was in various positions in our subjects, suggesting that LV twist analysis can be utilized for assessing fetal LV function during the prenatal period.
in Japanese pregnant women. The feasibility of LV twist analysis in the fetus was not associated with gestational age, or the age or body size of the mothers. LV twist analysis was not feasible in cases with an inappropriate position of the fetus or placenta, or with poor images of the fetal LV. We conclude that LV twist analysis is possible if there is a precise short-axis view of the LV, and there is no placenta in front of the fetus.

**Fetal echocardiography and 2DSTE:** Technical improvements in fetal ultrasonography have enabled prenatal CHD detection on routine screening examinations. In cases of suspected fetal CHD, pregnant women are referred for fetal echocardiography to assess morphologic and hemodynamic abnormalities. Deterioration of hemodynamic status may develop immediately after birth due to the transition from placental to pulmonary circulation. Therefore, prenatal evaluation of cardiac function, as well as the diagnosis of anatomical abnormalities, is essential to determine optimal delivery timing and prepare for extraterine treatment. Currently, fetal echocardiography is a mainstream imaging modality to assess fetal cardiac function during the prenatal period. Although echocardiography is an established imaging modality for assessing LV systolic and diastolic function in neonates, children, and adults, LV

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**Table I.** Comparison of Maternal Demographics between Pregnant Women for Whom LV Twist Analysis was Feasible and not Feasible

| Variable                | Feasible (n = 44) | Not feasible (n = 11) | P value |
|-------------------------|-------------------|-----------------------|---------|
| Age (years)             | 32.4 ± 4.68       | 31.4 ± 4.82           | 0.538   |
| Height (cm)             | 158 ± 4.23        | 158 ± 2.22            | 0.976   |
| Body weight (kg)        | 56.7 ± 9.30       | 55.4 ± 7.42           | 0.700   |
| Body mass index (kg/m²) | 22.5 ± 3.35       | 22.1 ± 3.48           | 0.774   |
| Gestational age (weeks) | 29.5 ± 3.69       | 29.7 ± 4.95           | 0.935   |
| FR/HR                   | 0.65 ± 0.11       | 0.72 ± 0.16           | 0.192   |

Data are expressed as mean ± SD. FR indicates frame rate; and HR, heart rate.

**Table II.** Fetal Demographics and Echocardiographic Parameters

| Variable                        | Mean ± SD         |
|---------------------------------|-------------------|
| Fetal demographics              |                   |
| Mean gestational age (weeks)    | 29.3 ± 3.78       |
| EFW (g)                         | 1468 ± 559        |
| HR (beats/minute)               | 147 ± 13.0        |
| Echocardiographic parameters    |                   |
| FR (Hz)                         | 102 ± 22.0        |
| FR/HR                           | 0.67 ± 0.12       |
| Depth (cm) *                    | 5.72 ± 2.12       |
| Cardiac size (TCD) (cm)         | 2.62 ± 0.47       |
| LV length (cm)                  | 2.09 ± 0.40       |
| LV end-diastolic dimension (mm) | 11.6 ± 2.35       |
| LV end-systolic dimension (mm)  | 7.67 ± 1.29       |
| LV ejection fraction (%)        | 66.4 ± 7.35       |
| LV twist analysis               |                   |
| Apical rotation (degrees)       | 7.88 ± 0.77       |
| Basal rotation (degrees)        | −3.68 ± 0.50      |
| Twist (degrees)                 | 11.1 ± 0.75       |

EFW indicates estimated fetal weight; HR, heart rate; FR, frame rate; LV, left ventricular, and TCD, total cardiac dimension. *Depth = distance from mother’s abdominal wall to the fetal heart. Demographic and echocardiographic parameters are presented as mean ± SD; apical and basal rotation and LV twist parameters are presented as mean ± SE.

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Figure 2. Feasibility of LV twist analysis. Among 55 recruited patients, LV twist analysis was feasible in 44 subjects (80%).
The change of apical rotation, basal rotation, and twist during a cardiac cycle in a representative case. First, the time from end-diastole to end-systole was measured as 220 ms in M-mode (left figure). Right figure demonstrates the time curve of rotational displacement of the basal and apical segments, and the calculated twist. Based on the time measured by M-mode, the timing of end-systole was defined on this graph.

Figure 3. 2DSTE images from the LV short-axis view in a representative case. The 2DSTE images clearly demonstrated LV rotational motion at the basal and apical short-axis view in cases with clear images.

Figure 4. The change of apical rotation, basal rotation, and twist during a cardiac cycle in a representative case. First, the time from end-diastole to end-systole was measured as 220 ms in M-mode (left figure). Right figure demonstrates the time curve of rotational displacement of the basal and apical segments, and the calculated twist. Based on the time measured by M-mode, the timing of end-systole was defined on this graph.

Functional parameters based on 2D or Doppler echocardiography are limited in the fetus because of the limited echocardiographic window or the fetus' position. 2DSTE is expected to overcome the limitations of 2D fetal echocardiography, and some previous studies reported the feasibility of 2DSTE in the fetus. Enzensberger, et al. reported that they were able to analyze right and LV longitudinal strain in 88% of fetuses at gestational ages between 19 and 37 weeks. Ishii, et al. reported that they were able to analyze circumferential strain in 69% of fetuses at gestational ages between 17 and 42 weeks, and their intra- and interobserver correlation coefficients were 0.78 and 0.64, respectively. The feasibility of LV twist measurement in our study was roughly intermediate between these previous reports. We might think that LV twist analysis is feasible for clinical practice in a substantial population, although there is still room for improvement.

