

รายงานวิจัยฉบับสมบูรณ์

โครงการการวินิจฉัยก่อนคลอดของปัญหาที่พบบ่อยที่สุดในทารกไทย
Prenatal Diagnosis of the Most Common Fetal Disorders in Thailand

ผู้วิจัย

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สังกัด

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สนับสนุนโดยสำนักงานคณะกรรมการการอุดมศึกษา
และสำนักงานกองทุนสนับสนุนการวิจัย

(ความเห็นในรายงานนี้เป็นของผู้วิจัย สกว. ไม่จำเป็นต้องเห็นด้วยเสมอไป)

บทคัดย่อ

(โปรดดูรายละเอียดของบทคัดย่อ หรือ abstract ของแต่ละผลงานในแต่ละเรื่องในส่วนของภาคผนวก สำหรับหนังสือทั้งสามเล่มและสื่อมัลติมีเดียโปรดดูรายละเอียดในหนังสือหรือแผ่นวีดีโอโดยตรง)

โครงการในสาขาวิชาเวชศาสตร์มารดาและทารก ซึ่งทำการศึกษาเกี่ยวกับปัญหาที่พบบ่อยที่สุดในทารกของประชากรไทย ซึ่งได้แก่ ภาวะซีดซีเขียวชนิดรุนแรง หัวใจพิการโดยกำเนิด กลุ่มอาการดาวน์ มีวัตถุประสงค์หลักในการค้นหาวิธีที่มีประสิทธิภาพของการวินิจฉัยก่อนคลอดเพื่อที่จะควบคุมโรคสำคัญดังกล่าว

ในระหว่างการศึกษา มีโครงการย่อย ๆ หลายโครงการซึ่งทั้งหมดมุ่งตรงที่การวินิจฉัยก่อนคลอดของโรคที่พบบ่อยในประเทศไทย โดยศึกษาเกี่ยวกับหัวใจทารกในครรภ์ และทารกซีดในครรภ์เป็นหลัก และยังรวมถึงประสิทธิภาพของการควบคุมโรคธาลัสซีเมียชนิดรุนแรง ศึกษาการทำงานของหัวใจและหลอดเลือดทารกในครรภ์สำหรับการประเมินเพื่อวินิจฉัยความผิดปกติต่อไป รวมทั้งศึกษาในรายที่ผิดปกติด้วย

หลายโครงการย่อยได้ทำการศึกษาถึงประสิทธิภาพของยุทธวิธีในการควบคุมโรคธาลัสซีเมียด้วยวิธีก่อนคลอด (โครงการ 1 & 7) ซึ่งทำให้ได้หลักฐานถึงประสิทธิภาพของระบบการคัดกรองและการวินิจฉัยก่อนคลอดที่อาจช่วยในการวางแผนการดูแลสุขภาพในระดับชาติในการควบคุมโรคนี้ นอกจากนี้ ยังมีการศึกษามาร์กเกอร์อัลตราซาวด์หลายตัวของโรคฮีโมโกลบินบาร์ที่ ได้ทำการศึกษาอย่างต่อเนื่องและกว้างขวาง ซึ่งช่วยให้ตรวจพบโรคดังกล่าวในครรภ์ได้เร็วขึ้น ตั้งแต่ไตรมาสแรก ซึ่งน่าจะมีการนำไปประยุกต์ใช้อย่างกว้างขวางในอนาคตอันใกล้นี้ โดยเฉพาะอย่างยิ่งการตรวจ cardiothoracic ratio and peak systolic velocity of middle cerebral artery เป็นต้น

โครงการที่ 1 และ 7 ที่ทีมงานได้ทำการศึกษาอย่างต่อเนื่องเกี่ยวกับรูปแบบใหม่ ๆ ของการควบคุมโรคธาลัสซีเมียด้วยยุทธวิธีก่อนคลอด ศึกษาถึงประสิทธิภาพในการตรวจคัดกรอง การวินิจฉัย ซึ่งนำไปสู่การวินิจฉัยได้เร็ว รวมทั้งการตรวจมาร์กเกอร์ทางอัลตราซาวด์ต่าง ๆ ที่ช่วยให้วินิจฉัยได้เร็วขึ้น ซึ่งผลการศึกษาได้แสดงให้เห็นชัดเจนว่าส่วนใหญ่ของโรคฮีโมโกลบินบาร์สามารถวินิจฉัยได้ด้วยอัลตราซาวด์ตั้งแต่ปลายไตรมาสแรก เป็นต้น

การศึกษาเกี่ยวกับโลหิตวิทยาของทารกในครรภ์ (โครงการ 2,3) ได้ทำให้ทราบข้อมูลพื้นฐานทางโลหิตวิทยาของทารกไทย สำหรับอ้างอิงเปรียบเทียบกับภาวะผิดปกติต่าง ๆ และยังได้พบมาร์กเกอร์ทางชีวภาพหลายอย่างที่จะช่วยในการวินิจฉัยภาวะซีด และนำไปสู่ความเข้าใจถึงพยาธิสรีรวิทยาของการทำงานของรก เช่น sFlt-1, placental growth factor ตลอดจนสารที่ช่วยบ่งชี้หัวใจทารกทำงานผิดปกติ เช่น cardiac troponin T และ N-terminal pro-B-type natriuretic peptide (nt-proBNP)

หัตถการการวินิจฉัยก่อนคลอด (โครงการ 4) เช่นการตัดชิ้นเนื้อรกตรวจดีเอ็นเอ หรือการเจาะเลือดสายสะดือทารกในครรภ์ ได้ทำการศึกษาหลายแง่มุมทั้งเทคนิคและความปลอดภัย หรือความเสี่ยง ซึ่งเป็นข้อมูลสำคัญสำหรับการให้คำปรึกษาแก่ผู้ตั้งครรภ์ที่ทารกมีความเสี่ยงต่อการเป็นโรค ตลอดจนเป็นข้อมูลประกอบการตัดสินใจเลือกวิธีการวินิจฉัย เป็นต้น

โครงการ 5,6 ได้ทำการศึกษาระบบหัวใจและหลอดเลือดของทารกในครรภ์ ทำให้ทราบค่ามาตรฐานต่าง ๆ ของทารกในทั้งในการทำงานและขนาดต่าง ๆ ตามอายุครรภ์ ซึ่งเป็นข้อมูลสำคัญสำหรับการประเมินภาวะผิดปกติของการทำงานของหัวใจและโครงสร้างของหัวใจทารก เช่น Tei index, excursion septum primum หรือขนาดของหลอดเลือดใหญ่ที่อายุครรภ์ต่าง ๆ เป็นต้น ยังได้ทำการศึกษากว้างขวางเกี่ยวกับมาร์คเกอร์ต่าง ๆ ของระบบหัวใจและหลอดเลือดที่แสดงออกในภาวะผิดปกติ โดยเฉพาะในทารกที่เป็นกลุ่มอาการดาวน์ เป็นต้น

สองโครงการสุดท้าย (**โครงการ 8 & 9**) เป็นความพยายามของทีมวิจัยในการสังเคราะห์องค์ความรู้จากผลงานวิจัยไปสู่การประยุกต์ใช้โดยการเรียบเรียงและสรุปองค์ความรู้ที่ทันสมัยเกี่ยวกับการวินิจฉัยก่อนคลอดของโรคที่พบบ่อยและที่เป็นปัญหาของคนไทย โดยอิงอยู่บนพื้นฐานความรู้เชิงประจักษ์ ในโครงการนี้สามารถผลิตตำราสามเล่ม (ทารกบวม น้ำ การตรวจหัวใจทารกในครรภ์ และหัตถการการวินิจฉัยก่อนคลอด) และมัลติมีเดีย 1 ชุด (ชุดปฏิบัติการการตรวจหัวใจทารกในครรภ์) โดยหวังว่าสื่อการเรียนการสอนนี้จะเอื้ออำนวยให้แพทย์ที่ปฏิบัติงานด้านนี้มีความรู้และทักษะสูงขึ้น และช่วยให้การวินิจฉัยก่อนคลอดมีประสิทธิภาพมากขึ้น

สรุปผล: โครงการนี้สร้างผลงานตีพิมพ์ในฐานข้อมูลสากล Scopus / PubMed จำนวน 33 เรื่อง ซึ่งมีผล impact รวม 59.6 พร้อมกับผลิตสื่อการเรียนการสอนสำหรับผู้เชี่ยวชาญโดยมีตำรา 3 เล่ม และสื่ออินเตอแอคทีฟมัลติมีเดีย 1 ชุด ในช่วงระยะเวลาที่ทำการศึกษพบว่าอัตราการวินิจฉัยก่อนคลอดของโรคหัวใจพิการโดยกำเนิดเพิ่มขึ้นเป็นสองเท่าตัว และสร้างนักวิจัยรุ่นใหม่ที่มีผลงานระดับนานาชาติมากกว่า 10 ท่าน

คำหลัก: การตั้งครรภ์, การวินิจฉัยก่อนคลอด, กลุ่มอาการดาวน์, ทารกในครรภ์, ธาลัสซีเมีย, อัลตราซาวด์, อัลตราซาวด์หัวใจ

Abstract

In the field of maternal-fetal medicine, the main fetal disorders (or the most common fetal abnormalities) in Thailand include severe fetal thalassemia syndrome, fetal congenital heart disease and fetal Down's syndrome. To overcome these major Thai fetal disorders, accurate and early prenatal diagnosis is imperative. Therefore, the main purposes of these projects focused on effective prenatal diagnosis for congenital heart disease and severe thalassemia.

During the study period, several minor projects studied on prenatal diagnosis of the common disease in Thailand focusing on fetal echocardiography and fetal thalassemia syndrome. The project included both effectiveness of prenatal diagnoses for congenital heart disease and severe thalassemia and construction of the reference ranges of fetal cardiac function and fetal hemodynamics as well as cardiac dimension.

Several new strategies for prenatal control of severe thalassemia were studied (**Project 1 & 7**). The outcomes of this project provide solid evidence which is probably useful to develop national guideline for the disease control. Additionally, various ultrasound markers for prenatal diagnosis of Hb Bart's diseases have also continuously investigated and several markers have been shown to be helpful and may be widely used in near future, especially cardiothoracic ratio and peak systolic velocity of middle cerebral artery even in the first trimester.

Fetal hematology has been extensively investigated in both normal fetuses and fetuses with anemia (**Project 2-3**). Several biochemical markers as well as sonomarkers have been shown to be helpful in diagnosis of fetal anemia. Additionally, several markers help us understand pathophysiology of placental dysfunction secondary to fetal anemia, as well as cardiac marker like troponin T and N-terminal pro-B-type natriuretic peptide (nt-proBNP)

Invasive prenatal diagnostic procedures (**Project 4**), especially cordocentesis, have been extensively investigated, and have been most comprehensively analyzed ever published. The information on risks and benefits of the procedures is helpful for patients counseling and choices of management among fetuses at risk of the disease.

The **project 5** have developed various reference ranges of fetal cardiac function and dimension as well as several hemodynamic parameters for Thai population such as Tei index, septum primum excursion, or various dimension of great arteries, which are baseline data, helpful for early prenatal diagnosis of fetal cardiovascular disease, and severe thalassemia syndrome. Finally, this project (**Project 6**) studied on

efficacy of various cardiac markers in identification of cardiovascular disease and fetal Down syndrome and hemoglobin Bart's disease. Various cardiac functions in fetal anemia and structural anomalies were evaluated, especially in fetal anemia and chromosomal abnormalities.

Project 8 & 9 are attempts to synthesis and distribute the important evidence-based knowledge concerning common disease for prenatal control for Thai physicians. Three books and one set of interactive DVD multimedia are developed, including hydrops fetalis, fetal cardiac examination (together with interactive multimedia), and invasive prenatal diagnosis. These academic materials are instructive, teaching how to implement research to more effective routine work, in the hope that they can improve performance in prenatal diagnosis.

Output summary: This project produces 33 publications (PubMed / Scopus database) with total impact factor of 59.6, three academic textbooks, one set of interactive multimedia. Moreover, the projects are associated with a two-fold increase in number of prenatal diagnoses of congenital heart disease and more than 10 young researchers have been developed.

Key Words: Down syndrome, Echocardiography, Fetus, Pregnancy, Prenatal diagnosis, Thalassemia, Ultrasound

Executive Summary

1. Project Title

Prenatal Diagnosis of the Most Common Fetal Disorders in Thailand

การวินิจฉัยก่อนคลอดของปัญหาที่พบบ่อยที่สุดในทารกไทย

The main projects are divided into 10 minor projects as follows:

1. New strategy for prenatal control of severe thalassemia: A multicenter study (*Chanane Wanapirak, et al*)
2. Maternal and fetal cardiac markers in fetal anemia focusing on homozygous alpha-thalassemia-1 (*Fuenglada Tongprasert, et al*)
3. Comparison of hematologic parameter among normal, beta- thalassemia trait, Hb E trait, Homozygous beta –thalassemia disease, and Beta-thalassemia/Hb E disease fetuses at mid-pregnancy (*Kasemsri Srisupundit, et al*)
4. Outcomes of pregnancies undergoing cordocentesis at mid-pregnancy: experience of 6,000 cases (*Wirawit Piyamongkol, et al*)
5. Normal reference range of the fetal heart and cardiac anomaly screening in low risk pregnancies (*Suchaya Luewan, et al*)
6. A comparison of cardiac function between fetuses with congenital heart defect and fetuses with anemia (*Theera Tongsong, et al*)
7. First trimester diagnosis of fetal Hb Bart's disease among pregnancies at risk (*Supatra Sirichotiykul, et al*)
8. Textbook: Fetal echocardiography
9. Interactive Multimedia E-learning: Workshop on fetal echocardiography

2. Head of the Project:

ศ. น.พ.ธีระ ทองสง ภาควิชาสูติ-นรีเวช คณะแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่

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Research Team: Prenatal Diagnostic Group (Maternal Fetal Medicine,

Chiang Mai University Thailand)

3. Research Field: Health Science

Clinical Applied Research in Maternal fetal medicine

วิจัยเชิงประยุกต์ทางคลินิก: สาขาเวชศาสตร์มารดาและทารก

4. Budgets 7,500,000 Baht

5. Study duration: 3 years

6. No any part of this project have been submitted for granting from other sources

7. Background and Rationale

(The details: please review in the separate individual projects:

Overall summary:

In the field of maternal-fetal medicine, the main fetal disorders (or the most common fetal abnormalities) in Thailand include severe fetal thalassemia syndrome, fetal congenital heart disease and fetal Down's syndrome.

Congenital heart defect is the most common fetal anomaly, with an incidence of 1 percent of births. Most of them are not diagnosed prenatally. Additionally this is one of the most common causes of perinatal death associated with anomalies. More than ten years we have studied and do several researches on this field (fetal echocardiography) in the hope that they may contribute to improvement in prenatal diagnosis and postnatal treatment of congenital heart defect in Thailand either via early diagnosis, intrauterine treatment especially cardiac failure due to anemia, referral to tertiary center for postnatal therapy or even option of termination of pregnancy for severe fetal morbidity when diagnosed in early gestation. We are the largest tertiary center in the northern part of Thailand taking care of fetuses in areas. Therefore we aim to improve the diagnoses in as early as possible by studying both in simplification for real practice and attempting to study on new technology and determine how to apply in Thai population.

Thalassemia is the most common hematologic genetic disease in Thailand. The prevalence of α -thalassemia-1, β -thalassemia, and HbE gene in our population is as high as 14%, 3-9%, and 13% respectively, leading to many births of children with severe thalassemia, including homozygous β -thalassemia, β -thalassemia/HbE, and Hb Bart's disease. The affected persons of the first two entities have low quality of life and have estimated average life expectancy of 10 and 30 years,

respectively while Hb Bart's hydropic fetuses have never survived and their mothers often suffer from obstetric complications such as pre-eclampsia, dystocia, postpartum hemorrhage due to a large placenta, and the psychological burden for carrying a nonviable fetus to term. Each year, our department faces about 20, 30-40, and 20-30 new cases of homozygous β -thalassemia, β -thalassemia/HbE and Hb Bart's disease, respectively. Therefore, these three entities of severe thalassemia need to be controlled, especially by prenatal approach. We continuously study and have launched several projects to control by prenatal approach. Though we have established the successful prenatal control of severe thalassemia, we have continuously developed a new or modified strategy based on our previous well-established strategy to be more effective. To be successful in control severe thalassemia in large-scaled population, we need to know what is the most appropriate thalassemia screening test and diagnostic tests for carrier status. Furthermore we need to establish the best approach for pregnancies at risk for Hb Bart's disease. We need to know whether ultrasound can replace cordocentesis, how long and how often serial ultrasound examinations are required and when non-invasive approach is the patient's choice.

To overcome these major Thai fetal disorders, accurate and early prenatal diagnosis is imperative. Therefore, the main purposes of these projects focus on effective prenatal diagnosis on aforementioned disorders.

8. Overview Objectives

(Please review in the separate projects:

1. To determine the effectiveness of the new strategy in prenatal control for severe thalassemia syndrome
2. To identify the effectiveness of non-invasive approach for early diagnosis of fetuses at risk of homozygous thalassemia-1
3. To determine the normal reference range of fetal hematology and fetal cardiology
4. To determine cordocentesis associated complications in real practice
5. To evaluate the effectiveness of fetal cardiac defect using STIC technology
6. To evaluate the efficacy of sonographic markers in the first trimester in predicting fetal homozygous alpha-thalassemia-1 in couples at risk
7. To assess fetal cardiac function in differentiating high and low-output heart failure
8. To determine the effectiveness of maternal serum biochemical markers (PIGF, sFlt-1 and PIGF/sFlt-1 ratio) for fetal anemia and fetal homozygous alpha thalassemia-1 disease detection.

9. To determine the effectiveness of fetal serum biochemical markers (cTnT and NT-proBNP) for fetal anemia detection.

9. Study Design

See in separate minor projects:

10. Project Output

- 33 international publications (PubMed) of high impact factor (total Impact factor 59.6)
- 3 Textbooks (fetal cardiac examination, invasive prenatal diagnosis, hydrops fetalis)
- 1 CD-interactive multimedia for training: Fetal cardiac examination
- New Researchers development, more than 20 researchers are involved in the projects and more than half are young researchers, including more than 10 new young researchers

Output ที่ได้จากโครงการ

1 ผลงานตีพิมพ์ในวารสารวิชาการนานาชาติ

ผลงานตีพิมพ์ระดับนานาชาติ จำนวน 33 เรื่อง ได้รับการตีพิมพ์ที่ปรากฏในฐานข้อมูลสากลแล้ว 27 เรื่อง และอยู่ระหว่างการตีพิมพ์ 6 เรื่อง ซึ่งทั้งหมดเป็นการตีพิมพ์ในฐานข้อมูล Scopus / Pubmed

- 1) Tongsong T, Wanapirak C, Piyamongkol W, Sirichotiyakul S, Tongprasert F, Srisupundit K, et al. Fetal ventricular shortening fraction in hydrops fetalis. *Obstet Gynecol* 2011 Jan;117(1):84-91. (Impact factor = 4.798)
- 2) Luewan S, Yanase Y, Tongprasert F, Srisupundit K, Tongsong T. Fetal cardiac dimensions at 14-40 weeks' gestation obtained using cardio-STIC-M. *Ultrasound Obstet Gynecol* 2011 Apr;37(4):416-22. (Impact factor = 3.557)
- 3) Tongprasert F, Srisupundit K, Luewan S, Wanapirak C, Tongsong T. Normal reference ranges of ductus venosus Doppler indices in the period from 14 to 40 weeks' gestation. *Gynecol Obstet Invest* 2012;73(1):32-7. (Impact factor = 1.103)
- 4) Tangshewinsirikul C, Wanapirak C, Piyamongkol W, Sirichotiyakul S, Tongsong T. Effect of cord puncture site in cordocentesis at mid-pregnancy on pregnancy outcomes. *Prenat Diagn* 2011 Sep;31(9):861-4. (Impact factor = 2.683)

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Total: Impact factor 59.634

2 หนังสือ Textbook: Prenatal control of severe thalassemia

1) ตำราทารกบวมน้ำ : Hydrops Fetalis

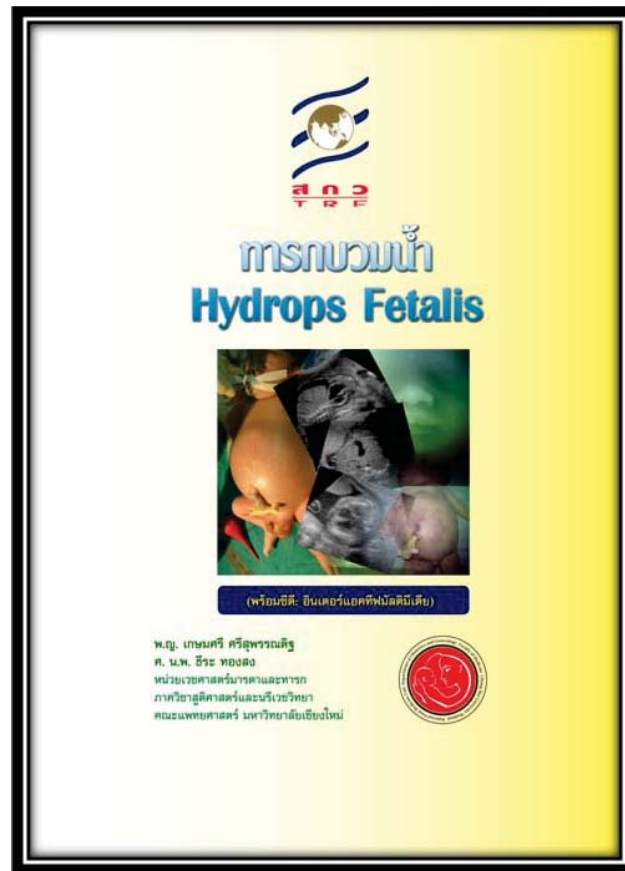
ชื่อตำรา ทารกบวมน้ำ : Hydrops Fetalis

บรรณาธิการ : เกษมศรี ศรีสุพรรณดิฐ, ธีระ ทองสง

ขนาดหน้าหนังสือ A4 จำนวน 234 หน้า

ISBN: 978-974-672-702-0

พิมพ์ครั้งที่ 1 จำนวน 1,000 เล่ม, 24 พฤษภาคม 2555



คำนำ: ภาวะทารกบวมน้ำนับเป็นปัญหาสำคัญทางสาธารณสุขของประเทศไทย การวินิจฉัยก่อนคลอดช่วยให้สามารถลดอุบัติการณ์นี้ได้อย่างมีประสิทธิภาพ ปัจจุบันมีการศึกษาวิจัยอย่างกว้างขวางเกี่ยวกับการวินิจฉัยก่อนคลอดตั้งแต่อาการอยู่ในระยะเริ่มต้น และมีการพัฒนาเทคนิคใหม่ๆ ทั้งในการวินิจฉัยและการดูแลรักษา ในกระบวนการนี้ จึงมีความจำเป็นที่แพทย์ผู้ปฏิบัติงานจะต้องมีความรู้ความเข้าใจและสามารถประยุกต์ใช้ได้ อย่างถูกต้อง ทีมงานจึงจัดทำตำราเล่มนี้ เพื่อให้แพทย์ที่มีหน้าที่ในการดูแลรักษาสตรีตั้งครรภ์ สามารถตรวจคัดกรอง วินิจฉัยได้อย่างถูกต้องตั้งแต่ระยะเริ่มแรก และให้การดูแลรักษาอย่างเหมาะสม และได้ทราบถึงความก้าวหน้าในวิชาการด้านนี้ ตำราเล่มนี้มีวัตถุประสงค์เพื่อ

- เผยแพร่ความรู้และแนวความคิดการป้องกันและวินิจฉัยทารกบวมน้ำตั้งแต่ระยะเริ่มต้น ด้วยข้อมูลเชิงประจักษ์ และทันสมัย
- เพื่อสร้างบรรยากาศทางวิชาการ การวิจัย การแลกเปลี่ยนความรู้ และความร่วมมือของประชาคมวิจัย
- เป็นการรวบรวมองค์ความรู้เพื่อเป็นที่ยอ้างอิงและนำไปสู่การวางแผนนโยบายในการควบคุมตั้งแต่ระดับชาติ ไปจนถึงระดับโรงพยาบาลชุมชนการจบบังเกิดผลงานวิจัยระดับนานาชาติจำนวนมาก และเป็นที่มาของหนังสือเล่มนี้

2) ตำราการตรวจหัวใจทารกในครรภ์

ชื่อตำรา : การตรวจหัวใจทารกในครรภ์ Fetal Cardiac Examination

บรรณาธิการ : สุขยา ลือวรรณ, วีระ ทองสง

ขนาดหน้าหนังสือ A4 จำนวน 174 หน้า

ISBN: 978-616-321-537-6

พิมพ์ครั้งที่ 1 จำนวน 1,000 เล่ม, 20 กันยายน 2555



คำนำ: เป็นตำราที่รวบรวมองค์ความรู้และเทคนิคในการตรวจหัวใจทารกในครรภ์ อาศัยหลักฐานการศึกษาและประสบการณ์ในการปฏิบัติงานและศึกษาวิจัยด้านนี้มากกว่า 15 ปีของทีมงาน มีการสะสมประสบการณ์และทรัพยากรการศึกษาเรียนรู้ในด้านนี้ในปริมาณมากพอสมควร จึงรวบรวมและนำเสนอแนวทางในการตรวจหัวใจทารกในครรภ์โดยอิงประสบการณ์และหลักฐานการศึกษา เพื่อแบ่งปันประสบการณ์กับท่านผู้ปฏิบัติงานด้านนี้ และส่งเสริมความรู้/ทักษะเชิงลึกในการตรวจหัวใจทารกในครรภ์ให้มีประสิทธิภาพมากยิ่งขึ้น ตำราเล่มนี้ประกอบด้วยความรู้วิทยาศาสตร์พื้นฐานที่เกี่ยวข้องกับหัวใจทารกทั้งกายวิภาค พัฒนาการ และสรีรวิทยาพอเป็นสังเขป (บทที่ 1-4) โดยเน้นส่วนสัมพันธ์กับการประยุกต์ใช้ในทางคลินิก ในส่วนเทคนิคและการวิเคราะห์ภาพอัลตราซาวด์อิงตามหลักฐานการศึกษาทางคลินิกและข้อแนะนำขององค์กรชั้นนำในทางอัลตราซาวด์ (บทที่ 5-9) และยังผนวกการตรวจขั้นสูงที่ช่วยเพิ่มขีดความสามารถในการตรวจให้มากขึ้นไปอีก (บทที่ 10-14) เช่น

การใช้อัลตราซาวด์สาม/สี่มิติในการตรวจหัวใจเป็นต้น มีความมุ่งหวังว่าตำรานี้จะมีส่วนช่วยเหลือหรือให้แนวทางในการประยุกต์เทคโนโลยีที่มีอยู่ทั่วไปให้เกิดประโยชน์สูงสุด มีส่วนเพิ่มพูนทักษะการประยุกต์ใช้ให้มีความสามารถในการวินิจฉัยโรคหัวใจพิการโดยกำเนิดได้ก่อนคลอดด้วยความมั่นใจสูง สามารถวินิจฉัยได้ตั้งแต่อายุครรภ์ยังน้อย หรือส่งตัวผู้ป่วยไปสู่การดูแลรักษาในสถานที่ที่มีความพร้อมมากกว่าเป็นต้น หรือแม้แต่การให้โอกาสทางเลือกในการยุติการตั้งครรภ์ตั้งแต่อายุครรภ์ยังน้อย (สำหรับรายที่ทารกมีความพิการชนิดรุนแรงที่อาจไม่สามารถมีชีวิตรอดได้)

3) ตำราหัตถการการวินิจฉัยก่อนคลอด

ชื่อตำรา : หัตถการการวินิจฉัยก่อนคลอด (Invasive Prenatal Diagnosis)

พ.ญ. เพ็ญสดา ทองประเสริฐ

ขนาดหน้าหนังสือ A5 จำนวน 158 หน้า

ISBN : 978-616-335-706-9

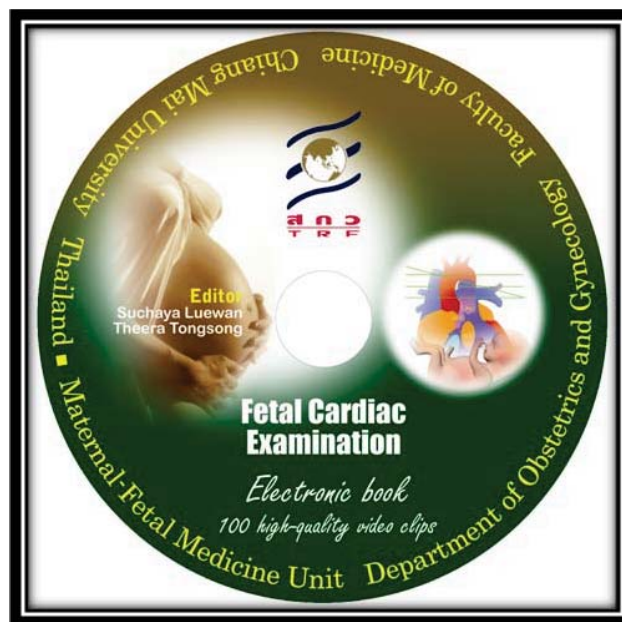
พิมพ์ครั้งที่ 1 จำนวน 1000 เล่ม กรกฎาคม 2556



คำนำ : เทคโนโลยีการตรวจวินิจฉัยก่อนคลอดได้รับการพัฒนาให้มีความก้าวหน้าอย่างมากในปัจจุบัน ทำให้การทำหัตถการการวินิจฉัยก่อนคลอดเพื่อตรวจวินิจฉัยโรคหรือภาวะต่าง ๆ ของทารกในครรภ์เป็นส่วนหนึ่งของการให้บริการการฝากครรภ์ โดยนับเป็นภาระงานหลักที่สำคัญยิ่งของแพทย์ผู้เชี่ยวชาญสาขาเวชศาสตร์มารดาและทารกที่จำเป็นต้องทราบรายละเอียดในทุกแง่มุมของการทำหัตถการโดยเฉพาะอย่างยิ่งเทคนิควิธีการทำหัตถการ และสามารถทำหัตถการสำเร็จได้อย่างปลอดภัยต่อมารดาและทารกในครรภ์ สำหรับสูติแพทย์และแพทย์เวชปฏิบัติทั่วไปควรทราบถึงข้อบ่งชี้ในการทำหัตถการ ภาวะแทรกซ้อน และอัตราการสูญเสียทารกภายหลังการทำหัตถการ เพื่อสามารถให้คำปรึกษาแนะนำและส่งต่อสตรีตั้งครรภ์ที่มีข้อบ่งชี้ในการทำหัตถการมายังสถานพยาบาลที่เปิดให้บริการการทำหัตถการการวินิจฉัยก่อนคลอด

ผู้พิมพ์หวังเป็นอย่างยิ่งว่าหนังสือเล่มนี้จะมีประโยชน์เพื่อใช้ประกอบการเรียนการสอนแก่นักศึกษาแพทย์ แพทย์ประจำบ้านสาขาสตรีศาสตร์และนรีเวชวิทยา และแพทย์ต่อยอดสาขาเวชศาสตร์มารดาและทารกให้มีความรู้ความเชี่ยวชาญเพิ่มมากขึ้น และเพื่อประโยชน์สูงสุดแก่สุขภาพของสตรีตั้งครรภ์และทารกต่อไป

3 Interactive Multimedia E-learning (E-Book): Fetal Cardiac Examination



สื่อมัลติมีเดียการเรียนการสอนเทคนิคในการตรวจหัวใจทารกในครรภ์ บรรจุในแผ่นดีวีดี 1 แผ่นประกอบด้วยเนื้อหา ลักษณะ tip & trick ในการฝึกปฏิบัติ ผ่านวิดีโอคุณภาพสูงของการตรวจหัวใจทารกในครรภ์ทั้งที่ปกติและผิดปกติกว่า 100 วิดีโอคลิป เน้น

- การตรวจหัวใจทารกปกติ ด้วยอัลตราซาวด์สองมิติ
- วิดีโอสาธิตการตรวจหัวใจทารกปกติในเพลนมาตรฐาน four-chamber view, outflow tract, three-vessel view, three-vessel-trachea view เป็นต้น

- ตัวอย่างวิดีโอคลิปของอาการแสดงต่าง ๆ ที่ผิดปกติของหัวใจทารก
- ผู้เรียนสามารถควบคุมวิดีโอคลิป เลือกเฟรมที่เหมาะสมได้ด้วยตนเอง
- ตัวอย่างวิดีโอคลิปของโรคหัวใจพิการโดยกำเนิด
- Tip & Trick ในการตรวจหัวใจทารกให้ได้ผลดี

4. การนำผลงานไปใช้ประโยชน์ในเชิงสาธารณะ

- ประโยชน์ที่เกิดขึ้นแก่การวิจัย :
 - มีผลเพิ่มจำนวนนักวิจัยรุ่นใหม่ที่มีผลงานระดับนานาชาติมากขึ้นเป็นจำนวนมาก ดังตัวอย่างที่เห็นได้ว่านักวิจัยในกลุ่มมีผลงานวิจัยเพิ่มขึ้นอย่างมาก
- ผลประโยชน์ในเชิงสาธารณะ
 - งานวิจัยด้านการวินิจฉัยก่อนคลอดเกี่ยวกับโรคหัวใจและโรคธาลัสซีเมียชนิดรุนแรง (ด้วยความร่วมมือของทีมงานเวชศาสตร์มารดาและทารก และห้องปฏิบัติการกุมารเวชศาสตร์) มีผลทำให้อุบัติการณ์ของการวินิจฉัยก่อนคลอดสูงขึ้นเป็นสองเท่า (ในช่วงที่ทำการศึกษา) ซึ่งคาดหวังว่าจะมีผลในวงกว้างต่อไปอย่างยั่งยืน นอกจากนี้โรคธาลัสซีเมียรายใหม่ในภูมิภาคแถบนี้ลดลงอย่างมากในปัจจุบัน จากการศึกษาเทคนิคในการคัดกรอง การทำการวินิจฉัยก่อนคลอด (โดยเฉพาะวิธีการเจาะเลือดสายสะดือทารก) โดยเฉพาะอย่างยิ่งปัญหาโรคธาลัสซีเมียชนิดรุนแรง ซึ่งมีอุบัติการณ์ที่ลดลงอย่างเป็นรูปธรรม
 - งานวิจัยด้านคลื่นเสียงความถี่สูงของทารกในครรภ์ ยังผลให้ความพิการโดยกำเนิดของทารกในครรภ์ โดยเฉพาะด้านหัวใจทารก และโรคฮีโมโกลบินบาร์ท ส่วนใหญ่ได้รับการวินิจฉัยและได้รับการดูแลเหมาะสมตั้งแต่อยู่ในครรภ์ และงานวิจัยจำนวนมากได้กลายเป็นพื้นฐานสำคัญของการพัฒนาสาขาวิชานี้ในประเทศไทย โดยเฉพาะอย่างยิ่งการศึกษาค้นคว้าเกี่ยวกับ โรคหัวใจพิการโดยกำเนิด (ซึ่งมีความผิดปกติของทารกในครรภ์ที่พบบ่อยมากที่สุด
- ผลประโยชน์ในเชิงนโยบาย
 - จากผลงานวิจัยยุทธวิธีในการควบคุมโรคธาลัสซีเมีย (Prenatal control of severe thalassemia: prenatal control) และวิจัยอื่น ๆ ที่เกี่ยวข้อง ได้กลายเป็นต้นแบบ หรือแหล่งอ้างอิงอย่างมีหลักฐานทางวิทยาศาสตร์ สำหรับกระทรวงสาธารณสุขในการนำไปประยุกต์ใช้ระดับประเทศ และเป็นที่ยอ้างอิงในการพัฒนานโยบายในการควบคุมโรคนี้ในทุกระดับ คาดว่าผลงานวิจัยเชิงคลินิกของทีมงานได้มีผลต่อระดับชาติในเชิงนโยบาย

5. รางวัลที่ได้รับระหว่างรับทุนส่งเสริมกลุ่มวิจัย

5.1 รางวัลระดับชาติ เช่น

- ชื่อนักวิจัย: (ทั้งกลุ่มวิจัย) รางวัลโครงการวิจัยเด่น สกว. ประจำปี 2552 จากโครงการ “ยุทธวิธีการควบคุมโรคธาลัสซีเมียชนิดรุนแรงก่อนคลอด”
- ชื่อนักวิจัย: ศ. นพ. วีระ ทองสง
 - รางวัลอาจารย์และนักวิจัยยอดเยี่ยมมหาวิทยาลัยเชียงใหม่ ประจำปี 2555 มหาวิทยาลัยเชียงใหม่ (รางวัลมหาวิทยาลัยเชียงใหม่ รางวัลข้างทองคำ)
 - เมธีวิจัยอาวุโส สกว. ปี 2553-2556
- ชื่อนักวิจัย: ร.ศ. นพ. ชเนนทร์ วนาภิรักษ์
 - โล่รางวัล นักวิจัยดีเด่นประจำปี 2552 มหาวิทยาลัยเชียงใหม่ (รางวัลมหาวิทยาลัยเชียงใหม่ รางวัลข้างทองคำ: เป็นรางวัลใหญ่ที่สุดของมหาวิทยาลัยเชียงใหม่สำหรับนักวิจัย ซึ่งมีปีละ 1 รางวัล)
- ชื่อนักวิจัย: ผ.ศ. พญ. เพ็ญลดา ทองประเสริฐ
 - รางวัลอาจารย์ดีเด่นประเภทอาจารย์รุ่นใหม่ดีเด่น (สอว.ประเทศไทย) สาขาวิทยาศาสตร์สุขภาพ จากสมาคมสถาบันการศึกษาระดับอุดมแห่งภูมิภาคเอเชียตะวันออกเฉียงใต้ ประจำปีประเทศไทย
 - โล่รางวัล นักวิจัยดีเด่นรุ่นใหม่ ประจำปี 2555 มหาวิทยาลัยเชียงใหม่ (รางวัลมหาวิทยาลัยเชียงใหม่ รางวัลข้างทองคำ)
- ชื่อนักวิจัย: อาจารย์ พญ. เกษมศรี ศรีสุพรรณดิฐ
 - โล่รางวัล นักวิจัยดีเด่นรุ่นใหม่ ประจำปี 2556 มหาวิทยาลัยเชียงใหม่ (รางวัลมหาวิทยาลัยเชียงใหม่ รางวัลข้างทองคำ)
- ชื่อนักวิจัย: ร.ศ. พญ. สุพัตรา ศิริโชติยะกุล
 - รางวัลผลงานวิจัยดีเด่น สาขาเวชศาสตร์มารดาและทารก ของราชวิทยาลัยสูติ-นรีแพทย์แห่งประเทศไทย ประจำปี 2556 เรื่อง “High resolution DNA melting analysis: an application for prenatal control of alpha-thalassemia”
 - รางวัลผลงานวิชาการดีเด่น เนื่องในการประชุมสัมมนาวิชาการธาลัสซีเมียแห่งชาติ ครั้งที่ 15 วันที่ 22-24 เมษายน พ.ศ. 2552

5.2 รางวัลระดับนานาชาติ เช่น

- ชื่อนักวิจัย: อาจารย์ พญ. สุชยา ลือวรรณ
 - The Best Oral presentation Awards ในการประชุมวิชาการ 22nd World Congress on Ultrasound in OB-GYN In area : Fetal heart โดยการนำเสนอเรื่อง Fetal myocardial performance (Tei) index in fetal hemoglobin Bart's disease (OC08.04) (S. Luewan, F. Tongprasert, K. Srisupundit, T. Tongsong) 9–13 September 2012, Copenhagen, Denmark

จึงเรียนมาเพื่อโปรดพิจารณา

ขอแสดงความนับถือ



ศาสตราจารย์นายแพทย์ธีระ ทองสง

ภาควิชาสูติศาสตร์และนรีเวชวิทยา

คณะแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่

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- 1) Tongsong T, Wanapirak C, Piyamongkol W, Sirichotiyakul S, Tongprasert F, Srisupundit K, et al. Fetal ventricular shortening fraction in hydrops fetalis. *Obstet Gynecol* 2011 Jan;117(1):84-91. (Impact factor = **4.798**)
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- 3) Tongprasert F, Srisupundit K, Luewan S, Wanapirak C, Tongsong T. Normal reference ranges of ductus venosus Doppler indices in the period from 14 to 40 weeks' gestation. *Gynecol Obstet Invest* 2012;73(1):32-7. (Impact factor = 1.103)
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- 15) Srisupundit K, Wanapirak C, Sirichotiyakul S, Tongprasert F, Luewan S, Traisrisilp K, et al. Hemoglobin levels and red blood cell indices in mid-gestational fetuses with beta-thalassemia/HbE, beta-thalassemia trait or Hb E trait and normal fetuses. *Prenat Diagn* 2013 Sep 13;1-4. (Impact factor 2.683)
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Fetal Ventricular Shortening Fraction in Hydrops Fetalis

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OBJECTIVE: To estimate fetal ventricular shortening fraction, representing cardiac contractility, derived from cardiospatiotemporal image correlation with M-mode display “STIC-M” in fetuses with hydrops fetalis secondary to high-output (fetal anemia) and low-output causes (congenital heart defects).

METHODS: A cross-sectional study was conducted in normal fetuses (group 1), fetuses with hemoglobin Bart’s disease with (group 2) and without (group 3) hydrops fetalis, and those with hydrops fetalis resulting from cardiac defects (group 4). Volume data sets of cardiospatiotemporal image correlations were acquired for each group for subsequent offline analysis with cardiospatiotemporal image correlation with M-mode display. Group 1 data were used to construct reference ranges of left and right ventricular shortening fraction for assessment of fetuses in the remaining groups.

RESULTS: A total of 606 measurements, 15–35 per week, were performed in normal fetuses to construct reference ranges as well as Z-scores of left and right ventricular shortening fraction. Both parameters were decreased with increasing gestation with weak correlation ($r^2=0.141$, $P<.001$ and $r^2=0.055$, $P<.001$, respectively). Shortening fraction did not significantly change among 111 fetuses with hemoglobin Bart’s disease with and without hydrops. However, left and right ventricular shortening fraction were significantly decreased (mean Z-scores 5 standard deviations and 8 standard deviations

below the mean, respectively) in 21 hydropic fetuses as a result of congenital heart defects ($P<.001$).

CONCLUSION: Fetuses with hydrops fetalis secondary to cardiac defects and anemia have a different pattern of shortening fraction. Hydrops fetalis resulting from cardiac defect is primarily caused by cardiac decompensation; whereas in fetal anemia, it is probably caused by hypervolemia with cardiac decompensation occurring when the cardiac compensatory mechanism is exhausted.

(*Obstet Gynecol* 2011;117:84–91)

DOI: 10.1097/AOG.0b013e3181fc3887

LEVEL OF EVIDENCE: II

Fetal cardiac contractility can be objectively assessed by measurement of the cardiac output using spectral Doppler analysis and shortening fraction of ventricles. Each of these techniques has its own limitations. Measurement of cardiac output by spectral Doppler is rarely used in clinical practice because it is technically difficult, time-consuming, and needs high expertise. Assessment of shortening fraction of ventricles is much simpler either using two-dimensional ultrasonography or M-mode for dimension shortening. Many authors^{1–7} propose an assessment of shortening fraction of ventricular dimension using M-mode (transverse diameter at end-diastole—diameter at end-systole/diameter in end-diastole) and construct nomograms for ventricular shortening fraction. However, measurement using M-mode is associated with some unique technical difficulties in exact placement of the M-mode cursor perpendicular to the interventricular septum and variation of ventricular diameters in different views through the ventricles. The difficulty in obtaining a proper plane before M-mode display and measurement may explain the different normative values among various studies.^{1–7} For example, the left ventricular shortening fraction is

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Supported by the Thailand Research Fund (TRF) as a part of TRF Senior Research Scholar.

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Financial Disclosure

The authors did not report any potential conflicts of interest.

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ISSN: 0029-7844/10



0.26, 0.33, and 0.48 during similar gestational age in the studies reported by Koyanagi et al,⁴ De Vore et al,² and Hsieh et al,³ respectively. In addition, some studies found no change of ventricular shortening fraction throughout pregnancy, whereas some showed a decrease³ or an increase⁸ with gestational age. Moreover, the nomograms of such studies were based on a small sample size, including only 13 to 104 fetuses. It seems that such difference is likely the result of nonperfect planes of measurements because it is difficult to get proper planes with two-dimensional ultrasonography attributable to the fact that the exact reliable plane needs to be controlled in all three dimensions of the heart. It is therefore very difficult to be sure that the interventricular septum is exactly horizontal in all axes. To be more accurate, therefore, we created our own reference ranges for shortening fraction using innovative cardiospatiotemporal image correlation with M-mode technology with a large sample size to use as a standard in evaluating shortening fraction of fetuses with hydrops fetalis secondary to fetal anemia and congenital heart defects or congestive heart failure.⁹ Shortening fraction may be a useful tool in differentiating causes of hydrops fetalis. The purpose of this study was to estimate fetal ventricular shortening fraction representing cardiac contractility derived from cardiospatiotemporal image correlation with M-mode in fetuses with hydrops fetalis secondary to high-output (fetal anemia) and low-output causes (congenital heart defect).

MATERIALS AND METHODS

This cross-sectional study was conducted in the Department of Obstetrics and Gynecology, Faculty of Medicine, Chiang Mai University, Thailand, between September 1, 2007, and March 31, 2010. This research was approved by the research ethics committee of our institute. The study consisted of two parts simultaneously performed. Part I was a construction of normal reference ranges of shortening fraction as a standard tool for subsequent assessment of shortening fraction in pathologic fetuses in part II. In part I, normal pregnant women attending the antenatal care clinic were invited to the study only once for each pregnancy. The inclusion criteria were: 1) gestational age between 14 and 40 weeks based on regular menstruation with accurate date of the last menstrual period and ultrasonography in the first half of pregnancies; and 2) low-risk pregnancies without known obstetric and medical complications. Exclusion criteria were: 1) multifetal pregnancy; 2) fetal anomalies or chromosome abnormalities; 3) abnormal intrauterine

growth (estimated fetal weight of less than 10th percentile or more than 90th percentile); and 4) cardiospatiotemporal image correlation with M-mode display volume data sets could not be satisfactorily obtained.

In part II, all fetuses with hemoglobin Bart's disease, usually diagnosed at 18–22 weeks by fetal hemoglobin typing either with or without hydropic changes and hydropic fetuses caused by cardiac defects were recruited. The prenatal diagnosis of hydrops fetalis was defined by demonstration of fluid collections in at least two fetal body compartments such as ascites, pleural effusion, pericardial effusion, or skin edema. These fetuses underwent detailed ultrasonography to identify associated abnormalities. Hydrops fetalis secondary to any causes other than hemoglobin Bart's disease and congenital heart defects were excluded. Unsatisfactory or poor-quality cardiospatiotemporal image correlation with M-mode display volume data sets were also excluded.

All ultrasonographic examinations were performed by the four authors, who are experts in fetal echocardiography. The examination technique was standardized through hands-on training and a conference before initiation of the study.

All fetuses underwent ultrasonographic examination, including fetal biometry and anatomic survey and cardiospatiotemporal image correlation using real-time machines Voluson E8 equipped with three-dimensional transabdominal, 2- to 4-MHz curvilinear transducers. The acquired volume data sets were stored for subsequent offline analysis with cardio-spatio-temporal image correlation with M-mode display (4D Views 9). To measure ventricular shortening fraction, the cardio-spatio-temporal image correlation with M-mode display examination was performed as follows: 1) volume acquisition: volume data sets were obtained as a standard technique acquired with the cardiac axis oriented horizontally, ultrasonographic beam perpendicular to the interventricular septum. The virtual cardiac volumes were stored in a hard disk for subsequent offline analysis; 2) offline volume data set processing: the stored volume data sets were adjusted for brightness, contrast, and speed to optimize the images for proper analysis. On multiplanar view, the best image of the four-chamber view was identified and displayed on panel A and then was maneuvered by moving the reference dot and rotating along the three orthogonal axes (x, y, and z) to get the interventricular septum to be in exactly the horizontal plane in the three panels. The proper multiplanar views for analysis are as



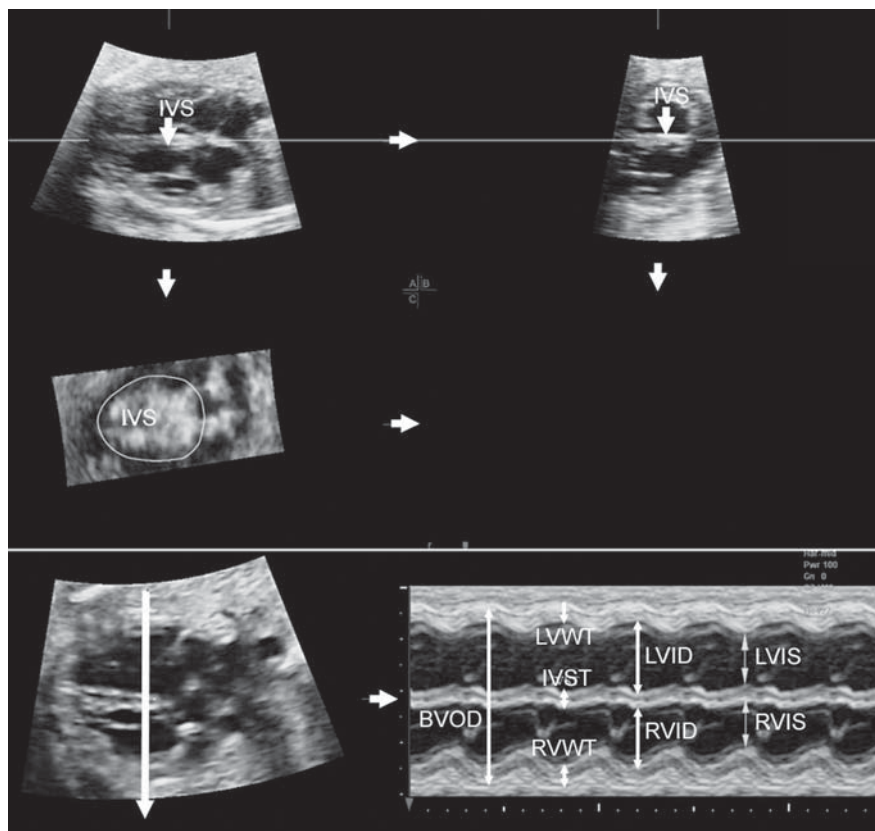


Fig. 1. Orientation of the four-chamber views to get the interventricular septum (IVS) to be exactly horizontal on the multiplanar view of a cardiospatiotemporal image correlation volume data set (*upper panel*) and cardiospatiotemporal image correlation with M-mode display with measurements of the cardiac dimension at end-diastole and end-systole on M-mode (*lower panel*). BVOD, biventricular outer dimension; LVWT, left ventricular wall thickness; IVST, interventricular septum thickness; RVWT, right ventricular wall thickness; LVID, left ventricular inner dimension; RVID, right ventricular inner dimension; LVIS, left ventricular inner diameter at end-systole; RVIS, right ventricular inner diameter at end-systole.