LV twist in fetuses: One of the advantages of LV twist is that it may provide both systolic and diastolic function in cases where the LV short-axis view can be obtained at two levels. LV twisting and untwisting motions are gener-
Table III. Inter- and Intra-observer Reproducibility of LV Twist Analysis

|                     | ICC (95% CI) | Bias (95% LOA) | P value |
|---------------------|-------------|----------------|---------|
| **Intra-observer reproducibility** |             |                |         |
| Apical rotation     | 0.47 (−0.17 to 0.83) | 0.03 (−6.16 to 6.16) | 0.070   |
| Basal rotation      | 0.79 (0.37 to 0.94)  | −0.19 (−3.38 to 3.00) | 0.002   |
| Twist               | 0.67 (0.03 to 0.91)  | 0.13 (−5.60 to 5.87) | 0.058   |
| **Inter-observer reproducibility** |             |                |         |
| Apical rotation     | 0.40 (−1.39 to 0.85) | 0.75 (−4.90 to 6.42) | 0.226   |
| Basal rotation      | 0.67 (0.12 to 0.90)  | 0.71 (−2.15 to 3.57) | 0.012   |
| Twist               | 0.64 (0.05 to 0.86)  | −1.71 (−3.35 to 2.38) | 0.018   |

ICC indicates intra-class correlation coefficient; LOA, limits of agreement; and CI, confidence interval.

Figure 5. Bland-Altman graphs demonstrating intra- and interobserver reproducibility of apical and basal rotation, and LV twist. Bland-Altman analysis demonstrated good agreement between the intra- and interobserver measurements using 2DSTE.

ated as a result of the dynamic interaction between obliquely oriented epicardial and endocardial fibers wound in opposite directions. LV twisting motion during systole plays an important role in the ejection of blood flow from the LV to the systemic circulation. The LV untwisting rate using 2DSTE is a surrogate marker of the time constant of LV pressure decay (tau) and is correlated with the LV intraventricular pressure gradient, which is a major contributor to active early LV filling during diastole that is independent of left atrial pressure. These rotational parameters could provide additional information on cardiac function beyond 2D echocardiographic parameters. In this study, we found a larger mean apical rotation of 7.88 ± 0.77 degrees and smaller mean basal rotation of −3.68 ± 0.50 degrees, generating a mean LV twist of 11.1 ± 0.75 degrees in our population. Similar rotational motions of the LV myocardium at both the apical and basal levels were observed in adults in previous studies. On the other hand, basal rotation was counterclockwise throughout most of systole in infancy but became more clockwise with growth. Maskatia, et al. reported gestational changes in fetal myocardial deformation using 2DSTE. The change in rotational motions of the LV myocardium before and after birth would be of interest in further stud-
ties.

In this study, the mean gestational week of our study fetuses was 29.3 ± 3.78 weeks. The gestational week influences on the fetal size and position, the placenta, and the amniotic fluid. In particular, amniotic fluid volume decreases after 30 or 32 weeks, and it may affect the image quality of fetal echocardiography. Changes of various conditions by gestational week may influence the LV twist’s feasibility and reproducibility. We need further study to address this issue.

**Reproducibility of LV twist measurements:** In this study, both intra- and interobserver ICC were moderate, indicating that the reproducibility of LV twist measurements still needs to be improved. In particular, we found that the fixed bias for the interobserver measurements was greater than that for the intraobserver measurements. The fixed bias for the interobserver measurements may be due to a difference in experience between the two investigators. LV twist analysis still requires manual tracing of the endocardial border and partially depends on the investigator’s proficiency level. The issue of operator-dependence of measurements is common with echocardiographic parameters and may be improved by training.

**Racial differences in fetal LV twist:** This is the first study examining the feasibility and reproducibility of LV twist measurements with 2DSTE in an Asian population, while one similar study was conducted in a Western population. As described in the discussion, there are racial differences in LV function and echocardiographic windows and this is one of advantages of our study. On the other hand, it also should be recognized there might be racial differences in the interpretation of our results.

**Limitations:** The interpretation of our study was limited by a small study population. Further study with a larger cohort is needed to confirm our results. Feasibility and reproducibility of 2DSTE depends on image quality and operators’ proficiency. Technological improvements in echocardiography and operator training may improve this limitation to some extent. However, 2DSTE feasibility and reproducibility are limited by poor images or inappropriate position of the fetus and placenta. It is necessary to recognize that 2DSTE measurement may not be applied in some fetuses at a certain rate. To assess twist measurements’ reproducibility, we used the same short-axis views recorded by a single operator and demonstrated that intra- and interobserver ICC of twist measurements were 0.67 and 0.64, respectively. However, interpretation of this reproducibility is limited by using the same images. Even in the same case, if images were recorded by different operators, this reproducibility might be different. There might be a small, but significant, variation in 2DSTE measurements among vendors and this should be considered when performing serial studies. Regrettably, we did not measure the LV untwisting rate with 2DSTE, which reflects LV diastolic function. Although LV untwisting rate was measured in the same manner in subjects in whom LV twist could be measured in previous studies, the feasibility and reproducibility of the fetal LV untwisting rate with 2DSTE needs to be examined in further studies. Finally, we included only normal fetuses in this study. Feasibility and reproducibility of LV twist analysis in the fetus with CHD needs to be examined in further studies.

**Conclusions**

In 80% of fetuses in various positions, LV twist could be measured using 2DSTE in a Japanese population. Measurements of LV twist are possible when precise short-axis views of the left ventricle can be obtained, and there is no placenta in front of the fetus. LV twist analysis in the fetus might bring new insight into CHD pathophysiology during the prenatal period. On the other hand, its reproducibility was moderate and needs improvement.

**Disclosures**

**Conflicts of interest:** There is no relationship with any industry.

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