Tongsong. Shortening Fraction in Hydrops Fetalis. Obstet Gynecol 2011.

follows: the interventricular septum in panel A must be in the same line with that in panel B (exactly horizontal) and total en face view of interventricular septum was displayed in panel C (Fig. 1, upper panel); 3) cardiospatiotemporal image correlation with M-mode display: when panel A was selected, the cardiospatiotemporal image correlation with M-mode line was placed perpendicular to the interventricular septum at the level of maximal ventricular dimension, usually just below level of the atrioventricular valves and the cardio-spatiotemporal image correlation with M-mode tracing was displayed (Fig. 1, lower panel); and 4) ventricular shortening fraction measurement: on cardiospatiotemporal image correlation with M-mode panels, the measurements were made at the end-diastole and the end-systole by placing the M-mode cursor at the level of the greatest dimension. The dimensions were measured included: left ventricular internal dimension, which was measured from the endocardium of the left ventricular wall to the endocardium of the left side of the interventricular septum; and right ventricular internal dimension, which was measured from the endocardium of the right ventricular wall to the endocardium of the

right side of the interventricular septum. These parameters were measured in the same tracings at both end-diastole and end-systole. The best measurements were selected and recorded for analysis. Left ventricle shortening fraction and right ventricle shortening fraction were calculated using equation: $\text{shortening fraction} = \frac{\text{dimension at end-diastole} - \text{dimension at end-systole}}{\text{dimension at end-diastole}}$.

In part I, regression analysis of right shortening fraction and left shortening fraction was performed to determine the best-fit equations as follows: gestational week, as an independent variable, and ventricular shortening fraction as dependent variables were used to generate the best-fit regression equations. Various built-in models, including linear, quadratic, cubic, exponential, and logarithmic models, were tested and compared. The simpler model was chosen if the more complex model did not result in significant improvement. The residuals were tested for normality of distribution using Kolmogorov-Smirnov and Shapiro-Wilk tests. If the distribution was not normal, the data were transformed and the new residuals were checked to determine whether they conformed to a normal distribution. Data were also examined to determine



whether the standard deviation of the residuals varied across the range of values for the independent variable using Levine's test. If a significant degree of heteroscedasticity was found, weighted regression of absolute residuals was used to adjust the standard deviation. To construct normal reference range including +1, 2, and 3 standard deviation curve with restricting the standard error of the limits of reference range to 10% of the standard deviation, a sample size of approximately 550 fetuses (14–40 weeks)¹⁰ was needed. The results in part II were related to the reference range data of left ventricle shortening fraction and right ventricle shortening fraction. The data were also compared between the group of hydrops fetalis resulting from hemoglobin Bart's disease and the group of that caused by congenital heart defects. Statistical analysis was performed using SPSS 17 for Windows and GraphPad Prism 5. Analysis of variance and Kruskal-Wallis nonparametric test were used to compare the mean and median of shortening fraction as well as Z-score as appropriate. Dunn's test was used for nonparametric multiple pairwise comparisons. *P* value of <.05 was considered statistically significant.

RESULTS

In part I, in construction of normal reference ranges of shortening fraction, a total of 606 measurements from 606 fetuses (group 1) were performed for left ventricle shortening fraction and right ventricle shortening fraction (Table 1). The mean number of measurements per week was 22.4 ranging from 15 to 35 measurements. The mean left ventricle shortening fraction and right ventricle shortening fraction was 0.32 ± 0.52 and 0.31 ± 0.49 , respectively. Both parameters slightly but significantly decreased with increasing gestational age. The weighted regression analysis, using weighted least square technique, reveals a fitted linear equation as follows:

Predicted mean left ventricle shortening fraction = $0.397 - 0.003\text{GA}(\text{week})$ ($r^2 = 0.141$, $P < .001$) and

Predicted mean right ventricle shortening fraction = $0.355 - 0.002\text{GA}(\text{week})$ ($r^2 = 0.055$, $P < .001$).

Predicted standard deviation (SD) was constant throughout gestation; predicted SD for left ventricle shortening fraction and right ventricle shortening fraction were 0.01989 and 0.011444, respectively.

Therefore, Z-scores for left ventricle shortening fraction or right ventricle shortening fraction for each measurement can be simply calculated using the equation: $Z \text{ score} = (\text{measurement} - \text{predicted mean}) / \text{predicted SD}$.

In part II, 63 fetuses with hemoglobin Bart's disease without hydrops fetalis (group 2), 48 fetuses with hemo-

Table 1. Distribution of Measurements Within Each Week in Normal Fetuses (Group 1)

Week of Gestation	Frequency	Percent	Valid Percent	Cumulative Percent
14	15	2.5	2.5	2.5
15	15	2.5	2.5	5.0
16	17	2.8	2.8	7.8
17	18	3.0	3.0	10.7
18	19	3.1	3.1	13.9
19	18	3.0	3.0	16.8
20	16	2.6	2.6	19.5
21	22	3.6	3.6	23.1
22	26	4.3	4.3	27.4
23	27	4.5	4.5	31.8
24	23	3.8	3.8	35.6
25	23	3.8	3.8	39.4
26	25	4.1	4.1	43.6
27	30	5.0	5.0	48.5
28	26	4.3	4.3	52.8
29	23	3.8	3.8	56.6
30	29	4.8	4.8	61.4
31	35	5.8	5.8	67.2
32	26	4.3	4.3	71.5
33	17	2.8	2.8	74.3
34	22	3.6	3.6	77.9
35	28	4.6	4.6	82.5
36	24	4.0	4.0	86.5
37	22	3.6	3.6	90.1
38	24	4.0	4.0	94.1
39	19	3.1	3.1	97.2
40	17	2.8	2.8	100.0
Total	606	100.0	100.0	

globin Bart's disease with hydrops fetalis (group 3; high-output hydrops fetalis), and 21 hydropic fetuses with congenital heart diseases (group 4; low-output hydrops fetalis) were successfully recorded for cardio-spatiotemporal image correlation. The groups and the various causes of congenital heart disease in group 4 are presented Table 2. The mean gestational ages for groups 2, 3, and 4 were 20.3 ± 5.1 weeks, 24.7 ± 5.3 weeks, and 25.7 ± 3.7 weeks, respectively.

All fetuses with hemoglobin Bart's disease either with or without hydrops fetalis had cardiomegaly but nearly all of them had normal shortening fraction. Because shortening fraction values significantly, although slightly, changed with gestational age, to compare left ventricle shortening fraction and right ventricle shortening fraction among the four groups, statistical control of gestational age was performed. Among the three groups (2, 3, and 4), analysis of covariance tests showed no significant effect of gestational age on left shortening fraction and right shortening fraction ($P > .05$). Post hoc test revealed that group 2 and group 3 were not significantly different ($P > .05$). On the contrary, left ventricle shortening



Table 2. Distribution of the Causes of Hydrops Fetalis

Causes	Number	Nulliparity*	Maternal Age (y) [†]	Gestational Age (wk) [‡]
Hemoglobin Bart's disease without hydrops fetalis	63	39	25.73±5.90	19.94±1.36
Hemoglobin Bart's disease with hydrops fetalis	48	16	24.38±5.13	24.93±5.44
Low-output hydrops fetalis	21	16	23.74±6.93	22.10±3.16
Ebstein's anomaly	5			
Hypoplasia left heart	4			
Atrial flutter or supraventricular tachycardia	3			
Heterotaxy	3			
Small heart syndrome	3			
Pulmonary stenosis	1			
Congenital heart block	1			
Tricuspid atresia	1			

Data are mean±standard deviation unless otherwise specified.

* χ^2 , $P>.05$.

[†] Analysis of variance test, $P>.05$.

[‡] Analysis of variance, $P<.05$.

fraction and right ventricle shortening fraction in hydropic fetuses with congenital heart defects were markedly decreased when compared with all other groups ($P<.001$) (Table 3; Fig. 2).

Likewise, Z-scores tended to be decreased in group 2 and in group 3 and even much more decreased in group 4, when compared with normal fetuses as shown in Table 4. Left ventricle shortening fraction and right ventricle shortening fraction of fetuses with hemoglobin Bart's disease were approximately one SD below the mean, whereas those of fetuses with hydrops fetalis secondary to congenital

heart defects were 5 SDs and 8 SDs below the mean, respectively. Medians and range of Z-scores for left shortening fraction and right shortening fraction were -0.4703 (-9.28 to 6.41) and -0.4817 (-16.22 to 11.72), respectively. Kruskal-Wallis test showed statistical significance between groups ($P<.001$). Dunn's posttests comparing the differences between each pair group showed significant differences between group 4 compared with group 1 ($P<.001$), group 4 compared with group 2 ($P<.001$), and group 4 compared with group 3 ($P<.001$), whereas there was no significance between the others ($P>.05$).

Table 3. A Comparison of Mean Difference of Left and Right Shortening Fraction After Control for Gestational Age Among Fetuses in the Four Groups

Main Groups	Compared Groups	Mean Difference LVSF (95% CI)	P	Mean Difference RVSF (95% CI)	P
Normal fetuses	Hemoglobin Bart's without hydrops	-0.003 (-0.015 to 0.009)	.644	-0.003 (-0.015 to 0.010)	.683
	Hemoglobin Bart's with hydrops	0.014 (-0.000 to 0.028)	.055	0.008 (-0.006 to 0.022)	.263
Hemoglobin Bart's without hydrops	Congenital heart disease	0.091 (0.070 to 0.111)	.000	0.084 (0.063 to 0.104)	.000
	Normal fetuses	0.003 (-0.009 to 0.111)	.644	0.003 (-0.010 to 0.015)	.683
	Hemoglobin Bart's with hydrops	0.017 (-0.001 to 0.035)	.068	0.010 (0 to 0.028)	.247
Hemoglobin Bart's with hydrops	Congenital heart disease	0.093 (-0.070 to 0.117)	.000	0.086 (0.063 to 0.109)	.000
	Normal fetuses	-0.014 (-0.028 to 0.000)	.055	-0.008 (-0.022 to 0.006)	.263
	Hemoglobin Bart's without hydrops	-0.017 (-0.035 to 0.001)	.068	-0.010 (-0.028 to 0.007)	.247
Congenital heart disease	Congenital heart disease	0.077 (-0.052 to 0.101)	.000	0.076 (0.052 to 0.100)	.000
	Normal fetuses	-0.091 (-0.111 to -0.070)	.000	-0.084 (-0.104 to -0.063)	.000
	Hemoglobin Bart's without hydrops	-0.093 (-0.117 to -0.070)	.000	-0.086 (-0.109 to -0.063)	.000
	Hemoglobin Bart's with hydrops	-0.077 (-0.101 to 0.052)	.000	-0.076 (0.100 to -0.052)	.000

LVSF, left ventricle shortening fraction; CI, confidence interval; RVSF, right ventricle shortening fraction.

Based on observed means; the mean difference is significant at the .05 level (Dunn's test).



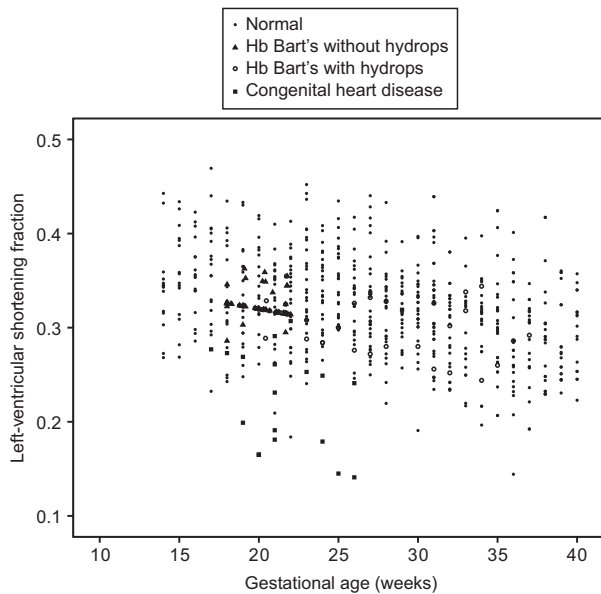


Fig. 2. The scattergrams of left ventricular shortening fraction for each gestational week among normal fetuses, fetuses with hemoglobin (Hb) Bart's disease with and without hydrops, and fetuses with congenital heart disease.

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DISCUSSION

Assessment of fetal cardiac contractility with M-mode was introduced by Wladimiroff et al⁶ as a simple objective quantification obtained by measuring the shortening fraction. However, standardization of measurement of the end-diastolic and the end-systolic diameter is difficult as a result of problems with two-dimensional M-mode alignment. As a result, in-

Table 4. A Comparison of Mean Z-Scores of Left and Right Shortening Fraction (as a Function of Gestational Age) Between Normal Fetuses and Hydropic Fetuses

Groups	No.	Mean Z-Score LVSF*	Mean Z-Score RVSF*
Normal fetuses	606	0.00	0.00
Fetuses with hemoglobin Bart's disease without hydrops fetal	63	-0.91	-0.84
Fetuses with hemoglobin Bart's disease with hydrops fetal	48	-1.06	-1.06
Fetuses with congenital heart disease	21	-5.32	-8.08

LVSF, left ventricle shortening fraction; RVSF, right ventricle shortening fraction.

* Analysis of variance test: statistical significance between groups ($P < 0.001$).

terobserver limits of agreement range approximately $\pm 15\%$.^{11,12} Nevertheless, the shortening fraction remains a widely used parameter of cardiac contractility. This may be helpful in evaluating contractility in abnormalities that may affect wall motion such as cardiomyopathies or congestive heart failure. With innovative three-dimensional cardiospatiotemporal image correlation M-mode, the problems of alignment and improper measurement have been resolved. This technique is simple, less time-consuming, and highly reliable in obtaining the proper orientation. Unlike measurement with two-dimensional ultrasonography in which we have to orientate a transducer to find the proper plane, with three-dimensional spatiotemporal image correlation offline analysis we can control and orientate the virtual heart to get the proper plane, not orientate the transducer. The interventricular septum can simply be maneuvered in the exact horizontal line in panels A and B and total en face view of the interventricular septum can be displayed in panel C, resulting in more reliable measurements.

Several fetal conditions such as congenital heart defects, anemia, or infection may lead to myocardial dysfunction and eventually cardiac decompensation resulting in hydrops fetalis. Therefore, evaluation of shortening fraction may be helpful in determining the degree of cardiac decompensation or differentiating causes of fetal hydrops. The extent of shortening fraction changes with gestational age may be small but significant. Therefore, to compare shortening fraction among fetuses with cardiac pathology, gestational age must be taken into account. Accordingly, we controlled for gestational age and used Z-scores to compare shortening fraction among fetuses at different gestational ages. Our results showed the different patterns of shortening fraction compromise between the group of hydrops fetalis associated with low cardiac output (cardiac defects) and the group with high cardiac output (fetal anemia). Low-output hydropic fetuses demonstrated impairment in contractility, whereas high-output hydropic fetuses had very minimally impaired cardiac contractility despite hyperdynamic circulation or hypervolemia, indicating that hydrops fetalis had developed before cardiac decompensation. This finding is contrary to a long-believed concept that hydrops fetalis secondary to anemia is a consequence of congestive heart failure.

Surprisingly, our study suggests that fetal anemia or hypervolemia may not be associated with significantly low shortening fraction. Most of these fetuses had normal shortening fraction, although there was a trend for low shortening fraction suggesting that hy-



drops fetal_{is} is not primarily caused by cardiac decompensation. Although long-lasting hypervolemia can lead to marked cardiomegaly, and finally to cardiac failure,¹³ cardiomegaly reflects rather high competency of cardiac compensation than cardiac failure. Despite hypervolemia, normal shortening fraction implies effective ventricular contractility or good adaptive response. Hypervolemia may be the result of a low blood viscosity resulting from anemia and effective cardiac contractility. On the contrary, in fetuses with cardiac defects and hydrops fetal_{is}, low shortening fraction indicates cardiac decompensation resulting from the cardiac pathology, leading to hydrops fetal_{is}, whereas hydrops fetal_{is} resulting from anemia is not caused by cardiac decompensation. We postulate that during the early stage of anemia, hydropic change can occur; the fetus effectively compensates by increasing the cardiac dimension and effective contractility to maintain peripheral perfusion. Congestive heart failure does not develop in most fetuses with high-output hydrops fetal_{is} if they are not in the end stage. This study implies that the normal fetal heart is capable of compensation to cope with anemic hypoxia. This is consistent with our observation that several fetuses with hemoglobin Bart's disease can show reactive nonstress test despite marked cardiomegaly without other signs of contractility compromise. Our findings indicate that in fetal anemia, local and systemic compensatory mechanisms are highly effective and may help the fetus to survive. On the other hand, some of these mechanisms at the same time increase the imbalance of Starling forces, resulting in enhanced interstitial fluid accumulation before the compensatory mechanisms become exhausted.

Although anemia in this study was uniformly lethal and the shortening fraction measurements are of no value, it could be a model for nonlethal causes of anemia such as Rh erythroblastosis or parvovirus B19. Although fetuses with hydrops fetal_{is} resulting from anemia may have poor prognoses overall, a better prognosis can still be expected if the shortening fraction is normal. In such cases, shortening fraction assessment may be used to differentiate hydropic fetuses with congestive heart failure (poor contractility) from ones without heart failure. Thus, shortening fraction assessment may be helpful in predicting the prognosis and in making a choice of management. However, this study is preliminary and further study of the clinical application is needed.

The limitation of this study is that resolution of the volume data set in several fetuses may be somewhat lower when compared with conventional two-

dimensional ultrasonographic resolution. Moreover, the problems of unsatisfactory acquisition can be encountered, especially as a result of fetal movement or maternal breathing. It is impossible to obtain good acquisition in many fetuses with very active movement for a long period or in fetuses in poor position. Additionally, even with a high-resolution machine, the small cardiac structures in the early second trimester could not be clearly demonstrated in several cases, leading to exclusion. Finally, because the examiners were aware of the underlying causes of the fetal hydrops, this could possibly lead to a potential source of bias in examination.

The strengths of this study include 1) the data were derived from a reliable technique, three-dimensional cardiospatiotemporal image correlation with M-mode display with offline analysis without time limit, enabling us to obtain the best image for measurement in all high-quality volume data sets; and 2) the large sample size per week of gestation to construct the reference ranges, providing a reliable tool for evaluation of shortening fraction in the pathologic groups.

In conclusion, fetuses with hydrops fetal_{is} secondary to cardiac causes and fetal anemia have a different pattern of shortening fraction. This study provides data that myocardial dysfunction and cardiac decompensation are a primary cause of hydrops fetal_{is} in fetuses with congenital heart defects, whereas hypervolemia may be a primary cause in fetuses with anemia with cardiac failure or decompensation occurring when the cardiac compensatory mechanism is exhausted.

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rev 12/2010



Normal Reference Ranges of Ductus Venosus Doppler Indices in the Period from 14 to 40 Weeks' Gestation

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Key Words

Fetus • Ductus venosus • Doppler ultrasound

Abstract

Objective: To construct reference ranges of ductus venosus (DV) Doppler indices in normal singleton pregnancies. **Methods:** A cross-sectional study was conducted on low-risk singleton pregnancies with accurate gestational age, and normal fetuses were enrolled into the study. Measurements of DV Doppler indices were determined by experienced sonographers. Poor quality images were excluded. **Results:** A total of 640 fetuses underwent DV measurements, ranging from 13 to 30 for each gestational week. The relationship between gestational age and DV Doppler indices was not linear. The best-fit regression equations are as follows: preload index = $(5.061 - 0.4882) \times \exp(-0.211 \times \text{GA}) + 0.4882$; peak velocity index for veins = $(7.498 - 0.5609) \times \exp(-0.2184 \times \text{GA}) + 0.5609$; pulsatility index for veins = $(17.9 - 0.6053) \times \exp(-0.2512 \times \text{GA}) + 0.6053$; systolic/atrial wave ratio = $(300 - 2.124) \times \exp(-0.3649 \times \text{GA}) + 2.124$. Notably, during the period from 14 to 20 weeks' gestation, all of the four indices rapidly decreased and they were relatively stable or slightly decreased after that. The nomogram tables for various percentile ranges were constructed. **Conclusion:** A nomogram

for DV Doppler indices for each gestational week during the period from 14 to 40 weeks' gestation was established. These reference ranges may be a useful non-invasive tool for evaluation of fetal cardiac function.

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Introduction

The ductus venosus (DV) originates from the umbilical vein before it turns to the right. It runs posteriorly and directly enters the inferior vena cava. It is an important fetal vessel that shunts oxygenated blood returning from the umbilical vein to the inferior vena cava and bypassing the liver. The introduction of the new color Doppler ultrasound technique has enabled hemodynamic assessment of the fetal venous system. Doppler flow measurements of DV have an increasingly important role in the evaluation of various fetal circulatory and cardiac conditions, permitting evaluation of cardiac preload. Therefore, DV Doppler indices may be helpful in the diagnosis of hypoxemia associated with intrauterine growth restriction [1, 2], fetal anemia [3, 4] and twin-to-twin transfusion syndrome [5], as well as congestive cardiac failure [6]. Indices commonly used to quantify DV waveforms include the

preload index (PLI), peak velocity index for veins (PVIV), pulsatility index for veins (PIV) and the systolic/atrial wave (S/a) ratio [7]. However, for the evaluation of effectiveness of these Doppler indices in the prediction of fetal cardiac function, it is essential that normal reference ranges are first constructed, and we should also have our own normative data for evaluation of fetuses in our own population. Several data have indicated that a different standard of ultrasound-based fetal growth is needed for different populations [8, 9]. Though normal reference ranges of the DV have been published, most were based on small sample size or late gestation, not including pregnancies in the early second trimester [7, 10–16]. Only a study reported by Bahlmann et al. [17] had adequate sample size and included pregnancies from 14 to 41 weeks of gestation. However, they did not determine the time-averaged maximum velocity (TAm_{ax}) due to lack of the required analysis instrument. This is an important parameter for PIV (S-a/TAm_{ax}) for evaluation of cardiac function. Therefore, we conducted this research to establish reference ranges of DV indices: PLI, PVIV, PIV and the S/a ratio, from 14 to 40 weeks of gestation, based on a large sample size.

Patients and Methods

This is a prospective, cross-sectional study conducted with ethical approval by the Ethic Committee of our institute, between June 1, 2007 and November 30, 2009. Low-risk pregnant women were recruited from our antenatal care clinic, and written informed consent was obtained. Inclusion criteria included: (1) accurate gestational age based on regular menstrual cycle with certain last menstrual period and consistent with sonographic fetal biometry in the first half of pregnancy, (2) gestational age between 14 and 40 weeks, and (3) no obstetric and medical complication. Exclusion criteria were: (1) abnormal fetal growth either fetal growth restriction (birth weight of less than 10th percentile) or macrosomia (birth weight of more than 90th percentile), (2) fetal anomalies, (3) multifetal pregnancy, and (4) satisfactory DV Doppler waveform could not be obtained. DV Doppler spectral waveforms were obtained using real-time equipment with color and pulsed Doppler capabilities, with the transabdominal 2–5-MHz curvilinear transducers Aloka SSD alpha-10 (Aloka, Tokyo, Japan) or Voluson E8 (GE Healthcare, Milwaukee, Wisc., USA). In each examination, a standard ultrasound examination (including fetal biometry and anatomical survey) was performed by three performers. Doppler measurements were obtained at a physiologic fetal heart rate of 120–160 bpm in the absence of maternal and fetal breathing movements. The DV Doppler waveforms were determined as follows: (1) the DV was visualized in a midsagittal scan or in an oblique transverse abdominal plane by color Doppler imaging. The DV was identified by color aliasing at the origin of this vessel. (2) The pulsed Doppler gate was placed in the distal portion of the um-

bilical sinus, preferably in the sagittal anterior or posterior insonation. (3) If such insonations were not possible, then an oblique transverse section through the fetal abdomen provided a good visualization of the DV inlet. (4) The size of the sample volume was adapted to the vessel diameter to reduce interference of velocities of the neighboring vessels. The sample volume was kept wide to ensure the recording of the maximum velocity during the cardiac cycle, usually a 1–2-mm sample gate, and (5) at least three consecutive high-quality waveforms with an insonation angle $\leq 30^\circ$ were used to measure the peak velocity during ventricular systole (S; S-wave) and diastole (D; D-wave), the lowest forward velocity during atrial contraction in late D (a; A-wave, and TAm_{ax}). The collected data were analyzed for four DV Doppler indices including PLI (S-a/S), PVIV (S-a/D), PIV (S-a/TAm_{ax}), and the S/a ratio. The predicted normative data were analyzed and constructed for the median, 5th, and 95th percentiles of DV Doppler indices for each gestational week. The best-fit regression equation was determined using the statistical package for the social sciences (SPSS) version 17.0 (Chicago, Ill., USA).

Results

A total of 694 singleton pregnant women were recruited into the study and 54 were excluded due to inability to obtain proper DV Doppler waveform, fetal structural anomalies, fetal growth abnormalities, abnormal chromosomes and unavailability of neonatal outcomes. Of the remaining 640 measurements, the average number of measurements per week was 22 ± 4.1 (mean $\pm$ SD), ranging from 13 to 30 per week. Mean maternal age was 27.8 ± 5.3 years (15–43). Median gestational age at delivery and birth weight were 38.0 weeks (95% CI: 35–40) and 3,005 g (95% CI: 2,250–3,649), respectively. Two hundred and ninety-seven women (46.4%) were nulliparous. The best regression model between gestational week and DV Doppler indices was observed to be non-linear. The best-fit equation functions for each index are as follows:

$$\begin{aligned} \text{PLI} &= 4.5728 \times \exp(-0.211 \times \text{GA}) + 0.4882 \\ \text{PVIV} &= 6.9371 \times \exp(-0.2184 \times \text{GA}) + 0.5609 \\ \text{PIV} &= 17.2947 \times \exp(-0.2512 \times \text{GA}) + 0.6053 \\ \text{S/a} &= 297.876 \times \exp(-0.3649 \times \text{GA}) + 2.124 \end{aligned}$$

Notably, during the period from 14 to 20 weeks' gestation, all of the four indices rapidly decreased and were relatively stable or slightly decreased after that as shown in figures 1–2. The table of reference ranges at various percentiles was constructed as shown in table 1.

Of 640 measurements, 60 fetuses (9.4%) showed no definite D peak during passive diastolic filling and 34 (5.3%) had no well-defined nadir. Of note, the TAm_{ax} during the entire cardiac cycle is 0.71 of the peak systolic velocity. The inter- and intraobserver mean differ-

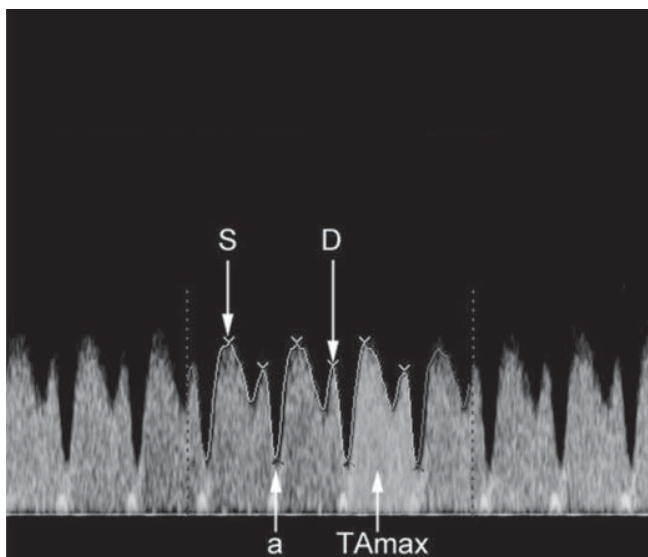


Fig. 1. DV Doppler spectral waveform consists of S, D, and a wave at 16 weeks of gestation. The S wave represents ventricular S, the D wave the early filling of ventricular D, and the a wave atrial contraction. The peak velocity for each waveform is indicated by arrows. TAmx is the area under the waveform (blue overlay).

ences for the measurements of all Doppler indices were not statistically different ($p > 0.05$), as presented in table 2.

Discussion

The DV with a sphincter-like action appears as a narrow vessel projecting a high-velocity jet posteriorly to reach the foramen ovale. The high peak velocity in the DV, comparable with arterial velocities, probably gives the blood sufficient momentum to reach the foramen ovale without extensive mixing with deoxygenated blood [18, 19]. Velocimetry of the DV carries new diagnostic possibilities to evaluate preload or cardiac function. The DV velocity pattern reflects the cardiac cycle with a peak during S, another during passive diastolic filling and a nadir during atrial contraction. Though our reference values and patterns may somewhat differ from those in previous reports [7, 10–16], they have a similar tendency through gestation. Some variation in reference ranges is seen, probably depending on equipment, insonation techniques, angle correction, and racial factors. These findings suggest that each population group may probably need its own normal reference range for clinical ap-

plication. We found a rather rapid decline in resistance in DV during the period from 10 to 20 weeks' gestation and a minimal decrease after that. Furthermore, we also observed that the nadir during atrial contraction in the first half of pregnancy was commonly close to zero, whereas none is close to zero in the second half of pregnancy in normal fetuses. Moreover, we noted that TAmx during the entire cardiac cycle is typically 0.7–0.8 of the maximum velocity in normal fetuses.

Some authors [10, 13] have measured blood flow as absolute velocity recordings. This seems to be more attractive, but it takes the extra challenge to record absolute velocities at a zero or low angle of insonation, a less important detail when using the waveform qualitative analysis. Although this may have research implications, it may not be practical for the clinician. Therefore, we have focused on qualitative assessment of the DV Doppler waveforms.

Our study included pregnancy in the early second trimester, which was not included in most previous studies. Our findings indicate that predicted DV indices using the linear equation reported in most studies are likely to be unreliable since the pattern of DV indices in the first half of pregnancy is apparently different from that in the second half. We found that during the period from 4 to 20 weeks' gestation, the four DV indices decreased relatively rapidly, whereas the DV indices were relatively constant or slightly decreased during the second half of pregnancy. Therefore, we could not reliably evaluate DV velocity in the first half of pregnancy using the linear equation from those reports, such as the nomogram constructed by Baschat [7]. However, the patterns of DV indices in this study are similar to those reported by Bahlmann et al. [17], though the equation is different.

Our experience shows that measurement of DV Doppler indices can be achieved in most women both in early and late pregnancy, and reproducibility is well acceptable as indicated by inter- and intraobserver variations. This is consistent with those reported in previous studies [17]. We have found higher variations in measurement in early gestation compared to late pregnancy; this is consistent with what was observed by Mavrides et al. [20] and Prefumo et al. [21], who showed that DV Doppler assessment during early pregnancy seems to have a reduced reproducibility.

Based on our experience, some observations in measurements of DV Doppler should be mentioned. We observed that about 10% of fetuses had no definite D peak during passive diastolic filling and about 5% had no well-defined nadir. This may reflect a wide range of geometri-

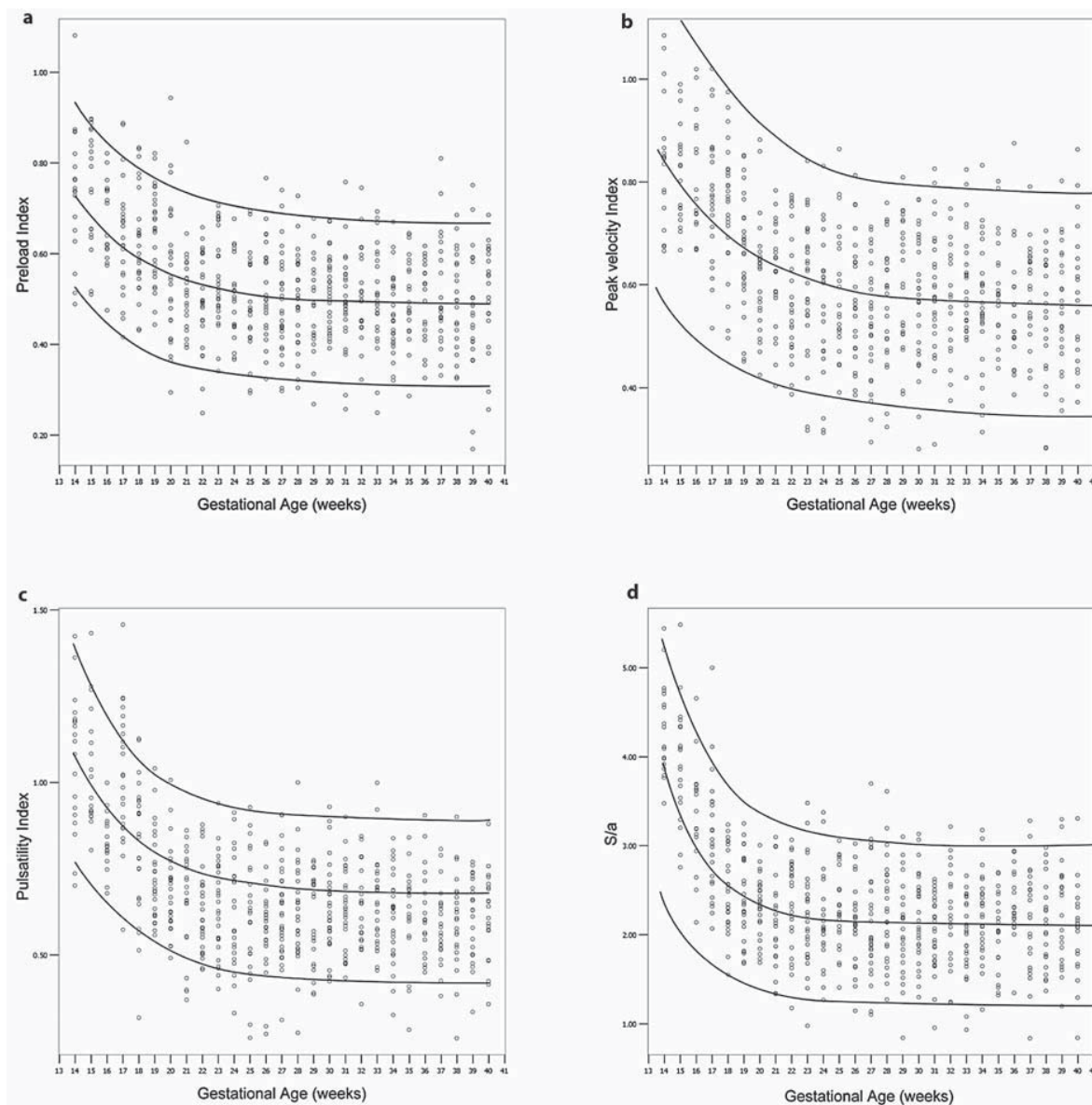


Fig. 2. Scattergrams of DV Doppler indices with predicted values at the 5th, 50th, and 95th percentiles for PLI (a), PVIV (b), PIV (c), and the S/a ratio (d).

cal normal variation. Squeezing of the DV outlet or the inferior vena cava at the level of the diaphragm will cause an increased wave reflection with less or no pulse wave transmitted into the DV resulting in non-pulsatile velocity recording at the isthmus [19].

The strengths of this study are: (1) the adequate sample size for each gestational week, and (2) that we also included fetuses in the early second trimester [14–20], unlike most other studies. Moreover, interobserver variations of the measurement are acceptable.

Table 1. Predicted DV Doppler indices (PLI, PVIV, PIV, and S/a ratio) for each gestational week with predicted values at the 5th, 50th, and 95th percentiles

Week	PLI			PVIV			PIV			S/a ratio		
	5th	50th	95th	5th	50th	95th	5th	50th	95th	5th	50th	95th
14	0.527	0.727	0.927	0.607	0.887	1.167	0.819	1.119	1.419	2.725	3.925	5.125
15	0.481	0.681	0.881	0.543	0.823	1.103	0.705	1.005	1.305	2.174	3.374	4.574
16	0.445	0.645	0.845	0.492	0.772	1.052	0.616	0.916	1.216	1.832	2.992	4.192
17	0.415	0.615	0.815	0.454	0.730	1.010	0.547	0.847	1.147	1.627	2.727	3.927
18	0.391	0.591	0.791	0.427	0.697	0.973	0.493	0.793	1.093	1.482	2.542	3.702
19	0.373	0.571	0.771	0.404	0.670	0.936	0.462	0.752	1.042	1.414	2.414	3.514
20	0.361	0.555	0.751	0.389	0.649	0.909	0.435	0.719	1.003	1.346	2.326	3.366
21	0.353	0.543	0.735	0.382	0.632	0.882	0.414	0.694	0.974	1.304	2.264	3.244
22	0.346	0.532	0.723	0.376	0.618	0.860	0.398	0.674	0.950	1.281	2.221	3.161
23	0.340	0.524	0.712	0.371	0.607	0.843	0.387	0.659	0.931	1.271	2.191	3.111
24	0.337	0.517	0.704	0.368	0.598	0.828	0.377	0.647	0.917	1.271	2.171	3.091
25	0.332	0.512	0.697	0.366	0.590	0.814	0.368	0.638	0.908	1.257	2.157	3.057
26	0.327	0.507	0.692	0.365	0.585	0.805	0.361	0.631	0.901	1.247	2.147	3.047
27	0.324	0.504	0.687	0.360	0.580	0.800	0.355	0.625	0.895	1.240	2.140	3.040
28	0.321	0.501	0.684	0.356	0.576	0.796	0.351	0.621	0.891	1.235	2.135	3.035
29	0.318	0.498	0.681	0.353	0.573	0.793	0.347	0.617	0.887	1.232	2.132	3.032
30	0.316	0.496	0.678	0.351	0.571	0.791	0.345	0.615	0.885	1.229	2.129	3.029
31	0.315	0.495	0.676	0.349	0.569	0.789	0.342	0.612	0.882	1.228	2.128	3.028
32	0.314	0.494	0.675	0.347	0.567	0.787	0.341	0.611	0.881	1.227	2.127	3.027
33	0.313	0.493	0.674	0.346	0.566	0.786	0.340	0.610	0.880	1.226	2.126	3.026
34	0.312	0.492	0.673	0.345	0.565	0.785	0.339	0.609	0.879	1.225	2.125	3.025
35	0.311	0.491	0.672	0.344	0.564	0.784	0.338	0.608	0.878	1.225	2.125	3.025
36	0.310	0.490	0.671	0.344	0.564	0.784	0.337	0.607	0.877	1.225	2.125	3.025
37	0.310	0.490	0.670	0.343	0.563	0.783	0.337	0.607	0.877	1.224	2.124	3.024
38	0.310	0.490	0.670	0.343	0.563	0.783	0.337	0.607	0.877	1.224	2.124	3.024
39	0.309	0.489	0.670	0.342	0.562	0.782	0.336	0.606	0.876	1.224	2.124	3.024
40	0.309	0.489	0.669	0.342	0.562	0.782	0.336	0.606	0.876	1.224	2.124	3.024

Table 2. Inter- and intraobserver variations for each DV Doppler index

	Paired differences					t	d.f.	p*
	mean	SD	SE	upper	lower			
Interobserver variation								
PLI	-0.00796	0.10170	0.01672	-0.04186	0.02595	-0.476	36	0.637
PVIV	0.03677	0.17703	0.02910	-0.02225	0.09579	1.263	36	0.215
PIV	-0.01173	0.17944	0.02950	-0.07155	0.04810	-0.398	36	0.693
Intraobserver variation								
PLI	0.00053	0.11535	0.01896	-0.03794	0.03899	0.028	36	0.978
PVIV	-0.01044	0.12400	0.02038	-0.05179	0.03090	-0.512	36	0.612
PIV	-0.01217	0.17888	0.02941	-0.07181	0.04747	-0.414	36	0.681

* Two-tailed significance.

The technical pitfalls in DV measurement should be mentioned here: this measurement is occasionally time-consuming and not always simple, especially when fetuses are in an improper position. Sometimes, sampling the velocity in a neighboring vein or including interference from the inferior vena cava may falsely be interpreted as abnormal DV waveforms. Moreover, the velocity in the hepatic veins tends to be more acute and, particularly during the second trimester, the veins have a zero or reversed velocity during atrial contraction. Our experience suggests that extreme caution must be taken in measuring DV Doppler indices, especially when performed by beginners.

We believe that this nomogram may serve as reference range for DV Doppler indices. This may be useful in assessment of fetal cardiac preload in cases of fetal growth

restriction or congenital heart defects, especially in combination with arterial Doppler measurement.

In conclusion, a nomogram of fetal DV Doppler indices during the period from 14 to 40 weeks' gestation is established. It may be helpful for the assessment of some fetal conditions associated with cardiac function, especially when fetal growth restriction, anemia or congenital heart defects are suspected. However, the effectiveness of this tool remains to be tested in further studies.

Acknowledgement

We wish to thank the National Research University Project under Thailand's Office of the Higher Education Commission for financial support.

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Fetal cardiac dimensions at 14–40 weeks' gestation obtained using cardio-STIC-M

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KEYWORDS: 4D ultrasound; fetal cardiac dimension; M-mode; spatiotemporal image correlation (STIC)

ABSTRACT

Objectives To establish normative reference ranges of fetal cardiac dimensions derived from volume datasets acquired using spatiotemporal image correlation with M-mode display (cardio-STIC-M).

Methods A cross-sectional study was undertaken on singleton pregnancies with normal fetuses and accurate gestational ages. Cardio-STIC volume datasets were acquired by experienced operators using a high-resolution ultrasound machine; these were maneuvered to obtain a four chamber-view with exactly horizontal interventricular septum (IVS). Cardiac dimensions were measured in STIC-M-mode using 4D View software.

Results A total of 657 measurements, at a rate of between 15 and 37 per week, were obtained. Normal reference ranges for biventricular outer diameter, left and right ventricular inner diameter, left and right ventricular wall thickness, IVS thickness, left to right ventricular diameter ratio and left and right ventricular shortening fractions were constructed based on best-fit equations as a function of gestational age, fetal head circumference and biparietal diameter. Thirty-four volume datasets of abnormal fetal hearts were also separately assessed, many of which showed abnormal cardiac dimensions.

Conclusions This is the first report on normal ranges of fetal cardiac dimensions constructed using the new cardio-STIC-M technology. Preliminary evaluation suggests that these reference ranges may be a useful tool in the assessment of fetal cardiac abnormalities. Copyright © 2011 ISUOG. Published by John Wiley & Sons, Ltd.

INTRODUCTION

Measurement of fetal heart dimensions plays an important role in the prenatal diagnosis of various cardiac

defects including hypoplastic left and right heart. Additionally, measuring cardiac dimensions is useful in the evaluation of fetuses with cardiac failure due to fetal anemia secondary to Rh alloimmunization or hemoglobin Bart's disease^{1,2}. Moreover, an assessment of ventricular systolic function using area shortening fraction (SF) is helpful in the diagnosis of fetal cardiac failure^{3–9}. However, to objectively diagnose cardiac abnormalities early in gestation it is necessary that normal reference ranges of fetal cardiac dimensions for each gestational week must first be established. In fact, several studies have published nomograms of cardiac dimensions using two-dimensional (2D) M-mode^{8–12} or B-mode^{13–15} ultrasound. However, these normal reference ranges are based on only a small sample size for each gestational week and do not include fetuses of less than 19 weeks' gestation. Furthermore, the measurements in all of these reports using either M-mode or B-mode are based on 2D ultrasound examination, in which it is often very difficult to obtain the accurate plane for measurement. With 2D measurements it is very difficult to be sure that the interventricular septum (IVS) is exactly horizontal or symmetric in all axes. Additionally, biventricular diameter, which is a useful parameter for the early diagnosis of cardiomegaly, has only been reported in the studies of DeVore *et al.*^{9,12}.

With cardio-spatiotemporal image correlation (cardio-STIC), a volume dataset of the fetal heart can easily be maneuvered to obtain the correct cardiac plane in the three orthogonal planes^{16–18}. For example, we can take advantage of its ability to increase measurement accuracy by adjusting the z-axis slider to achieve an ideal four-chamber view, and cardio-STIC volume datasets can now be displayed in M-mode, permitting more accurate measurement of fetal cardiac dimensions and making it easier to determine the exact point of end-diastole or systole than is possible with cine-loop playback.

The objective of this study was to establish normal reference ranges of fetal cardiac dimensions at 14–40 weeks'

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Accepted: 15 November 2010

gestation derived from cardio-STIC volume datasets using STIC-M-mode.

PATIENTS AND METHODS

This cross-sectional study was undertaken between September 1, 2007 and April 30, 2010 at the Department of Obstetrics and Gynecology, Maharaj Nakorn Chiang Mai Hospital, Faculty of Medicine, Chiang Mai University, Thailand. Pregnant women attending the antenatal care clinic were recruited into the study after providing written informed consent. Inclusion criteria included: (a) correct gestational age based on fetal biometry before 20 weeks' gestation and regular menstruation prior to pregnancy with known date of the last menstrual period; (b) gestational age between 14 and 40 weeks; and (c) low-risk pregnancy without known obstetric and medical complications. Exclusion criteria included: (a) multiple pregnancies; (b) fetal anomalies; (c) abnormal fetal growth – either small-for-gestational age (smaller than 10th centile for each gestational week) or macrosomia (larger than 90th centile); and (d) four-dimensional (4D)-STIC volume datasets could not be obtained satisfactorily. In addition, 4D-STIC datasets were obtained from a number of fetuses with cardiac anomalies.

All ultrasound examinations were performed by experienced operators using real-time equipment with 4D-STIC (Voluson E8; GE Healthcare Ultrasound, Milwaukee, WI, USA) with transabdominal 2–4-MHz curvilinear transducers. During the examination, a routine standard ultrasound scan, including fetal anomaly screening and fetal biometry, was first performed. The acquired volume datasets were stored for subsequent analysis with offline cardio-STIC-M (4D View software version 9; GE Healthcare Ultrasound). (The volume dataset acquisitions were started in 2007, but the offline analysis with STIC-M was started in 2009, when STIC-M first became available in our department.) To assess cardiac dimensions cardio-STIC-M examination was performed as follows:

1. Volume acquisition: Volume datasets of the fetal heart from the four-chamber view, in which the cardiac axis was oriented horizontally and with the ultrasound beam perpendicular to the IVS, were obtained by transverse sweeps through the fetal thorax. Women were asked to hold their breath during volume acquisition (lasting 7.5–12.5 s) which was performed in the absence of fetal movement.
2. Volume processing: The volume datasets underwent image processing such as speed or brightness adjustment to optimize tissue contrast resolution as appropriate for analysis.
3. Offline volume dataset analysis: Multiplanar slicing analysis was subsequently performed. Dynamic images of the fetal heart were simultaneously visualized in the three orthogonal planes (axial, coronal and sagittal). Images of the four-chamber view were adjusted and displayed by moving the reference dot and rotating the fetal heart around the three orthogonal axes (x, y and

z) to position the IVS in the exactly horizontal plane in all three panels. The IVS in Panel A must be in the same line as that in Panel B; a total en-face view of the IVS was displayed in Panel C (Figure 1).

4. STIC-M display: In Panel A, the M-mode cursor was placed perpendicular to the IVS at the level of maximal transverse diameter, in which the atrioventricular (AV) valve motion was identified during ventricular diastole and the M-mode tracing was displayed (Figure 1).
5. Cardiac size measurement: The following measurements were made at end-diastole on M-mode tracings: biventricular outer diameter (BVOD), which was measured from the epicardium of the left ventricle to the epicardium of the right ventricle; left ventricular internal diameter (LVID), which was measured from the endocardium of the left ventricular wall to the endocardium of the left side of the ventricular septum; and right ventricular internal diameter (RVID), which was measured from the endocardium of the right ventricular wall to the endocardium of the right side of the ventricular septum. The thickness of the left ventricular wall (LVWT), right ventricular wall (RVWT) and the IVS (IVST) were also measured in the same plane. LVID and RVID were also measured during end-systole for calculation of left and right ventricular SFs (LVSF and RVSF, respectively). SF was calculated using the formula (transverse diameter at end-diastole – diameter at end-systole)/diameter at end-diastole). The best measurements were selected and recorded for analysis.

This research was approved by the research ethics committee of our institute, and was financially supported by the Thailand Research Fund.

Statistical analysis

Regression analysis of each cardiac dimension was performed to determine the best-fit equation, which was then used for construction of reference intervals, using the statistical package for the social sciences (SPSS) software version 17.0 (SPSS Inc., Chicago, IL, USA) and STATA version 10.0 (Statacorp, College Station, TX, USA). Analysis of reference ranges was performed in the manner recommended by Royston and Wright¹⁹. In brief, polynomial regression models were fitted separately to the mean and SD of the cardiac dimensions (dependent variables) as functions of gestational age, head circumference (HC) and biparietal diameter (BPD) (independent variables) to generate the best-fit regression equations. The models were selected by successive testing of the highest-order coefficient of cubic, quadratic and linear models for significant difference from zero (the coefficient not less than twice its standard error).

The assumption of normality and the goodness of fit of each regression model were checked by examination of the scatter patterns of points relative to fitted means and SDs, expressed as Z-scores ((measured value – estimated mean)/estimated SD). Z-scores were assessed for normality by QQ plots and the Shapiro–Francia *W'* test.

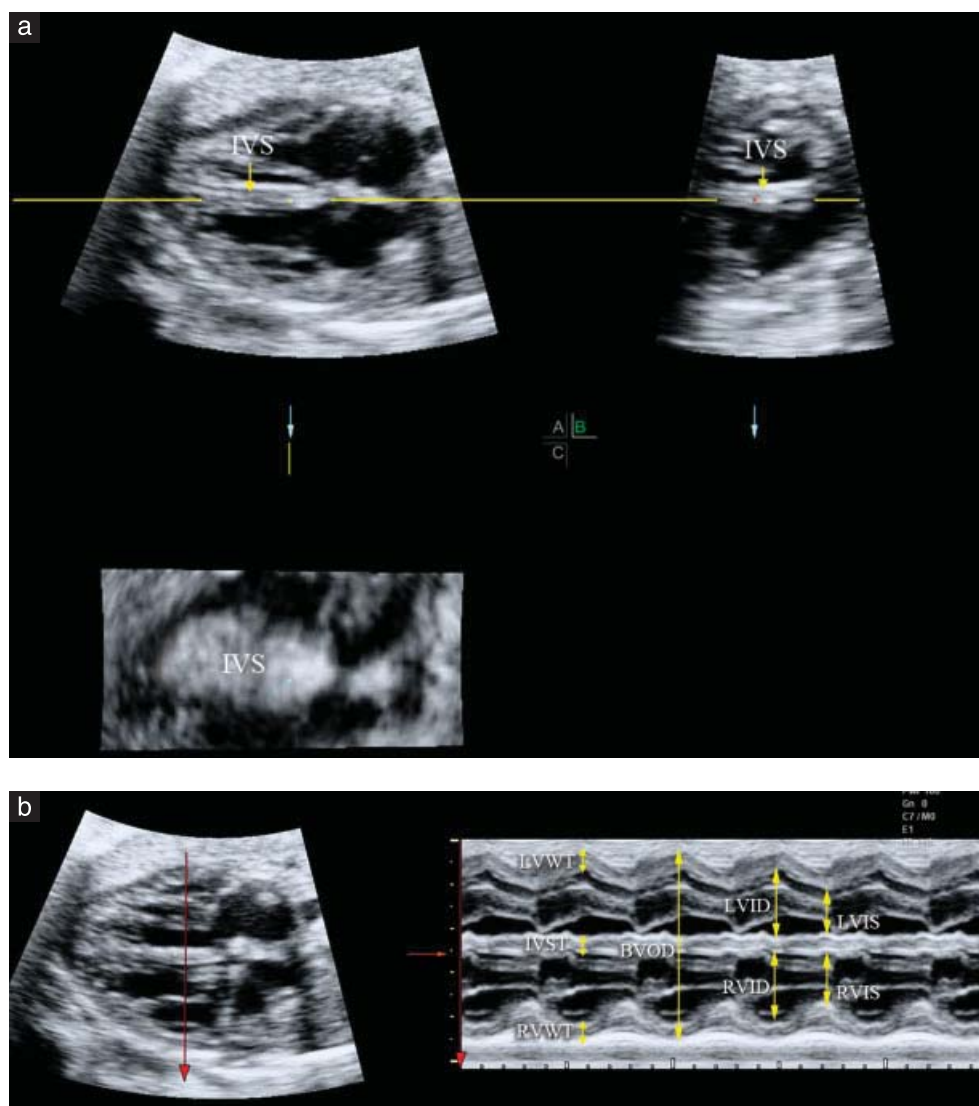


Figure 1 (a) Using spatiotemporal image correlation (cardio-STIC), volume datasets of the fetal heart were obtained from the four-chamber view, which was displayed in Panel A. This image was maneuvered so that the interventricular septum (IVS) was exactly horizontal and the reference dot was placed so that the IVS was displayed in three orthogonal planes in the multiplanar view, the *en-face* view being obtained in Panel C. (b) Measurements of the cardiac dimensions during end-diastole were obtained in M-mode (cardio-STIC-M). BVOD, biventricular outer diameter; IVST, IVS thickness; LVID, left ventricular internal diameter at end-diastole; LVIS, left ventricular internal diameter at end-systole; LVWT, left ventricular wall thickness; RVID, right ventricular internal diameter at end-diastole; RVIS, right ventricular internal diameter at end-systole; RVWT, right ventricular wall thickness.

Tables were constructed for the mean and 5th and 95th centiles for each cardiac variable, using gestational age, HC and BPD as the reference (centiles = estimated mean $\pm$ (1.645 $\times$ SD)), where both mean and SD were obtained using the regression formulae. Correlation coefficients and associated *P*-values for the relationships of the cardiac dimension were calculated. For statistical significance, a type I error probability (α) was set to be less than 0.05.

RESULTS

A total of 751 women in whom volume acquisition had been successful were recruited. Of these, 60 were later excluded because of poor volume datasets, most of which had been obtained at 14–16 weeks (28 cases) and 36–40 weeks of gestation (21 cases). Of those remaining 34 had cardiac abnormalities and were excluded from

the statistical analysis, leaving 657 volume datasets for cardio-STIC-M analysis. The number of measurements for each gestational week ranged from 15 to 37. Regression analysis showed that the best-fit equations relating gestational age, HC and BPD to the cardiac parameters were defined by quadratic functions except for the SFs (which were linear) and IVST (which was linear for HC and BPD), as shown in Table 1. The SDs for Z-score calculation are also provided in Table 1. Each final model was confirmed for adequacy of its fitted curve by demonstrating a good scatter pattern of points relative to the Z-scores (fewer than 10% beyond the 5th and 95th centile range), and normalcy of the Z-scores was confirmed by normal histogram distribution, QQ plots and Shapiro–Francia *W'* test. Normative reference ranges of end-diastolic BVOD, LVID, RVID, LVWT, RVWT, IVST, LVIS and RVIS at each week of gestational age

Table 1 Summary of regression analysis of cardiac measurements and ratios against gestational age (GA), head circumference (HC) and biparietal diameter (BPD)

Dimension	Estimated mean	Estimated SD	r (P)
Function of GA (in weeks)			
BVOD (cm)	$-2.3624 + (0.2769 \times \text{GA}) - (0.0030 \times \text{GA}^2)$	$-0.0154 + (0.0102 \times \text{GA})$	0.951 (<0.001)
LVID (cm)	$-0.9149 + (0.1054 \times \text{GA}) - (0.0012 \times \text{GA}^2)$	$-0.0182 + (0.0056 \times \text{GA})$	0.899 (<0.001)
RVID (cm)	$-1.0024 + (0.1107 \times \text{GA}) - (0.0012 \times \text{GA}^2)$	$-0.0378 + (0.0067 \times \text{GA})$	0.913 (<0.001)
LV:RV ratio	$1.0210 - (0.0026 \times \text{GA})$	$0.0421 + (0.0016 \times \text{GA})$	0.216 (<0.001)
IVST (cm)	$-0.1332 + (0.0183 \times \text{GA}) - (0.0002 \times \text{GA}^2)$	$-0.0020 + (0.0011 \times \text{GA})$	0.917 (<0.001)
LVWT (cm)	$-0.1600 + (0.0218 \times \text{GA}) - (0.0002 \times \text{GA}^2)$	$-0.0032 + (0.0015 \times \text{GA})$	0.859 (<0.001)
RVWT (cm)	$-0.1518 + (0.0207 \times \text{GA}) - (0.0002 \times \text{GA}^2)$	$-0.0084 + (0.0016 \times \text{GA})$	0.875 (<0.001)
LVSF	$0.3964 - (0.0027 \times \text{GA})$	$0.0490 - (0.00007 \times \text{GA})$	0.381 (<0.001)
RVSF	$0.3547 - (0.0016 \times \text{GA})$	$0.0388 + (0.00025 \times \text{GA})$	0.237 (<0.001)
Function of HC (in cm)			
BVOD (cm)	$-0.4256 + (0.1503 \times \text{HC}) - (0.0006 \times \text{HC}^2)$	$0.0291 + (0.0097 \times \text{HC})$	0.950 (<0.001)
LVID (cm)	$-0.2179 + (0.0609 \times \text{HC}) - (0.0004 \times \text{HC}^2)$	$-0.0024 + (0.0057 \times \text{HC})$	0.898 (<0.001)
RVID (cm)	$-0.2071 + (0.0580 \times \text{HC}) + (0.0002 \times \text{HC}^2)$	$-0.0170 + (0.0067 \times \text{HC})$	0.913 (<0.001)
LV:RV ratio	$0.9527 + (0.0033 \times \text{HC}) - (0.0001 \times \text{HC}^2)$	$0.0407 + (0.0017 \times \text{HC})$	0.224 (<0.001)
IVST (cm)	$-0.0032 + (0.0098 \times \text{HC})$	$0.0015 + (0.0011 \times \text{HC})$	0.917 (<0.001)
LVWT (cm)	$-0.0046 + (0.0115 \times \text{HC}) - (0.0005 \times \text{HC}^2)$	$0.0015 + (0.0015 \times \text{HC})$	0.857 (<0.001)
RVWT (cm)	$-0.0016 + (0.0108 \times \text{HC}) - (0.00001 \times \text{HC}^2)$	$-0.0039 + (0.0017 \times \text{HC})$	0.875 (<0.001)
LVSF	$0.3876 - (0.0027 \times \text{HC})$	$0.0506 - (0.0001 \times \text{HC})$	0.365 (<0.001)
RVSF	$0.3510 - (0.0016 \times \text{HC})$	$0.0395 + (0.0025 \times \text{HC})$	0.335 (<0.001)
Function of BPD (in cm)			
BVOD (cm)	$-0.8278 + (0.7088 \times \text{BPD}) - (0.0213 \times \text{BPD}^2)$	$0.0274 + (0.0364 \times \text{BPD})$	0.950 (<0.001)
LVID (cm)	$-0.3593 + (0.2788 \times \text{BPD}) - (0.0099 \times \text{BPD}^2)$	$-0.0028 + (0.0213 \times \text{BPD})$	0.898 (<0.001)
RVID (cm)	$-0.3761 + (0.2791 \times \text{BPD}) - (0.0080 \times \text{BPD}^2)$	$-0.0148 + (0.0247 \times \text{BPD})$	0.911 (<0.001)
LV:RV ratio	$0.9627 + (0.0078 \times \text{BPD}) - (0.0014 \times \text{BPD}^2)$	$0.0428 + (0.0064 \times \text{BPD})$	0.214 (<0.001)
IVST (cm)	$0.0006 + (0.0360 \times \text{BPD})$	$0.0015 + (0.0041 \times \text{BPD})$	0.916 (<0.001)
LVWT (cm)	$-0.0351 + (0.0541 \times \text{BPD}) - (0.0015 \times \text{BPD}^2)$	$0.0018 + (0.0054 \times \text{BPD})$	0.858 (<0.001)
RVWT (cm)	$-0.0319 + (0.0516 \times \text{BPD}) - (0.0011 \times \text{BPD}^2)$	$-0.0026 + (0.0060 \times \text{BPD})$	0.875 (<0.001)
LVSF	$0.3880 - (0.0101 \times \text{BPD})$	$0.0489 - (0.0002 \times \text{BPD})$	0.372 (<0.001)
RVSF	$0.3508 - (0.0060 \times \text{BPD})$	$0.0390 + (0.0010 \times \text{BPD})$	0.236 (<0.001)

BVOD, biventricular outer diameter; IVST, interventricular septum thickness; LVID, left ventricular inner diameter; LVSF, left ventricular shortening fraction; LVWT, left ventricular wall thickness; LV:RV ratio, left to right ventricular inner diameter ratio; RVID, right ventricular inner diameter; RVSF, right ventricular shortening fraction; RVWT, right ventricular wall thickness.

are presented in Table S1. The ratio of the end-diastolic LVID to the end-diastolic RVID for each gestational week was also calculated and is presented as mean and 5th and 95th centiles. All of these cardiac dimensions were also compared with fetal HC and BPD as presented in Tables S2 and S3, respectively. The mean LVID was slightly but significantly lower than that of RVID (mean difference = 0.072 mm; paired *t*-test, *P* < 0.001). The mean LVSF for all gestational ages (14–40 weeks) was 0.321 ± 0.051 (range, 0.14–0.47; 5th and 95th centiles, 0.26 and 0.41, respectively) whereas the mean RVSF was 0.311 ± 0.048 (range, 0.17–0.46; 5th and 95th centiles, 0.25 and 0.40, respectively). The overall SF of the two sides showed a minimal but statistically significant difference (paired *t*-test, *P* < 0.001). The LVSF:RVSF ratio (1.06 ± 0.23 ; range, 0.41–1.87) had a tendency to decrease with gestational age, but this was not statistically significant.

During the study period volume datasets were acquired from 34 fetuses with cardiac abnormalities, as shown in Table 2 and Figure 2. Cardiomegaly secondary to hemoglobin Bart's disease was the most common disorder among the recorded volume datasets. When abnormal cardiac dimensions were defined as those less than the 5th centile or more than the 95th centile for each gestational week, all 11 fetuses with hydrops fetalis due

to hemoglobin Bart's disease showed abnormally enlarged LVID, RVID and BVOD, whereas abnormal SF was found in only three out of 11 cases. All three fetuses with left heart hypoplasia demonstrated abnormally small left ventricular diameters. Likewise, two cases with right heart hypoplasia were associated with abnormally small right ventricles. All fetuses with double outlet right ventricle and left heart hypoplasia showed abnormally low left to right ventricular inner diameter ratios. It is interesting that nearly all of the fetuses with tetralogy of Fallot (five out of six) showed no abnormal measurement of the dimensions considered.

DISCUSSION

Several techniques for assessing cardiac dimensions have been published^{20,21}. 2D ultrasound is prone to providing an incorrect plane of the exact four-chamber view, even if the ultrasound beam is precisely perpendicular to the IVS, since it can be tilted towards or away from the examiner on the z-axis, resulting in asymmetry of left- and right-sidedness of the heart, and leading to inaccurate measurement of cardiac dimensions. This may partly explain the different ranges seen in the different studies, which are derived from either 2D M-mode^{9,12} or 2D

Table 2 Number of cases with abnormal cardiac dimensions, derived from spatiotemporal image correlation volume datasets with M-mode display, amongst 34 fetuses with cardiac pathology

Pathology (n)	LVID percentile		RVID percentile		LV:RV ratio percentile		BVOD percentile		LVSF percentile		RVSF percentile	
	< 5 th	> 95 th	< 5 th	> 95 th	< 5 th	> 95 th	< 5 th	> 95 th	< 5 th	> 95 th	< 5 th	> 95 th
Coarctation of aorta (n = 3)	—	—	—	—	—	—	—	—	1	1	—	—
Atrioventricular defect (n = 1)	1	—	1	—	—	—	1	—	—	—	—	—
Double outlet right ventricle (n = 4)	4	—	—	3	4	—	—	—	1	—	—	—
Ebstein's anomaly (n = 2)	—	—	2	—	—	2	—	—	—	—	—	—
Cardiomegaly* (n = 11)	—	11	—	11	1	1	—	11	3	—	3	—
Right isomerism (n = 1)	—	1	—	1	—	—	—	1	—	—	—	—
Hypoplastic left heart (n = 3)	3	—	—	1	3	—	2	—	1	—	—	2
Hypoplastic right heart (n = 2)	—	1	2	—	—	2	2	—	—	—	2	—
Tetralogy of Fallot (n = 6)	—	1	—	1	—	—	—	1	—	—	—	—
Ventricular septal defect (n = 1)	—	—	—	—	—	—	—	—	—	—	—	—

*Secondary to hemoglobin Bart's disease. BVOD, biventricular outer diameter; LVID, left ventricular inner diameter; LVSF, left ventricular shortening fraction; LV:RV ratio, left to right ventricular inner diameter ratio; RVID, right ventricular inner diameter; RVSF, right ventricular shortening fraction.

B-mode^{13–15,22}. Fortunately, this problem has now been resolved by the new cardio-STIC-M.

The normative reference ranges derived from three-dimensional (3D) cardio-STIC may possibly be more reliable. With 3D cardio-STIC, the IVS can simply be maneuvered into the exactly horizontal line in Panels A and B, while the total en-face view of the IVS is displayed in Panel C^{16,18}. The level of the AV valves as used in some series^{10,23,24} may not be the best plane for the evaluation of cardiac size, since this is not the plane of greatest transverse diameter and it is difficult to distinguish the inner ventricular wall from the attachment of the AV valves, resulting in unreliable measurements. Therefore in this study the level of measurement was based on the maximum transverse diameter, just below the AV valve orifices as described by Sharland and Allan¹³, which is easily demonstrated by adjustment of the STIC-M volume datasets. At this level the ventricular walls are readily distinguished from the relatively anechoic chambers.

This study showed that all the cardiac dimensions, apart from SF, increase steadily throughout gestation. The growth pattern of all dimensions, apart from SF, is better described by a quadratic function than a linear function, as reported in previous studies^{10,24}. This finding is similar to those studied with 2D M-mode ultrasound^{9,12}, although our reference range values seem to be slightly lower. The normative data in this study are somewhat different from those of several previous reports^{8,9,13,15}, but similar to those reported by Shapiro *et al.*¹⁴. However, Shapiro *et al.* used simple 2D B-mode instead of M-mode, and the nomogram of biventricular diameter is not available in their report¹⁴. Our extensive experience suggests that assessment of biventricular diameter is very useful in the early detection of fetal cardiomegaly, the earliest sign of hydrops fetalis²⁵. In particular, our results show a decrease in the left:right ventricular ratio from 0.99 at 14 weeks' gestation to 0.90 at 40 weeks. This is different from the results reported by DeVore *et al.*⁹ and Wladimiroff *et al.*⁸, which showed no change in the ratio

with gestational age. However the change pattern in this study is consistent with those reported by Shapiro *et al.*¹⁴ and Firpo *et al.*¹⁵, although the change is less in this study.

A review of normal LVSF and RVSF values as measured by different researchers reveals marked variation^{3–9}, ranging from 0.26⁴ to 0.48⁵ for the left ventricle and 0.21⁶ to 0.42⁵ for the right ventricle. Some researchers found a small decrease in ventricular SF with advancing gestational age⁵, but most found no significant effect of gestational age^{6,9}. We found a small but statistically significant decrease in both LVSF and RVSF. The mean SFs for each gestational age in this study are rather low in comparison to most of those in the study by Hsieh *et al.*⁵, but relatively high when compared with those reported by Wladimiroff and McGhie⁷ and Sikkil *et al.*⁶. The different ranges of normal values in the various studies may partly reflect the different imaging techniques used, or may be partly associated with ethnic factors.

Since the relationship between SF and gestational age is subtle, previous investigations may have been underpowered to detect it. However, although SF may be associated with gestational age, such a minimal *statistically* significant change may be not *clinically* significant, and it may be assumed that SF is relatively constant throughout pregnancy. We believe that it may be possible to use overall SF for all fetuses (LVSF, 0.32 ± 0.52 ; RVSF, 0.31 ± 0.49) to evaluate fetal cardiac contractility as an age-independent tool.

Unfortunately, not all fetuses with cardiac abnormalities found during the study period were included in this study. Therefore the number of volume datasets with cardiac defects was too small for analysis. However, despite the small sample size of the pathologic groups, the findings presented here suggest that measurement of cardiac dimensions may be helpful in the prenatal diagnosis of various abnormalities, including hypoplastic heart, Ebstein's anomaly, double outlet right ventricle and cardiomegaly secondary to various causes. As already mentioned, nearly all fetuses with tetralogy of Fallot showed no abnormalities in all dimensions, indicating that

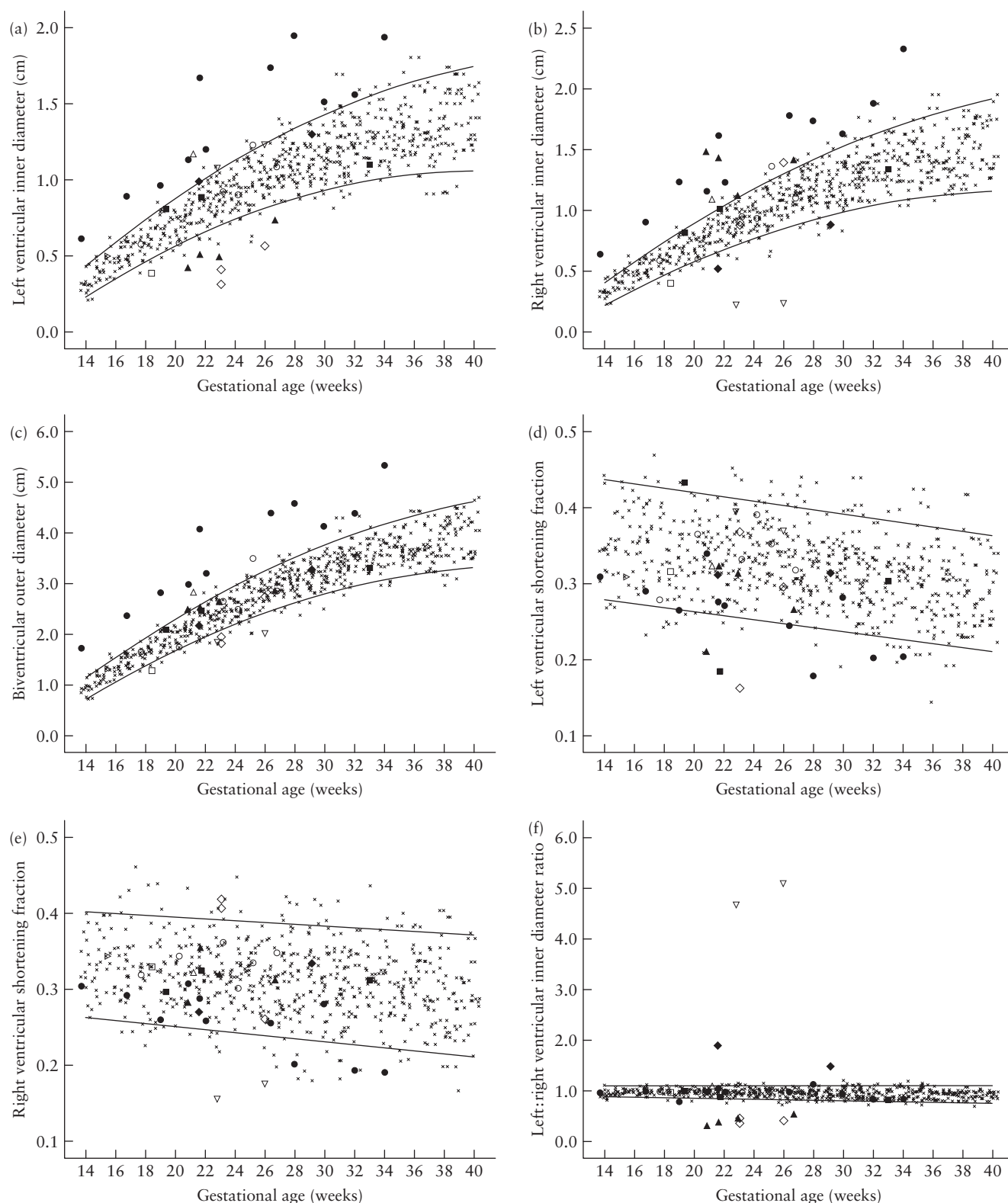


Figure 2 Scatterplots of cardiac measurements and ratios at end-diastole against gestational age with estimated 5th and 95th centiles: (a) left ventricular inner diameter; (b) right ventricular inner diameter; (c) biventricular outer diameter; (d) left ventricular shortening fraction; (e) right ventricular shortening fraction; (f) left:right ventricular inner diameter ratio. Measurements obtained from abnormal fetuses are also plotted. ×, normal; ■, coarctation of aorta; ◆, Ebstein's anomaly; △, right isomerism; ●, hemoglobin Bart's disease; □, atrioventricular canal; ◇, hypoplastic left heart; ▽, hypoplastic right heart; ○, tetralogy of Fallot; ▲, double outlet right ventricle; ▷, ventricular septal defect.

the measurement may not be helpful for the diagnosis of this disorder. Notably, an abnormal SF was commonly seen in hypoplastic ventricles. An abnormal SF as an indicator of cardiac failure among fetal hearts with obvious

hydrops fetalis due to hemoglobin Bart's disease was found in only three out of 11 cases, and all three cases occurred in late pregnancy. This finding suggests that cardiomegaly in early hydrops fetalis related to fetal anemia

may not be caused by cardiac failure but by some other mechanism of hypervolemia. This may be evidence for a new insight that in fetuses with high cardiac output due to anemia, myocardial dysfunction and cardiac decompensation do not constitute a primary cause of hydrops fetalis but are a consequence only when the cardiac compensatory mechanism is exhausted.

One limitation of this study is that resolution of the virtual cardiac cycle might be somewhat decreased in several cases secondary to maternal or fetal movement and fetal position. Additionally, in some cases early in the second trimester the small cardiac structures could not be clearly visualized, especially before 16 weeks, and were excluded because of poor quality.

The strengths of this study include: (a) a large sample size, ranging from 15 to 37 examinations per gestational week; (b) reliable gestational age, based on both accurate last menstrual period and early ultrasound; (c) data derived from 3D-cardio-STIC-M together with offline analysis without time limit, allowing us to obtain the best image for measurement in all satisfactory volume datasets; (d) our normative tables include fetuses at early gestation (from 14 weeks), unlike those in most other studies; and (e) our data include biventricular outer diameter, which is unavailable in most other studies.

ACKNOWLEDGMENTS

The authors wish to thank the Thailand Research Fund (TRF) as a part of TRF Senior Research Scholar and the National Research University Project under Thailand's Office of the Higher Education Commission for financially supporting this research.

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SUPPORTING INFORMATION ON THE INTERNET

Tables S1–S3 may be found in the online version of this article.

Effect of cord puncture site in cordocentesis at mid-pregnancy on pregnancy outcomes

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Objectives To compare the procedure and pregnancy outcomes of pregnancies undergoing cordocentesis between a group of cord insertion puncture and that of free loop puncture.

Materials and Methods Pregnancies undergoing cordocenteses meeting the following inclusion criteria were recruited: (1) singleton pregnancies at gestational ages of 18–22 weeks; (2) no fetal abnormalities; (3) the procedures performed by experienced operators; and (4) the procedures of no placenta penetration. The recruited cases were classified into two groups: a group of cordocenteses with puncture site at placental cord insertion (Group 1) and those with puncture site at cord free loop (Group 2). The primary outcomes to be compared were rates of fetal loss, newborns with low birth weight and preterm birth. The secondary outcomes included a rate of successful procedures, umbilical cord bleeding, fetal bradycardia, maternal blood contamination, and duration of the procedure time.

Results Of 6147 cordocenteses recruited, 2214 met inclusion criteria. The mean duration to complete the procedure in Group 1 was significantly shorter than that in Group 2 (4.5 ± 5.4 vs 6.7 ± 8.5 min, Student's *t*-test; $p = 0.001$). The mean birth weight and gestational age at delivery were comparable between the two groups. No significant differences between the two groups were seen in rates of fetal loss, low birth weight, preterm birth, successful procedures, umbilical cord bleeding, or fetal bradycardia. However, the rate of maternal blood contamination was significantly higher in the group of puncture at the cord insertion site.

Conclusion Of cordocenteses performed based on accessibility and quality of visualization, cord insertion puncture may cause more maternal blood contamination but free loop puncture may be associated with more difficult procedures, while the safety was comparable. Copyright © 2011 John Wiley & Sons, Ltd.

KEY WORDS: cord puncture; cordocentesis; fetal outcome; prenatal diagnosis

INTRODUCTION

Cordocentesis or percutaneous umbilical blood sampling is a procedure to obtain fetal blood from umbilical vessels by puncturing the needle through maternal abdominal wall, under ultrasound guidance. Cordocentesis was first established in 1983 (Daffos *et al.*, 1985). The procedure has rapidly gained acceptance with demonstration of its safety (Weiner and Okamura, 1996a; Tongsong *et al.*, 2001; Liao *et al.*, 2006a) directly leading to the development of fetal medicine. This procedure is performed by using spinal needle puncture through maternal abdominal wall to access the umbilical cord. However, the procedure can be associated with some significant risks to the fetuses, risk of fetal loss (procedure-related fetal loss) of about 0.9–3.2% (Buscaglia *et al.*, 1996; Weiner and Okamura, 1996b). Several factors may contribute to the higher risk such as placenta penetration, levels of experience of performers, maternal abdominal wall thickness, etc. Whether different techniques such as puncture target site are associated with fetal

risk or not has yet to be explored. In terms of technical aspects, two practical approaches to choose a target site, cord insertion as a target of first choice and accessibility regardless of location. Several authors prefer the first approach. However, our practice is based on the latter one. In the western countries, the preferred location for cord puncture is the placental origin, where the cord is relatively fixed. However, from our experience, the best location is the most accessible, usually at a free loop of umbilical cord (Tongsong *et al.*, 2000; Liao *et al.*, 2006b). Indeed, sometimes the placental insertion of the umbilical cord is at the posterior uterine wall, which may be too deep to reach, or the fetus may be lying over the cord, making cord puncture more dangerous. Although several studies on cordocentesis-associated complications have been published, no study has specifically evaluated the effect of puncture site on pregnancy outcomes. Moreover, those previous studies were flawed by heterogeneous study population in terms of various gestational weeks and lack of control in techniques especially status of placenta penetration, as well as levels of the performer experience. The objective of this study was to compare the procedure and pregnancy outcomes between pregnancies undergoing cordocentesis with cord puncture at insertion site to those with puncture at free loop.

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MATERIALS AND METHODS

This prospective study was conducted at a single center, Maharaj Nakorn Chiang Mai Hospital, Chiang Mai, Thailand, between September 1989 and December 2010. All consecutive cases of cordocenteses were prospectively recorded and followed up for pregnancy outcomes. Baseline characteristics of the patients and procedural details were recorded, for example: procedure time, placenta penetration, puncture site, the presence of umbilical cord bleeding, duration of bleeding, or bradycardia. The data were computerized stored for subsequent analysis. The inclusion criteria for analysis included (1) singleton pregnancies at 18–22 weeks of gestation; (2) no fetal structural anomalies and chromosomal abnormalities; (3) the procedures performed by experienced operators defined by real practice of cordocentesis of more than 50 cases; and (4) the procedures performed without placenta penetration. The exclusion criteria were (1) fetuses with subsequently proven to have anomalies, or chromosomal abnormalities or any serious diseases, for example, hydrops fetalis, required termination of pregnancy; (2) no complete data of pregnancy outcomes or loss to follow-up, or (3) pregnant women with previous cordocentesis, or amniocentesis or chorionic villus sampling (CVS) in the current pregnancy.

The techniques of cordocentesis in this study were described in our previous report (Tongsong *et al.*, 2000). All procedures were performed using free-hand technique using a 22-gauge needle tracked by imaging the tip and shaft with transabdominal ultrasound guidance, using the convex transducer of 3.5 MHz (model E8 GE medical system, WI, USA; and model SSD 620, 680EX, SSD 1700; SSD 5000, alpha-10 Aloka, Tokyo, Japan).

In our practice, the umbilical cord puncture site was chosen based on the accessibility and quality of visualization, with an attempt to avoid placenta penetration, if possible. We preferred to target the easiest location for direct approach rather than confine ourselves to the umbilical cord insertion site. Cordocenteses in this study were done under local anesthesia without prophylactic antibiotics and no administration of neuromuscular antagonist to eliminate fetal movement. Failed cordocentesis was not definitely defined by duration of the procedure but based on operators' consideration. Typically, pregnancies with severe fetal diseases ended up with pregnancy termination, while pregnancies with normal results of prenatal diagnosis were treated as a routine standard prenatal care.

Cordocenteses meeting the inclusion criteria were divided into two groups: those with puncture at placental cord insertion (Group 1) and those with puncture at cord free loop (Group 2). In comparing the two groups, the primary outcomes were rates of fetal loss, low birth weight (<2500 g), and preterm birth (<37 complete weeks of gestation). The secondary outcomes included rate of successful procedures, fetal bradycardia, and umbilical cord bleeding, which was based on impression by the performers at the time of performing, maternal blood contamination, and duration of the procedure time (from the puncture needle in and out of the maternal

skin). Fetal loss referred to a pregnancy ending up with spontaneous abortion or perinatal death, including stillbirth and neonatal death. Perinatal death is a death of infant born after 20 weeks and ending at 28 complete days after birth. This research was approved by the research ethics committee of our institute.

Statistical analysis

Pearson's Chi-square test was used to test the difference in the rates of fetal loss, low birth weight, preterm birth, successful procedures, umbilical cord bleeding, fetal bradycardia, and maternal blood contamination between both groups. Student's *t*-test was used to compare means of duration of the procedure time, birth weight, and gestational age of delivery between both groups. The analysis used the statistical software for the social sciences (SPSS) version 17.0 (Chicago, IL, USA).

RESULTS

During the study period, September 1989 to December 2010, a total of 6147 procedures were performed at 18–22 weeks of gestation. To gain high homogeneity of the study population and reduce potential confounding factors as many as possible, 3933 cases were excluded as follows: twin pregnancies (55), loss to follow-up/incomplete data (1272), procedures performed during early learning curve experience of the operators (1123), cases with prior cordocentesis/amniocentesis or CVS (66), fetal structural/chromosomal abnormalities (802), and procedures with placenta penetration (615). Finally, 2214 procedures were available for analysis. Of these, 559 had puncture site at placental cord insertion (Group 1) and 1655 were punctured at the umbilical cord free loop (Group 2). The most common indications for cordocentesis were pregnancies at risk of fetal severe thalassemia syndrome and fetal chromosomal abnormalities. The indications for cordocentesis are presented in Table 1. Each indication was equally distributed in the two groups. Table 2 displays the distribution of placental locations in the two groups: anterior placentas were more common in Group 1, posterior placentas in Group 2. The means of maternal age and gestational age at cordocentesis between both groups were not significantly different (30.8 ± 7.8 vs 31.2 ± 8.2 years; $p = 0.26$; and 19.5 ± 1.1 vs 19.6 ± 1.1 weeks; $p = 0.88$, respectively). The characteristics of pregnancy outcomes and procedure-related complications are presented in Table 3. Regarding technical aspect, the mean duration to complete the procedure in Group 1 was significantly shorter than that in Group 2 (4.5 ± 5.4 vs 6.7 ± 8.5 min, $p = 0.001$). The mean birth weight and gestational age at delivery were comparable between the two groups. No significant differences between the two groups were seen in rates of fetal loss, low birth weight, preterm birth, successful or failed procedures, umbilical cord bleeding, and fetal bradycardia. However, the rate of maternal blood contamination was significantly higher in group of puncture at the cord insertion site (2.3% vs

PLACENTAL PENETRATION DURING CORDOCENTESIS

Table 1—Indications for cordocentesis

	Group 1		Group 2		Odds ratio	95% confidence interval
	<i>n</i> = 559	%	<i>n</i> = 1655	%		
Risk of fetal Hb Bart's disease	120	21.5	306	18.5	1.20	0.95–1.53
Risk of fetal beta-thalassemia major	92	16.5	201	12.2	1.43	1.09–1.86
Risk of fetal beta-thalassemia/HbE	150	26.8	447	27.0	0.99	0.80–1.23
Mixed Risk of fetal severe thalassemia	4	0.7	15	0.9	0.79	0.26–2.38
Rapid karyotyping	97	17.4	400	24.2	0.66	0.51–0.84
Risk of fetal severe thalassemia + chromosome	93	16.6	276	16.7	0.99	0.77–1.29
Others	3	0.5	10	0.6	0.89	0.24–3.24

Table 2—Placental location

	Group 1		Group 2		Odds ratio	95% confidence interval
	<i>n</i> = 559	%	<i>n</i> = 1655	%		
Anterior	186	33.3	428	25.9	1.44	1.17–1.77
Antero-posterior	4	0.72	20	1.2	0.74	0.28–1.98
Posterior	221	39.5	1039	62.8	0.39	0.32–0.47
Posterior lateral	8	1.4	22	1.3	1.21	0.56–2.65
Lateral	2	0.4	22	1.3	0.40	0.12–1.34
Posterior fundus	1	0.2	7	0.4	0.85	0.17–4.08
Fundus	15	2.7	78	4.7	0.60	0.35–1.03
Other	122	21.8	39	2.4	11.9	8.13–17.36

Table 3—Comparisons of outcomes between the group of cord insertion puncture (Group 1) and free loop puncture (Group 2)

	Group 1 (n = 559)		Group 2 (1655)		<i>p</i> -value*		
	Median	Interval	Median	Interval			
Time (min)	3	1–80	2	1–20	<0.001		
	Mean	Standard deviation	Mean	Standard deviation	<i>p</i> -value**		
Birth weight (g)	2980.2	509.7	2986.1	556.9	0.824		
GA_delivery (weeks)	38.1	2.2	38.1	2.2	0.701		
	<i>n</i>	Percentage	<i>n</i>	Percentage	<i>p</i> -value#	Odds ratio	95% confidence interval
Fetal loss	16	2.9	37	2.2	0.40	1.29	0.71–2.34
Maternal contamination	13	2.3	10	0.6	0.001	3.92	1.71–8.98
Success rate	551	98.6	1636	99.1	0.29	0.63	0.27–1.50
Significant bleeding	159	28.4	453	27.4	0.62	1.05	0.85–1.30
Bradycardia	33	5.9	69	4.2	0.09	1.44	0.94–2.21
Low birth weight	59	10.6	217	13.1	0.11	0.78	0.58–1.06
Preterm birth	67	12.0	225	13.6	0.33	0.89	0.65–1.16

* Mann–Whitney *U*-test,** Student's *t*-test,

Chi-square test.

0.6%, $p = 0.001$). Of 53 fetal losses, the mean $\pm$ SD gestational age was comparable between the two groups (32.8 ± 6.9 vs 31.5 ± 6.7 , Student's *t*-test: $p = 0.551$).

DISCUSSION

To compare effects of puncture site between free loop and cord insertion on pregnancy and procedure outcomes, potential confounding factors on such outcomes

should be excluded or controlled to give reliable results. Therefore, in this study, we tried to control all obviously known confounders including gestational age, levels of experience of performers, and placenta penetration. Theoretically, penetration of the placenta during cordocentesis can cause injury to fetal and placental vasculature, resulting in bleeding and thrombosis in the placenta or intervillous space as well as fetal hemorrhage from chorionic vessels. Thereby, placenta penetration

can lead to adverse pregnancy outcomes such as spontaneous abortion or preterm labor. We thus preferred to exclude the procedures involving placenta penetration. Twin pregnancies and other potential confounders that may affect pregnancy outcomes were also excluded. In addition, gestational age which can definitely effect on procedural complications was controlled by including only pregnancy at 18–22 weeks of gestation.

For technical aspects, puncture site at cord insertion took significantly less time to complete procedure than that at free loop. This implies that the procedure with puncture site at cord insertion, without placenta penetration, may be easier than the procedure at free loop puncture. This may be explained by the fact that cord insertion target is usually anchored more securely than free loop target due to less effect from fetal movements. Some authors (Daffos, 1989; Antsaklis *et al.*, 1998; Joy *et al.*, 2009), prefer a cord insertion target to a free loop because of its stability and always try to avoid free loop puncture if possible. On our point of view, that may be not the best choice if a cord insertion target is located in the area of less accessible and our study does not support the concepts of a strong attempt to get the insertion target. Note that rate of posterior placental location is significantly lower in Group 1. This is likely due to the fact that, based on accessibility, several areas of free loop sites were more accessible than cord insertion sites.

On the basis of accessibility and visualization, the safety of cordocentesis puncture at free loop and cord insertion was comparable in term of umbilical cord bleeding, preterm birth, low birth weight, and fetal loss. However, the target site at free loop may be associated with more difficulty, when defined by prolonged procedures. A disadvantage of cord insertion puncture was a significantly increased chance of maternal blood contamination, although this was a rare occurrence when performed by experienced hands. Of note, puncture at a free loop did not eliminate maternal blood contamination, although occurring with a lower rate. This might be associated with trauma to the nearby placenta or spiral vessels in the myometrium, while withdrawing back or blind injury during the needle tip out of the scene. Of note, percentage of cord bleeding was similar. It may be concluded that rate of cord vessel injury is not increased with a free loop puncture when performed by experienced hands.

The weakness of this study might include no evaluation of the abdominal wall thickness, which made the procedure more difficult, leading to adverse outcomes. However, this was likely to have only a minimal effect, as it did not influence on making decision on selecting the target site. Difficulty secondary to thick abdominal wall may presumably distribute into both groups equally. Moreover, although our sample size was large enough to gain power in evaluation of several outcomes, but it was probably still small for assessment a rare occurring event like fetal loss rate. In addition, the results of safety evaluation according to puncture site in this study could not be applied to practice of which cord insertion is the main target of first choice, as mentioned above.

The strength of this study includes large sample size from a single center, which gains enough power to assess such a rare outcome like fetal loss rate. In addition, strongly potential confounding factors on adverse pregnancy outcomes were excluded, in particular placenta penetration which was likely to influence on adverse pregnancy and procedure outcomes. Moreover, cases recruited to the study were relatively homogeneous in term of gestational age, 18–22 weeks of gestation and the levels of experience. Certainly, levels of expertise can affect the success rate of procedures and pregnancy outcomes. Cordocentesis of the first 50 cases of learning curve takes more time (Tongsong, *et al.*, 2000), reflecting theoretical risk associated with low experience. Therefore, procedures in early learning curve might be a confounder for the study outcomes and we, thus, excluded them from the study.

In conclusion, cordocentesis performed based on the best accessibility and quality of visualization, puncture sites at cord insertion and free loop were associated with comparable safety in term of rates of pregnancy outcomes. However, cord insertion puncture may cause more maternal blood contamination. This study supports the practice based on accessibility and quality of visualization.

ACKNOWLEDGEMENTS

The authors wish to thank Thai Research Fund and the National Research University Project under Thailand's Office of the Higher Education Commission for financial support.

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Comparisons of outcomes after cordocentesis at mid-pregnancy between singleton and twin pregnancies

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Objective To compare the outcomes after mid-pregnancy cordocentesis between singleton and twin pregnancies.

Methods We compared immediate complications and pregnancy outcomes between a cohort of twin pregnancies undergoing mid-pregnancy diagnostic cordocentesis between the years 1989 and 2010, and a control group of singleton pregnancies matched based on maternal age and years of the procedure with a ratio of 1 : 3.

Results Among 6147 pregnancies undergoing cordocentesis during the study period, 122 twin fetuses met the study criteria and were matched with 336 singleton controls. Success rate of sampling was slightly higher in singleton than in twin gestations (98.8% vs 97.3%), and time required for the procedure was significantly longer in the twin group (8.1 vs 6.3 min, $p = 0.02$). Immediate complications, such as fetal bradycardia and transient bleeding from the puncture site, were significantly higher in twins (13% vs 6.0%, $p = 0.001$; and 34.8% vs 26.1%, $p = 0.03$, respectively). However, fetal loss rate within 2 weeks of cordocentesis was not different (1.4% and 1.1%, $p = 0.42$).

Conclusion Immediate complications following cordocentesis are significantly higher in twin pregnancies, but fetal loss rate within 2 weeks of the procedure is comparable. Copyright © 2011 John Wiley & Sons, Ltd.

KEY WORDS: cordocentesis; pregnancy outcome; prenatal diagnosis; fetal blood sampling; twins; multifetal pregnancies

INTRODUCTION

Cordocentesis is an invasive prenatal diagnostic procedure aimed at obtaining a fetal blood sample from the umbilical cord for diagnostic purposes. Though there are alternative means of invasive prenatal diagnosis with lower complication rates, such as amniocentesis and chorionic villus sampling, cordocentesis is still an important procedure in maternal-fetal medicine. Moreover, cordocentesis may be the only one option in certain situations such as fetal blood analysis for biochemical or hematologic studies, and for intrauterine therapy or intrauterine blood transfusion. Many studies on cordocentesis in singleton pregnancies have been published (Kanokpongsukdi *et al.*, 1985; Rudigoz *et al.*, 1990; Bell and Weiner, 1993; Wax and Blakemore, 1993; Megerian and Ludomirsky, 1994; Pardi *et al.*, 1994; Wilson *et al.*, 1994; Bernaschek *et al.*, 1995; Donner *et al.*, 1995; Tongsong *et al.*, 2000; Tongsong *et al.*, 2001). However, studies about this procedure in twin pregnancies have been reported in only a very limited number, and at various gestational ages (Cox *et al.*, 1987; Okamura *et al.*, 1994; Antsaklis *et al.*, 2003; Weisz and Rodeck, 2005; Tongprasert *et al.*, 2007), including one from our center (Tongprasert *et al.*, 2007). However, all of the previous studies are case series with descriptive analysis

and included only a small number of cases. Obviously, several series are needed to add to the existing body of knowledge in literatures to permit a comprehensive analysis of this invasive procedure. To date, no study has been reported comparing complications of cordocentesis and pregnancy outcomes between procedures performed on singleton and twin pregnancies. The objective of this study was to compare the procedure and pregnancy outcomes after cordocentesis at mid-pregnancy between singleton and twin pregnancies from a single tertiary center.

METHODS

The computerized prospective database of cordocenteses at the Maternal Fetal Medicine Unit, Maharaj Nakorn Chiang Mai Hospital, Chiang Mai University, was accessed, which contains data of all women who underwent cordocentesis at mid-pregnancy between January 1989 and December 2010. In our practice, all patients with proper indications for cordocentesis were counseled regarding the risk of fetal loss and other complications related to the procedure and then provided written informed consent. The inclusion criteria for this study consisted of (1) singleton or twin pregnancies at gestational age of 18 to 22 weeks, (2) cordocenteses performed by experienced operators, defined as having done more than 50 procedures on actual patients and (3) final outcomes of pregnancies available. Repeated

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cordocenteses and procedures after amniocentesis or chorionic villous sampling in the same pregnancy were excluded from analysis. We also excluded pregnancies which resulted in termination of pregnancy because of severe fetal thalassemia disease, chromosomal abnormality or major structural anomaly. Each twin fetus was matched in a 1:3 ratio with a control (singleton fetus) based on maternal age and year of the procedure. The twin pregnancy and the matched control underwent cordocentesis in the same year and had the same maternal age, within a range of 2 years. Patient characteristics, indications for cordocentesis, technical aspects and complications of the procedure were compared. This study was conducted with approval of the ethical committee, Faculty of Medicine, Chiang Mai University.

Cordocenteses were performed under real time ultrasound guidance using transabdominal 3.5-MHz curvilinear transducer equipped with machine either model SSD 620, 680EX, SSD 1700; SSD 5000, alpha 10 Aloka, Tokyo, Japan or E8 GE medical system, Wisconsin, USA. Detailed ultrasound scan was done to determine the number of fetuses, viability, gestational age, fetal biometry and anomaly scan, as well as placenta and umbilical cord locations. Cordocentesis was done under aseptic, free-hand technique and carried out as outpatient setting. A 22-gauge spinal needle was inserted under local anesthesia. The puncture site, either near the placental cord insertion or a free-floating loop, was identified based on the accessibility and quality of visualization, with an attempt to avoid placental penetration. Fetal blood was aspirated into 1-mL disposable syringes, and was sent to the laboratory for the pertinent tests. After the needle was removed, bleeding from the puncture site and the fetal heart rate were assessed. Presence of fetal bradycardia and bleeding at the puncture site were recorded, including duration of bradycardia or bleeding. Bleeding from the puncture site was classified as transient or prolonged based on the duration less or greater than 1 min. In case of twin pregnancies, the same procedure was repeated for the second twin. After 20 min of bed rest, the patients were discharged home without any tocolysis or antibiotics. Rh-negative women received anti-D immunoglobulin prophylaxis. If necessary, acid elution test was used to differentiate fetal and maternal blood before laboratory analysis using standard technique as previously described (Rujiwetpongstorn *et al.*, 2005). The singleton-continuing pregnancies were followed with standard antenatal care, whereas twin pregnancies were taken care at high-risk pregnancy unit until delivery. Outcomes of cordocentesis were prospectively recorded in the database of the Maternal Fetal Medicine Unit.

For the purpose of analysis, all available data in the twin group were used for comparison, whereas for pregnancy outcomes we excluded the twin pregnancies with affected fetuses which underwent termination of pregnancy or feticide. The main outcome measures for comparison of procedural techniques were rates of fetal bradycardia, umbilical cord bleeding, duration of the procedures and success rates of sampling. With regard to pregnancy outcomes, we compared the fetal loss rate within two weeks of the procedures.

STATISTICAL ANALYSIS

The analysis used the statistical software for the social sciences version 17.0 (Chicago, IL, USA). McNemar chi-square test was used to compare categorical variables, whereas continuous variables were compared using paired *t*-test.

RESULTS

During the 21-year period, a total of 6147 pregnancies underwent mid-pregnancy cordocentesis. Among them, 56 sets (112 fetuses) of twins and 336 matched controls meeting inclusion criteria with completed data for analysis were recruited. The mean ($\pm$ SD) maternal age was 28.5 ± 6.4 (15–44) years and mean gestational age at the time of cordocentesis was 19.5 ± 1.6 (18–22) weeks. Technical aspects of cordocentesis compared between the singleton and twin groups are presented in Table 1. Success rates of sampling were 98.8% vs 97.3% (p -value = 0.001) in the control and twin groups, respectively. Duration of the procedure in each fetus was significantly longer in the group of twins than in singletons (8.1 ± 7.0 vs 6.3 ± 7.6 min, p -value 0.025). Immediate complications such as fetal bradycardia (13% vs 6.0%, p -value 0.001) and transient bleeding from the puncture site (34.8% vs 26%, p -value 0.375) were higher among twins than singletons.

Among the twin group, 12 fetuses (six pregnancies) were electively terminated and six underwent selective feticide due to severe fetal disease. The remaining 94 fetuses in this group continued pregnancy to delivery. After excluding the fetuses which underwent termination or feticide and their matched singleton fetuses in the control group, outcomes of pregnancies between the two groups were compared, as shown in Table 2. One of the fetuses following selective feticide died within 2 weeks of the procedure. Fetal loss rate within 2 weeks of cordocenteses was not significantly different between the two groups (1.4 and 1.1%, p -value 0.42), including one fetal loss in the control group occurring within 72 h after the procedures. As expected, the rates of low birth weight and preterm births were significantly higher in the group of twins.

DISCUSSION

Our study on cordocentesis in twins provides new insights on several aspects of this invasive prenatal diagnostic procedure. We have found that the time needed to complete the procedure for each fetus is significantly longer and the success rate is slightly lower in the twin group. Though the 2-min difference may not be clinically significant given the quite comparable success rates, this may imply more difficulty and theoretically higher risk of complications. This may be due to a limitation in the selection of the target puncture site, due to space constraint and crowding. Similarly, the incidence of bleeding from the puncture site, which may signify the

CORDOCENTESIS IN TWIN PREGNANCIES

Table 1—Comparison of technical aspects of cordocentesis procedures and immediate complications between singleton and twin groups

Technical aspects and immediate complications	Singleton (336 fetuses)	Twin (112 fetuses)	<i>p</i> -Value
Indications for cordocentesis			
• Risk of thalassemia major disease	253 (75.3%)	88 (78.6%)	0.17
• Karyotyping	22 (6.5%)	4 (3.6%)	—
• Karyotype and thalassemia major	59 (17.6%)	20 (17.9%)	—
• Others	2 (0.6%)	0	—
Anterior placenta (fetuses, %)	147 (43.8)	44 (39.3)	<0.005
Puncture site (fetuses, %)	—	—	0.09
• Free-floating loop	270 (80.4%)	88 (78.6%)	—
• Cord insertion	66 (19.6%)	24 (21.4%)	—
Duration of procedure; minutes (mean ± SD)	6.3 ± 7.6	8.1 ± 6.76	0.02
Success rate of sampling (fetuses, %)	329 (98.8)	109 (97.3)	0.001
Fetal bradycardia (fetuses, %)	20 (6.0)	14 (13)	0.001
• Missing data	—	4	—
Bleeding from puncture site (fetuses, %)			
• Prolonged bleeding	7 (2.7%)	0	—
• Transient bleeding	69 (26.1%)	31 (34.8%)	0.37
• No bleeding	188 (71.2%)	58 (65.2%)	—
• Missing data	72	23	—

Table 2—Outcome of mid-pregnancy cordocentesis, compared between group of singleton and twin

Outcomes	Singleton (282 fetuses)	Twin (94 fetuses)	<i>p</i> -Value
Fetal loss within 2 weeks of cordocentesis (fetuses, %)	4 (1.4)	1 (1.1)	0.42
Birth weight, grams (mean ± SD)	3037 ± 489	2279 ± 605	NA
Low birth weight infant (fetuses, %)	27 (9.6)	38 (40.4)	NA
Gestational age at delivery, weeks (mean ± SD)	38.3 ± 4.3	35.3 ± 3.1	NA
Preterm birth (fetuses, %)	34 (12.1)	42 (44.7)	NA

NA, not applicable, test for significant difference was not performed due to irrelevance.

technical difficulty, was also significantly more common in twins. Though no prolonged bleeding was observed in the twins in our series, this immediate complication may affect outcomes after cordocentesis. Fetal bradycardia, though usually temporary, may also have adverse effects on pregnancy outcomes. In our study, immediate fetal bradycardia following cordocentesis occurred more often in the twin than the singleton group, but most of such bradycardias were benign and transient. However, severe and persistent fetal bradycardias could affect the outcomes of cordocentesis such as fetal loss after the procedure. Bradycardias may be related to the difficulty of the procedure as described above. Alternatively, this finding may signify less placental reserve in some twins compared with singletons. Taken together, our findings suggest that cordocentesis in twin gestations may be more technically difficult. Other technical aspects such as puncture site were comparable between the two groups. In our practice, the puncture site for cordocentesis is chosen based on accessibility and quality of visualization whether in singleton or twin pregnancies. Consequently, the puncture site for cordocentesis (either at the cord insertion or at a free-floating loop of cord) was not significantly different between the two groups.

One of the main concerns for cordocentesis is the fetal loss rate. In our series, the overall fetal loss rate within 2 weeks of the procedure, including the procedure-related fetal loss and background loss, was not

significantly different between the two groups, suggesting that cordocentesis in twin pregnancies performed by experienced operators, and for certain indications, may not increase the risk of fetal loss over that performed in a singleton pregnancies. Though ours is a rather large series of twin pregnancies, the sample size may still be too small to allow adequate statistical power to address such a rare complication.

To date, only few studies on cordocentesis in twin pregnancies have been published. The data from previous studies and ours are summarized in Table 3. Compared with previous studies (Cox *et al.*, 1987; Antsaklis *et al.*, 2003), fetal loss within 2 weeks of the procedure in our study occurred at the lowest rate (1.1%), whereas the success rate of fetal blood sampling was comparable. However, several aspects of the techniques do not allow proper comparison of the results. The time frames of database collection, especially in the study by Cox *et al.* (1987), could have affected the quality of the ultrasound imaging. In the largest study reported by Antsaklis *et al.* (2003), the fetal loss rate was 8.2% but the series also included fetal blood samplings via fetoscopy, which is a more invasive technique of fetal blood sampling.

The strength of our study includes a rather large sample size from a single center with high homogeneity in term of gestational age and expertise levels of the operators. We excluded the procedures in the first 50 cases of each operator to limit the confounding effect of

Table 3—Comparison between The previous studies and present study about cordocentesis in twin pregnancies

	Cox <i>et al.</i> (1987)	Antsaklis <i>et al.</i> (2003)	Our study (2011)
Period (years)	No data	1977–2000 (23)	1989–2010 (21)
Total pregnancies (<i>n</i>)	19	89	56
Total fetuses sampled (<i>n</i>)	34	178	112
Gestational age (week) at the time of cordocentesis (mean and range)	18–34	20.3 (18–29)	19.5 (18–22)
Success rate of sampling (%)	33/34 (97.0)	100	109/112 (97.3)
Technique of cordocentesis	Needle guided by ultrasound	Fetoscopy (the first 28 cases, before 1985) and ultrasound-guided free-hand technique	Ultrasound-guided free-hand technique
Averaged-time used (min)	2.67	No data	8.14
Procedure-related complications			
• Bleeding at puncture site (%)	No data	No data	31/112 (34.8)
• Fetal bradycardia (%)	No data	No data	14/112 (13)
• Fetal loss within 2 weeks (%)	4/33 (12.1)	9/110 (8.2)	1/94 (1.1)
Preterm delivery [<i>n</i> (%)]	9/21 (42.9)	24/53 (45.3)	42/94 (44.7)

the learning curve, resulting in minimal inter-observer variation of skill.

In conclusion, our study suggests that immediate risks secondary to cordocentesis are significantly higher in twin pregnancies than in singletons in terms of procedure duration, transient fetal bradycardia and bleeding from the puncture site. Nevertheless, the fetal loss rate within 2 weeks of cordocentesis is not different between the two groups, provided the procedure is performed by experienced operators and within the limits of the indications for the procedure included in our study.

ACKNOWLEDGEMENTS

The authors wish to thank Thai Research Fund and the National Research University Project under Thailand's Office of the Higher Education Commission for financial support.

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Reference Ranges for the Fetal Cardiac Circumference Derived by Cardio–Spatiotemporal Image Correlation From 14 to 40 Weeks' Gestation

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Objectives—The purpose of this study was to construct reference ranges for the fetal cardiac circumference derived from volume data sets obtained by cardio–spatiotemporal image correlation.

Methods—A prospective descriptive study was conducted on normal singleton pregnancies with certain dates from 14 to 40 weeks' gestation. All underwent cardio–spatiotemporal image correlation to acquire volume data sets for subsequent analysis. Cardiac circumferences were measured offline in a multiplanar view with **4-dimensional imaging software**. The reference ranges were constructed against gestational weeks and the biparietal diameter as independent variables, using regression models for both the mean and SD.

Results—A total of 678 satisfactory volumes were analyzed. Normal reference ranges for predicting means and SDs of the fetal cardiac circumference were established based on best-fitted equations. The mean cardiac circumference (millimeters) was modeled as a function of gestational age (weeks) and biparietal diameter (centimeters) as follows: cardiac circumference = $-53.11 + 6.56 \times \text{gestational age} - 0.035 \times \text{gestational age}^2$ (SD = $0.67 + 0.18 \times \text{gestational age}$) and $-17.60 + 17.68 \times \text{biparietal diameter}$ (SD = $1.651 + 0.61 \times \text{biparietal diameter}$). Equations for z score calculation were also provided, and percentile charts for predicting the cardiac circumference at various points of gestational age and biparietal diameter were constructed.

Conclusions—Normal reference ranges and z scores for the fetal cardiac circumference have been provided. These normative data may be useful tools for assessment of fetal cardiac size, especially in cardiomegaly due to fetal anemia.

Key Words—fetal cardiac circumference; 4-dimensional sonography; prenatal; reference range; spatiotemporal image correlation; z scores

Received February 10, 2011, from the Department of Obstetrics and Gynecology, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand. Revision requested March 2, 2011. Revised manuscript accepted for publication April 6, 2011.

This work was supported by the Thai Research Fund and the National Research University Project under Thailand's Office of the Higher Education Commission.

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Assessment of fetal cardiac size is essential in prenatal diagnosis of various cardiac anomalies, especially cardiomegaly secondary to fetal anemia of various causes. For example, our experience shows that the cardiac diameter for cardiac size assessment is very helpful in early diagnosis of cardiomegaly due to hemoglobin Bart disease.¹ Theoretically, measurement of the cardiac circumference is certainly more accurate than the cardiac diameter because it better represents the cardiac size in all dimensions. In the past, we rarely measured the cardiac circumference because it is more time-consuming and subject to measurement error because of a poor border outline, especially in early gestation. Therefore, a only few studies on cardiac circumference have been

published.^{2–6} Additionally, most included only short gestational periods or small sample sizes. Moreover, only a study reported by Lee et al⁵ provided *z* scores as a quantitative assessment for clinical purposes. Importantly, *z* scores allow the examiner to quantify the cardiac size without the limitations of traditional confidence intervals. However, the study by Lee et al⁵ was based on a retrospective database assessment, and their *z* score reference ranges were derived from 2-dimensional fetal echocardiography, with which it is relatively difficult to get a precise plane for a cardiac study compared to 4-dimensional sonography with spatiotemporal image correlation (cardio–spatiotemporal image correlation).

With **cardio–spatiotemporal image correlation**, the focused structures can be shown in all dimensions, leading to more precise measurement.⁷ Therefore, we conducted this prospective study to develop *z* scores and percentile reference ranges for the fetal cardiac circumference derived from cardio–spatiotemporal image correlation. To our best knowledge, reference ranges for the cardiac circumference derived from cardio–spatiotemporal image correlation have not been reported previously.

Materials and Methods

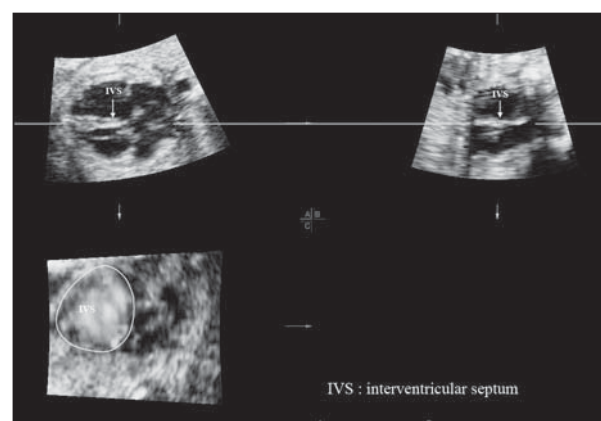
This prospective descriptive study was conducted between September 1, 2007, and October 31, 2010, at Maharaj Nakorn Chiang Mai Hospital, Chiang Mai University, with approval from the institute's Ethics Committee. Normal singleton pregnancies were recruited from the antenatal care unit, and written informed consent was obtained. The inclusion criteria were as follows: (1) gestational age between 14 and 40 weeks, (2) accurate gestational age based on sonographic fetal biometric measurements in the first half of pregnancy and consistent with a reliable menstrual period, and (3) low-risk pregnancies without known medical or obstetric complications. The exclusion criteria were as follows: (1) multifetal pregnancies, (2) fetal anomalies, (3) abnormal fetal growth (fetal growth restriction [<10 th percentile] or macrosomia [>90 th percentile]), and (4) inability to obtain satisfactory cardio–spatiotemporal image correlation volume data sets.

All patients underwent standard sonographic examinations, including fetal biometric measurements with an anatomic survey, and cardio–spatiotemporal image correlation volumes were acquired using a real-time machine with 2- to 5-MHz curvilinear transabdominal transducers (Voluson E8; GE Healthcare, Milwaukee, WI). The volume data sets were acquired with transverse sweeps through the fetal chest, which included the proper apical

and transverse 4-chamber views. Acquisition times ranged from 7.5 to 15 seconds, and acquisition angles ranged from 20° to 40° depending on the gestational age and cardiac size. All of the cardio–spatiotemporal image correlation volumes were stored in the ultrasound machine's hard drive for subsequent offline analysis. The volume data set analysis was systematically performed to identify the exact 4-chamber view, according to instructions described elsewhere,^{8,9} using 4D View version 9.0 software (GE Healthcare, Zipf, Austria), as shown in Figure 1.

The analysis is summarized as follows: Dynamic images of the fetal heart were simultaneously visualized in the 3 orthogonal planes on panels A, B, and C of the multiplanar views. Images of the 4-chamber view were minutely maneuvered by moving the reference dot and rotating the fetal heart around the 3 orthogonal axes (*x*, *y*, and *z*) to get the interventricular septum in the exact horizontal plane in all 3 panels. The proper image could be checked by showing that the interventricular septum in panel A was on the same line as in panel B, and a total en face view of the interventricular septum was visualized in panel C (Figure 1). The typical 4-chamber view in panel A was frozen at end-diastole and then was displayed and magnified as a single image on the screen. The cardiac circumference was measured at end-diastole using the area point or area trace function in 4D View.

Regression analysis of the fetal cardiac circumference was performed to identify the best-fitted equation using SPSS version 17.0 software (SPSS Inc, Chicago, IL). The procedure followed instructions published by Royston and Wright.¹⁰ Briefly, regression models were fitted separately to the mean and SD of the cardiac circumference (depen-



dent variables) to generate the best-fitted regression equations. The best-fitted models for the SD were derived from regression of scaled absolute residuals which were obtained as: scaled absolute residual = $1.25 \times$ absolute (measured value – predicted value). Normality and goodness-of-fit regression models were assessed by examination of the scatter patterns of points relative to fitted means and SDs, expressed as z scores (measured value – estimated mean/estimated SD). The z scores were tested for normality using the Shapiro-Wilk W test and Q-Q plots. Percentile curves and tables of reference ranges were constructed by the formula percentile = mean + $K \times$ SD, where K was the corresponding percentile of the standard Gaussian distribution.

Results

A total of 710 cardio-spatiotemporal image correlation volumes were successfully acquired and available for offline analysis; however, only 678 volume data sets from 678 fetuses had satisfactory cardiac circumference measurements. The mean maternal age $\pm$ SD was 26.74 ± 6.31 years (range, 15–43 years), and most of them (395 [58.26%]) were nulliparous. The number of participants at each gestational age is shown in Table 1.

A quadratic regression model was the best description of the predicted mean of the cardiac circumference based on gestational weeks, whereas linear regression was the best-fitted model for predicting the mean based on the biparietal diameter and SD of both independent variables, as presented in Table 2 and Figures 2 and 3. These equations were validated by constructing a z score for each variable. The normalcy of the z scores was evident in a Q-Q plot. The Shapiro-Wilk W test showed normality in the distribution ($P = .58$). Additionally, the z scores were evenly distributed above and below 0 across the entire range of gestational ages and biparietal diameters and had a standard normal distribution. The z scores that were outside the range of ± 2 SD did not differ significantly from the expected 10% of the values (Figure 4). Percentile models for the cardiac circumference as a function of gestational age in weeks and biparietal diameter in centimeters are shown in Tables 1 and 3, respectively.

The z score for each actual cardiac circumference value can be calculated as follows: z score = (measured cardiac circumference value – predicted value)/predicted SD. Example of a z score calculation: actual measured biparietal diameter = 4.0 cm; cardiac circumference = 68 mm. Using the formula in Table 2: predicted cardiac circumference = $-17.60 + (17.68 \times 4.0) = 53.12$ mm; predicted SD of car-

diac circumference = $1.6512 + (0.61 \times 4.0) = 4.07$ mm; and z score = $(68 - 53)/4 = 3.75$.

Therefore, the fetuses had an enlarged cardiac circumference at 3.75 SD above the predicted mean cardiac circumference for a fetus whose biparietal diameter was 4 cm (out of the normal range).

Discussion

Although our cardiac diameter measurement is simple, more practical, reproducible, less time-consuming, and very helpful for early diagnosis of cardiomegaly secondary to various causes, especially fetal anemia,¹ theoretically, the cardiac circumference better represents the fetal heart size than the cardiac diameter. However, in practice, the measurement is more time-consuming and subject to error in cases with a poor cardiac border. Currently, high-resolution sonography allows tracing of the heart perimeter with higher reliability than ever before. Normal reference ranges for the cardiac circumference are very limited, especially z

Table 1. Fetal Cardiac Circumference as a Function of Gestational Age

GA, wk	n	Cardiac Circumference, mm						
		2.5th	5th	10th	50th	90th	95th	97.5th
14	15	26	27	28	32	36	37	38
15	16	31	32	33	38	42	43	44
16	17	36	37	39	43	48	49	50
17	20	41	42	44	48	53	54	56
18	21	46	47	49	54	59	60	61
19	19	51	52	54	59	64	66	67
20	19	56	57	59	64	70	71	73
21	24	61	62	64	69	75	77	78
22	27	66	67	69	75	80	82	83
23	28	70	72	73	80	86	87	89
24	28	75	76	78	84	91	93	94
25	27	79	81	83	89	96	98	99
26	26	84	85	87	94	101	103	104
27	33	88	90	92	99	106	108	109
28	34	93	94	96	103	111	113	114
29	31	97	99	101	108	115	118	119
30	35	101	103	105	113	120	122	124
31	38	105	107	109	117	125	127	129
32	28	109	111	113	121	130	132	134
33	22	113	115	117	126	134	136	138
34	24	117	119	121	130	139	141	143
35	31	121	123	125	134	143	145	148
36	26	125	127	129	138	147	150	152
37	27	128	130	133	142	151	154	156
38	26	132	134	137	146	156	158	161
39	19	135	138	140	150	160	163	165
40	17	139	141	144	154	164	167	169

GA indicates gestational age; and n, sample size for each gestational week.

Table 2. Regression Models for Prediction of the Mean and SD of the Fetal Cardiac Circumference Based on Gestational Age and Biparietal Diameter

Parameter	Model Derived From Regression Analysis	r
Gestational age (GA), wk		
Cardiac circumference, mm	$-53.11 + 6.56 \times \text{GA} - 0.035 \times \text{GA}^2$	0.97
SD of cardiac circumference, mm	$0.67 + 0.18 \times \text{GA}$	0.28
Biparietal diameter (BPD), cm		
Cardiac circumference, mm	$-17.60 + 17.68 \times \text{BPD}$	0.97
SD of cardiac circumference, mm	$1.651 + 0.61 \times \text{BPD}$	0.25

Figure 2. Quadratic function relationship between gestational age and the cardiac circumference. The lines represent the 2.5th, 5th, 10th, 50th, 90th, 95th, and 97.5th percentiles.

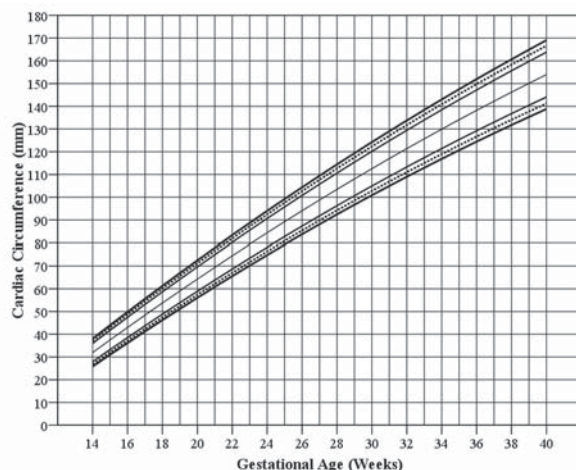
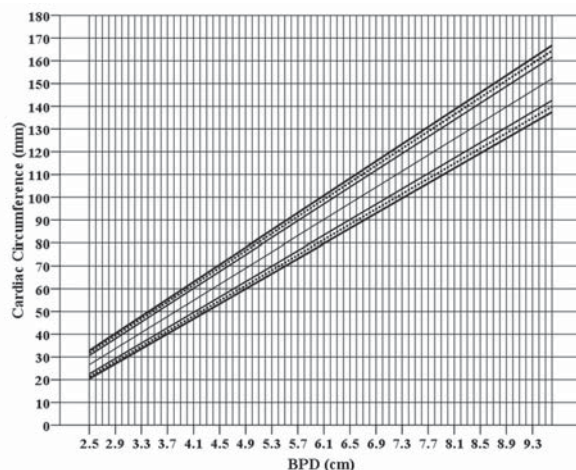


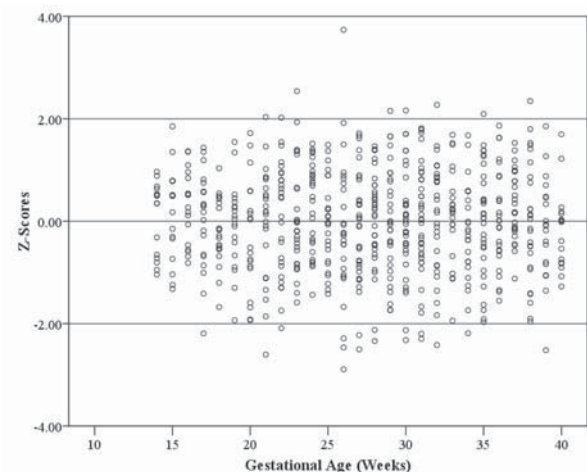
Figure 3. Linear relationship between the biparietal diameter (BPD) and cardiac circumference. The lines represent the 2.5th, 5th, 10th, 50th, 90th, 95th, and 97.5th percentiles.



scores for quantitative assessment of fetal cardiac size. On the basis of this study and previous reports,^{2,3,5,11} the cardiac size increases steadily through gestational age. However, our results are somewhat different from those of other studies in that the best-fitted model for gestational age was a quadratic function rather than linear, as in other studies. We found that the SD of the cardiac circumference was greater with increasing gestational age. Therefore, we had to analyze and regress the SD separately. To our knowledge, SD analysis was not performed in most previous studies; however, it is critically important for z score assessment.^{10,12}

In addition to providing z score assessment, another main difference from other studies was that our data were derived from cardio-spatiotemporal image correlation. With conventional 2-dimensional sonography, it is difficult to get a proper plane in the 4-chamber view, even with the ultrasound beam exactly perpendicular to the interventricular septum, because the depicted image on the screen, although appearing to be perfect in the x- and y-axes, can be tilted toward or away from the examiner in the

Figure 4. Distribution of calculated z scores for the cardiac circumference against gestational age, indicating an adequate level of fit.



z-axis, resulting in asymmetry of the left and right sides of the heart and leading to an inaccurate circumference. Obtaining a perfect 4-chamber view is often time-consuming and sometimes impossible. With the innovative cardio-spatiotemporal image correlation, this problem has been solved. It is very simple, less time-consuming, and highly reliable for getting the proper orientation. Unlike an examination with 2-dimensional sonography, in which we have to orient the transducer to visualize the proper plane, with the offline analysis of cardio-spatiotemporal image correlation, we can control and orient the virtual heart volume to get the proper plane and not have to orient the transducer. The interventricular septum can be simply maneuvered to be on the exact horizontal line in panels A and

B, and a total en face view can be displayed in panel C, resulting in more reliable measurements.

In this study, we used the area point or area trace function available in 4D View version 9.0 to trace the cardiac outline as meticulously as we wanted. We chose this technique because the heart shape is too complex, not symmetrically geometric, to be accurately assessed with a simple eclipse tool or computed from two diameters, as in previous studies.^{5,11}

One limitation of the cardio-spatiotemporal image correlation used in this study was that the resolution of the volume data sets may have been somewhat compromised in some cases because of fetal movement and maternal breathing. Additionally, even with a high-resolution ultra-

Table 3. Fetal Cardiac Circumference as a Function of Biparietal Diameter

BPD, cm	Cardiac Circumference, mm							BPD, cm	Cardiac Circumference, mm						
	2.5th	5th	10th	50th	90th	95th	97.5th		2.5th	5th	10th	50th	90th	95th	97.5th
2.5	20	21	23	27	31	32	33	6.1	80	81	83	90	97	99	101
2.6	22	23	24	28	33	34	35	6.2	81	83	85	92	99	101	103
2.7	24	25	26	30	34	36	37	6.3	83	85	87	94	101	103	105
2.8	25	26	28	32	36	37	39	6.4	85	87	89	96	103	105	106
2.9	27	28	29	34	38	39	40	6.5	86	88	90	97	105	107	108
3.0	29	30	31	35	40	41	42	6.6	88	90	92	99	106	108	110
3.1	30	31	33	37	42	43	44	6.7	90	92	94	101	108	110	112
3.2	32	33	34	39	44	45	46	6.8	91	93	95	103	110	112	114
3.3	34	35	36	41	45	47	48	6.9	93	95	97	104	112	114	116
3.4	35	36	38	43	47	49	50	7.0	95	97	99	106	114	116	118
3.5	37	38	39	44	49	51	52	7.1	96	98	100	108	116	118	120
3.6	39	40	41	46	51	52	54	7.2	98	100	102	110	117	120	122
3.7	40	41	43	48	53	54	55	7.3	100	102	104	112	119	122	123
3.8	42	43	45	50	55	56	57	7.4	101	103	105	113	121	123	125
3.9	44	45	46	51	57	58	59	7.5	103	105	107	115	123	125	127
4.0	45	46	48	53	58	60	61	7.6	105	107	109	117	125	127	129
4.1	47	48	50	55	60	62	63	7.7	106	108	110	119	127	129	131
4.2	48	50	51	57	62	64	65	7.8	108	110	112	120	129	131	133
4.3	50	51	53	58	64	65	67	7.9	109	112	114	122	130	133	135
4.4	52	53	55	60	66	67	69	8.0	111	113	116	124	132	135	137
4.5	53	55	56	62	68	69	71	8.1	113	115	117	126	134	136	139
4.6	55	56	58	64	69	71	72	8.2	114	117	119	127	136	138	140
4.7	57	58	60	66	71	73	74	8.3	116	118	121	129	138	140	142
4.8	58	60	61	67	73	75	76	8.4	118	120	122	131	140	142	144
4.9	60	61	63	69	75	77	78	8.5	119	122	124	133	141	144	146
5.0	62	63	65	71	77	79	80	8.6	121	123	126	134	143	146	148
5.1	63	65	67	73	79	80	82	8.7	123	125	127	136	145	148	150
5.2	65	66	68	74	81	82	84	8.8	124	127	129	138	147	150	152
5.3	67	68	70	76	82	84	86	8.9	126	128	131	140	149	151	154
5.4	68	70	72	78	84	86	88	9.0	128	130	132	142	151	153	155
5.5	70	71	73	80	86	88	89	9.1	129	132	134	143	153	155	157
5.6	72	73	75	81	88	90	91	9.2	131	133	136	145	154	157	159
5.7	73	75	77	83	90	92	93	9.3	133	135	138	147	156	159	161
5.8	75	76	78	85	92	93	95	9.4	134	137	139	149	158	161	163
5.9	77	78	80	87	93	95	97	9.5	136	138	141	150	160	163	165
6.0	78	80	82	89	95	97	99	9.6	138	140	143	152	162	164	167

BPD indicates biparietal diameter.

sound machine, the small cardiac structures early in the second trimester could not be clearly distinguished from surrounding structures, and several volume data sets in early gestation were excluded because of unsatisfactory quality for analysis. Moreover, it was sometimes impossible to obtain satisfactory volumes in fetuses with very active and long movement or when they were poorly accessible, especially in a supine position.

The strengths of this study included first its large sample size (678), ranging from 15 to 38 examinations per gestational week; constructing a normal reference range with a +1 to 3 SD curve and restricting the SE of the limits of the reference range to 10% of the SD requires a sample size of about 550 fetuses (14–40 weeks).¹⁰ Second, measurement based on offline analysis with cardio–spatiotemporal image correlation did not have a time limit, allowing us to obtain reliable planes of exact 4-chamber views. Third, our normative tables included fetuses in early and late gestation, unlike in most previous reports. Finally, *z* scores for quantitative evaluation were also provided, and the *z* score distribution against gestational age and biparietal diameter indicated an adequate model fit.

In conclusion, reference ranges for the fetal cardiac circumference at each gestational age and biparietal diameter from 14 to 40 weeks were constructed on the basis of cardio–spatiotemporal image correlation volume data sets. These reference ranges may be useful tools for assessment of fetal cardiac size, especially when fetal cardiomegaly is suspected. However, the effectiveness of this fetal parameter is yet to be validated by further studies.

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Normal Reference Ranges of Inferior Vena Cava Doppler Indices From 14 to 40 Weeks of Gestation

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Received 15 January 2011; accepted 14 November 2011

ABSTRACT: *Objective:* To establish normal reference ranges of inferior vena cava (IVC) Doppler velocities in normal singleton pregnancies from 14 to 40 weeks.

Methods: A prospective descriptive study was conducted on normal singleton pregnancies with confirmed gestational age. The sonographic measurements of IVC Doppler indices, including preload index, peak velocity index for vein, and pulsatility index for vein, were determined by experienced sonographers.

Results: A total of 645 measurements were performed, ranging from 12 to 30 measurements available for each gestational week. The relationship between gestational age and IVC Doppler indices was not linear. The best-fitted regression equations for the indices are as follows:

$$\text{Preload index} = 66.579 \times \exp(-0.35 \times \text{GA}) + 0.183$$

$$\begin{aligned} \text{Peak velocity index of vein} &= 420.58 \\ &\times \exp(-0.350 \times \text{GA}) + 1.620 \end{aligned}$$

$$\begin{aligned} \text{Pulsatility index of vein} &= 565.81 \\ &\times \exp(-0.35 \times \text{GA}) + 1.690 \end{aligned}$$

(GA = gestational age in week)

All IVC Doppler indices rapidly declined from 14 to 20 weeks of gestation and then they were relatively constant or slightly decreased throughout the rest of pregnancy. The tables of normal reference ranges were constructed.

Conclusions: Normal reference ranges of IVC Doppler indices for each gestational week from 14 to 40 weeks were constructed. This normative data may be a useful noninvasive tool in evaluation of fetal cardiac

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Keywords: fetus; inferior vena cava; Doppler ultrasound; prenatal diagnosis

The inferior vena cava (IVC) drains blood from the lower extremities and abdominal cavity and returns it to the right atrium. Assessment of IVC blood flow can provide information relating to the compliance of the right ventricle. The introduction of color Doppler equipment has enabled hemodynamic assessment of the fetal venous system. Doppler flow measurements of IVC have an increasingly important role in the evaluation of various fetal circulatory and cardiac conditions, permitting evaluation of cardiac preload. Therefore, IVC Doppler indices are helpful in the assessment of cardiac function, especially in cardiac compromise secondary to fetal growth restriction,^{1,2} or congenital heart defects.³ During ventricular systole, pressure in the right atrium is lower than that in the IVC, resulting in forward flow from the IVC into the right atrium, represented by the S wave. At the completion of ventricular systole, the tricuspid valve opens, allowing blood to flow from the right atrium into the right ventricle during early, passive filling phase. This results in forward flow from the IVC as blood continues to flow into the right atrium, which is represented by the D wave. At the end of the rapid filling phase of diastolic filling, the right atrium contracts against a partially filled right ventricle. Because of the stiffness of the right ventricle, there is high resistance to blood entering the right ventricle. This results in the

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VOL. 00, NO. 0, MONTH 2011

negative a wave of IVC waveform. Indices commonly used to quantify IVC Doppler velocity include the preload index (PLI; a/S), peak velocity index for vein (PVIV; $S-a/D$), and pulsatility index for vein (PIV; $S-a/Tamx$).⁴ An abnormal pattern of these IVC indices can represent hemodynamic deterioration in the compromised fetal heart. However, for clinical use of IVC Doppler indices, it is essential that normal reference ranges first be established. To the best of our knowledge, few studies on reference ranges of the IVC Doppler indices have been published.^{4–8} Most were based on a small sample size of 35–60 fetuses. The two largest series reported by Axt-Flidner et al⁸ and Baschat⁴ consisted of sample sizes of 329 and 237 pregnancies, respectively, and excluded pregnancies in the early second trimester. Therefore, we conducted this research to establish nomograms of IVC indices, PLI, PVIV, and pulsatility index for veins, from 14 to 40 weeks of gestation, based on a large sample size.

MATERIALS AND METHODS

This prospective study was conducted with approval by the Ethic Committee. Normal singleton pregnancies were recruited with written informed consents. Inclusion criteria were 1) accurate gestational age based on reliable menstrual period and consistent with sonographic fetal biometry in the first half of pregnancies, 2) gestational age of 14–40 weeks, and 3) no obstetric and medical complication. Exclusion criteria were 1) fetal anomalies, 2) fetal growth restriction or macrosomia, 3) multifetal pregnancy, 4) poor quality imaging. IVC Doppler indices were measured using real-time equipment with color and pulsed Doppler capabilities, with transabdominal 2–5 MHz curvilinear transducers (Voluson E8; GE Healthcare, Milwaukee, WI; or SSD alpha-10; Aloka, Tokyo, Japan). In each examination, a standard ultrasound examination, including fetal anomaly screening and fetal biometry, was performed by four experienced perinatologists. Each participant had only one examination. Doppler measurements were obtained at a normal fetal heart rate of 120 to 160 bpm in the absence of maternal and fetal breathing. The measurement of IVC Doppler indices was performed as follows: 1) flow velocity waveforms from the IVC were obtained in a longitudinal section, 2) the sample volume was placed over the IVC immediately distal to its widening and entrance into the right atrium. The right hepatic vein was visualized to be sure the sam-

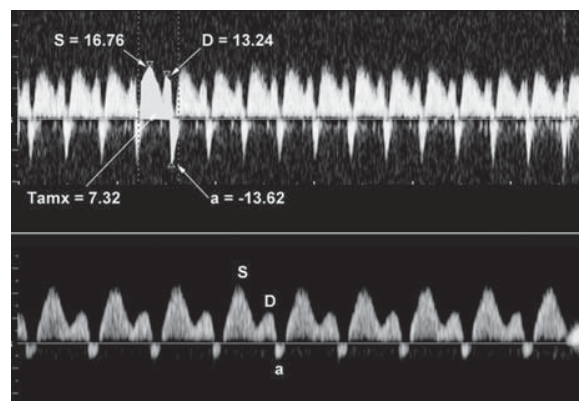


FIGURE 1. Inferior vena cava Doppler waveforms at 14 (upper) and 21 (lower) gestational weeks. S wave represents ventricular systole (forward flow, 16.76 cm/s). D wave represents the early filling of ventricular diastole (forward flow, 13.24 cm/s). a wave represents atrial contraction (reverse flow, 13.62 cm/s). TAmx is the time average maximal velocity (forward flow, 7.32 cm/s, yellow overlay) Note a prominent a wave at 14 weeks and smaller a wave at 21 weeks.

pling gate was not placed on this vein, 3) at least three consecutive high-quality waveforms were used to calculate automatically the PLI (a/S), PVIV ($S-a/D$), and pulsatility index ($S-a/Tamx$) (Figure 1). The collected data were analyzed using the Statistical Package for the Social Sciences (SPSS), version 17.0 (Chicago, IL). The distribution of IVC indices were tested for normality using the Kolomogorov-Smirnoff test. Regression analysis was used to express the correlation between IVC Doppler indices and gestational ages. The best-fit regression equation was determined and a nomogram of IVC Doppler indices was constructed for the 5th, 50th, and 95th percentile for each gestational week.

RESULTS

A total of 694 women were recruited into the study and 49 were excluded due to fetal growth disorders,^{1,4} fetal structural abnormalities,⁵ or chromosomal abnormalities.³ Of the remaining 645 measurements, the average number of measurements available for each week of gestational age was 21 ± 5 (mean $\pm$ SD), ranging from 12 to 30. Kolomogorov-Smirnoff test showed normal distribution. The mean ($\pm$ SD) maternal age was 28 ± 5 years (range, 15–43 years). Three hundred six of the 645 (47.4%) women studied were nulliparous. The best regression model between gestational week and IVC Doppler indices was observed to be nonlinear. The best-fitted equation functions for each index are as follows:

INFERIOR VENA CAVA DOPPLER INDICES

$$\begin{aligned} \text{Preload index} &= 66.957 \times \exp(-0.35 \times \text{GA}) \\ &\quad + 0.183 \quad (r^2 = 0.46) \end{aligned}$$

$$\begin{aligned} \text{Peak velocity index of vein} &= 420.58 \\ &\quad \times \exp(-0.350 \times \text{GA}) + 1.620 \quad (r^2 = 0.68) \end{aligned}$$

$$\begin{aligned} \text{Pulsatility index of vein} &= 565.81 \\ &\quad \times \exp(-0.35 \times \text{GA}) + 1.690 \quad (r^2 = 0.74) \end{aligned}$$

(GA = gestational week)

All IVC Doppler indices rapidly declined from 14 to 20 weeks of gestation and then they were relatively constant or slightly decreased throughout the rest of the pregnancy as shown in Figure 2. Table 1 shows the normal reference ranges constructed at various percentiles.

DISCUSSION

The IVC velocity pattern reflects the cardiac cycle with a forward flow peak during ventricular systole (S), another during passive diastolic filling (D), and a retrograde flow peak during atrial contraction (a). Therefore, IVC Doppler velocimetry can be informative for assessment of central venous pressure or atrial pressure and it carries new diagnostic possibilities to evaluate preload or cardiac function in clinical practice. For example, Rizzo et al.⁹ found that acidosis in growth-restricted fetuses can be noninvasively identified by Doppler measurements of the IVC, especially by the use of preload index.

Notably, our reference values and the patterns of IVC Doppler velocity apparently differ from those in previous reports. Axt-Fliedner et al.⁸ and Baschat⁴ showed a linear relationship between the IVC indices and gestational age, whereas the nonlinear equation was observed in this study. Not surprisingly, those studies included pregnancies in the second half of the pregnancy where the IVC Doppler indices are rather constant. In fact, we found the same pattern in the second half and the means as well as 95th percentile values were comparable. However, a rather rapid decline in all IVC Doppler indices or resistance in IVC during 10–20 weeks of gestation was clearly shown in this study. Our findings indicate that predicted IVC indices using the linear or quadratic equation reported in most studies are likely to be unreliable for early gestation because the pattern of IVC indices in the first half of the pregnancy is markedly different from that in the second half. Based on this study, during 14–20 weeks, the three IVC Doppler indi-

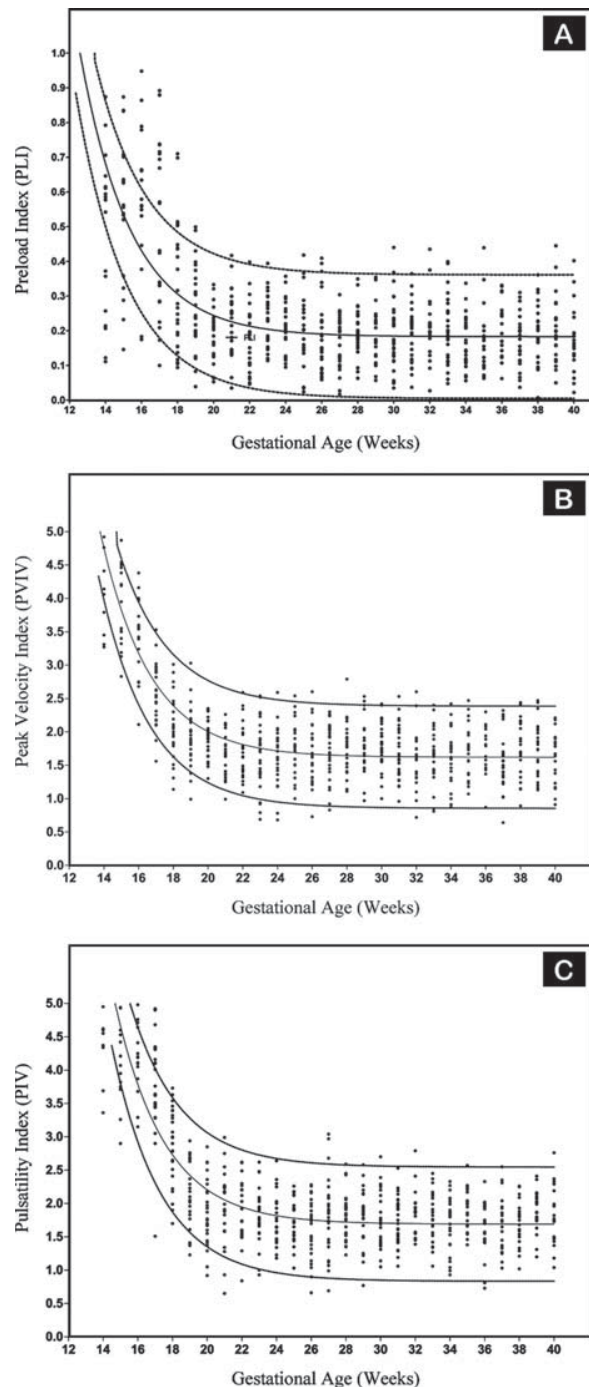


FIGURE 2. Scattergrams of inferior vena cava Doppler indices with predicted values at 5th, 50th, and 95th percentiles. **(A)** Preload index (PLI). **(B)** Peak velocity index for vein (PVIV). **(C)** Pulsatility index for vein (PIV).

ces showed a relatively rapid decrease, whereas they were relatively constant or slightly decreased during the second half. Therefore, we could not evaluate reliably IVC velocity or cardiac function in the first half of the pregnancy by using the best-fitted equations from those

TABLE 1
Doppler Indices (preload index, peak velocity index for vein, and pulsatility index for vein) in the IVC by Gestational Age

Gestational Age (weeks)	Preload Index			Peak Velocity Index for Vein			Pulsatility Index for Vein		
	5th Percentile	50th Percentile	95th Percentile	5th Percentile	50th Percentile	95th Percentile	5th Percentile	50th Percentile	95th Percentile
14	0.47	0.68	0.90	3.87	4.75	5.63	4.94	5.90	6.86
15	0.32	0.53	0.75	2.95	3.83	4.71	3.70	4.66	5.62
16	0.21	0.43	0.65	2.30	3.18	4.06	2.82	3.78	4.74
17	0.17	0.36	0.55	1.84	2.72	3.60	2.20	3.16	4.12
18	0.12	0.31	0.50	1.51	2.39	3.27	1.77	2.73	3.69
19	0.08	0.27	0.46	1.28	2.16	3.04	1.46	2.42	3.38
20	0.06	0.24	0.42	1.20	2.00	2.80	1.37	2.21	3.05
21	0.06	0.23	0.40	1.09	1.89	2.69	1.21	2.05	2.89
22	0.04	0.21	0.38	1.01	1.81	2.61	1.11	1.95	2.79
23	0.03	0.20	0.37	0.95	1.75	2.55	1.03	1.87	2.71
24	0.03	0.20	0.37	0.92	1.72	2.52	0.98	1.82	2.66
25	0.02	0.19	0.36	0.89	1.69	2.49	0.94	1.78	2.62
26	0.02	0.19	0.36	0.87	1.67	2.47	0.91	1.75	2.59
27	0.02	0.19	0.36	0.85	1.65	2.45	0.90	1.74	2.58
28	0.02	0.19	0.36	0.84	1.64	2.44	0.88	1.72	2.56
29	0.02	0.19	0.36	0.84	1.64	2.44	0.87	1.71	2.55
30	0.02	0.19	0.36	0.83	1.63	2.43	0.87	1.71	2.55
31	0.01	0.18	0.35	0.83	1.63	2.43	0.86	1.70	2.54
32	0.01	0.18	0.35	0.83	1.63	2.43	0.86	1.70	2.54
33	0.01	0.18	0.35	0.82	1.62	2.42	0.86	1.70	2.54
34	0.01	0.18	0.35	0.82	1.62	2.42	0.85	1.69	2.53
35	0.01	0.18	0.35	0.82	1.62	2.42	0.85	1.69	2.53
36	0.01	0.18	0.35	0.82	1.62	2.42	0.85	1.69	2.53
37	0.01	0.18	0.35	0.82	1.62	2.42	0.85	1.69	2.53
38	0.01	0.18	0.35	0.82	1.62	2.42	0.85	1.69	2.53
39	0.01	0.18	0.35	0.82	1.62	2.42	0.85	1.69	2.53
40	0.01	0.18	0.35	0.82	1.62	2.42	0.85	1.69	2.53

reports, such as the nomograms constructed by Axt-Fliedner et al⁸ and Baschat.⁴

In contrast to the trend reported by Baschat,⁴ who found a slightly increase in PLI with gestational age, we observed a slight decrease in PLI with advancing gestational age. This is probably due to a significant increase in time-averaged velocity (Tamx) and a significant decrease in percentage retrograde flow (a wave) with increasing gestational age as observed by our team (Figure 1), as well as by Huisman et al⁵ and Wladimiroff et al.⁶ Of interest, a relatively rapid decrease in IVC Doppler indices from 14 to 20 weeks of gestation may be associated with the increasing cardiac peak E wave/A wave ratio. Therefore, changes in IVC Doppler indices represent a marked increase in cardiac compliance during this period.

Some authors⁸ measured IVC Doppler waveforms as absolute velocity recordings. This is time-consuming and takes the extra challenge to record absolute velocities at a zero or low angle of insonation, which is not so important for the waveform qualitative analysis like in this study. We preferred qualitative assessment of the relationship among the three velocity components (S, D, and a).

In conclusion, the nomograms of fetal IVC Doppler indices from 14 to 40 weeks are established. This may be useful in the assessment of some fetal conditions associated with cardiac function disorders, especially when fetal growth restriction, anemia, or congenital heart defects are suspected.

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Case Report

Prenatal Sonographic Diagnosis of Congenital Varicella Syndrome

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Received 1 August 2011; accepted 28 December 2011

ABSTRACT: Congenital varicella syndrome is a rare disorder occurring in less than 1% of maternal varicella during early pregnancy but is associated with high fetal morbidity and mortality. This case report aimed to describe the sonographic features of congenital varicella syndrome following maternal varicella. Well-documented maternal chicken pox was made at 12 weeks of gestation and prenatal ultrasound was performed at 16 weeks. Striking sonographic features included hydropic changes and disseminated calcifications in multiple organs, especially liver and myocardium. Elective termination of pregnancy was done at 17 weeks. The presence of disseminated calcifications could suggest the diagnosis of congenital varicella syndrome. © 2012 Wiley Periodicals, Inc. *J Clin Ultrasound* 00:000–000, 2012; Published online in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/jcu.21891

Keywords: fetus; prenatal diagnosis; varicella; ultrasound

Varicella zoster virus (VZV) is a member of the herpesvirus family found throughout the world. It is highly infectious and causes chickenpox as the primary infection and herpes zoster when it reactivates. Varicella affecting women during pregnancy or neonates is rare. Primary VZV infection in the pregnant woman results in the birth of a normal newborn in most cases. Rarely, the outcome is congenital varicella syndrome and/or fetal death. VZV may be transmitted across the placenta and cause intrauterine infection at any time during gestation. However, the spectrum of clinical course correlates with

the gestational age of the fetus. The highest risk for congenital varicella syndrome is in the first 20 weeks of gestation, but the overall risk in the first 20 weeks is <1%.¹ Harger et al² reported that the frequency of congenital varicella syndrome is low (0.4%, 95% CI 0.1–2.4) in a prospectively study among 231 infants of mothers infected during pregnancy who had satisfactory follow-up data.

The features of congenital varicella syndrome may include chorioretinitis, microphthalmia cerebral cortical atrophy, growth restriction, hydronephrosis, and skin or bone defects.³ In this report, we describe the sonographic (US) features of congenital varicella syndrome following maternal varicella.

CASE REPORT

A 23-year-old primigravid woman was clinically diagnosed with chickenpox at 12 weeks of gestation by internists. She experienced constitutional symptoms of fever and malaise. Three days later, she developed the onset of rash characterized by superficial vesicular lesions with a erythematous base occurring in a cluster in various stages from maculopapular to vesicular or some crusted pustules, mostly involving the trunk and face. Her clinical manifestations disappeared a week later. Her serology revealed elevated IgG and IgM levels using enzyme-linked immunosorbent assay. The woman was referred to the high-risk antenatal care clinic for proper counseling on maternal exposure of varicella at 14 weeks of gestation.

On US examination performed at 16 weeks of gestation using a SonoAce 8000EX scanner and a transabdominal 3.5-MHz curvilinear transducer (Samsung-Medison, Seoul, Korea), a single viable fetus with a normal amount of amniotic fluid and

Additional Supporting Information may be found in the online version of this article.

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VOL. 00, NO. 0, MONTH 2012

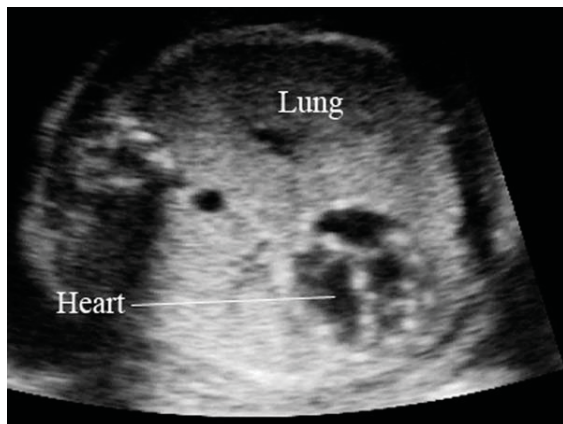


FIGURE 1. Sonogram of the fetal chest at the level of four-chamber view shows numerous calcifications in the myocardium and relatively small cardiac size.



FIGURE 2. Sonogram of the fetal upper abdomen shows ascites and numerous calcifications scattered throughout the liver.

three-vessel umbilical cord was demonstrated. Fetal biometry was compatible with 16 weeks of gestation. All extremities appeared to be normal in both biometry and morphology but fetal movement was observed to be subjectively decreased. Hydropic changes were evidenced by obvious ascites, subcutaneous edema, and a relatively thickened (3-cm thickness) placenta. Intracranial structures showing normal but few spots of calcification at the outer border of lateral ventricles were demonstrated. The lateral ventricular width was within the normal range (7 mm of atrial hemispheric width). The heart showed numerous echogenic foci in the myocardium (Figure 1, Supporting Information Video Clip 1), but the contractility was normal. Intracardiac echogenic foci and cardiac structural anomalies were not observed. However, the cardiac size was relatively small, with a cardiothoracic diameter ratio of 30%. Numerous intrahepatic calcifications were scattered throughout the liver (Figure 2, Supporting Information Video Clip 2). The other structures were unremarkable.

After comprehensive counseling, the couple chose to have elective termination of the pregnancy at 17 weeks of gestation, resulting in a male normally formed but hydropic abortus, with no limb or skin deformities. Abortal heart blood analysis demonstrated elevated IgG and IgM antibodies against varicella zoster. However, autopsy was not performed.

DISCUSSION

As mentioned above, a pregnant woman who contracts chickenpox carries a risk of fetal infection that varies from 1% to 20%.⁴ Infection during the first or second trimester may result in fetal

anomalies or varicella embryopathy. When the infection occurs perinatally, the fetus usually does not have structural abnormalities but may develop a fulminant neonatal infection. The highest risk of congenital varicella syndrome is between 13 and 20 weeks, probably 2%. Approximately one-third of affected infants die within the neonatal period, and survivors have severe abnormalities.⁴ Less than 2% of infants born to mothers infected with VZV during pregnancy develop congenital varicella syndrome. Embryopathy involves multiple organs with variable severity. In some cases, this syndrome may only be detected by serial ultrasound, because a latency period between maternal infection and the sonographic detection of fetal anomalies varies from 5 to 19 weeks.⁵

Common sonographic findings of the syndrome may be summarized as follows⁴: cutaneous scars, musculoskeletal deformities, cerebral anomalies, ocular anomalies, intestinal and hepatic echogenic foci, and hydrops fetalis. Fetal demise, growth restriction, and polyhydramnios can also occur. Skin lesions in a dermatomal distribution are typical with varicella embryopathy but are not always present. Musculoskeletal abnormalities may be related to scar formation, leading to limb contractures and hypoplasia. Cerebral anomalies visualized by ultrasound include ventriculomegaly, microcephaly with polymicrogyria, and porencephaly. Congenital cataract and microphthalmos are the most common ocular lesions. The hyperechoic foci within the fetal liver have been identified as calcifications on autopsy.

Unfortunately, histologic evidence of varicella at perinatal autopsy was not available in this case. However, typical clinical manifestation in the mother and fetal serology as well as sonographic findings of the fetus might be strong enough to make a diagnosis of congenital vari-

PRENATAL DIAGNOSIS OF VARICELLA

cella syndrome in our case. This syndrome may be diagnosed by nucleic acid amplification methods on amniotic fluid⁶ but this technique is not available in our setting.

Our findings suggest that on ultrasound examination of pregnancies at risk of the syndrome, attention must be paid to identify miliary calcifications and hydropic changes. Alternatively, because microcalcifications in multiple organs are rare, the presence of these findings should always be considered a possible fetal varicella infection despite no typical limb defects as commonly seen in this syndrome.

The VZV is a neutropic virus that probably causes direct lesion to the nerve cells but the true mechanism explaining the diversity and multiplicity of the lesions remains unclear. Fetal malformations are believed to be associated with subsequent reactivation of the virus in the fetus, not directly related to maternal VZV. After a latent period, VZV in the sensory ganglia of the posterior roots of the spinal cord reactivate, replicate, and result in cell destruction of nerve tissue, which may account for limb denervation, leading to the formation of lesions at the skin as well as bone hypoplasia.⁷ The sonographic findings observed in this case may be somewhat contradictory to this hypothesis, because we found no skin or limb deformities that are commonly seen in other reports. Disseminated calcifications without cutaneous or limb deformities in our cases indicate that other mechanism like hematogenous spreading may play a role as well. Calcifications are associated with tissue necrosis caused by VZV infection,⁸ mainly demonstrated in the liver and brain but very rarely noted in the fetal heart. To the best of our knowledge, only two cases of calcifications in the myocardium have been described in the literature.^{9,10}

Surprisingly, the findings in this case were similar to that reported by Apuzzio et al,¹¹ who suspected that inadvertent vaccination with varicella vaccine during early pregnancy might be associated with miliary fetal tissue calcifications and hydrops. Therefore, our case is evidence supporting their hypothesis on the cause-effect of varicella vaccine and congenital varicella syndrome.

Based on literature review and this report, maternal manifestation combined with US findings of disseminated calcifications in multiple organs and hydrops fetalis may suggest congenital varicella syndrome.

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ORIGINAL ARTICLE

Effect of placenta penetration during cordocentesis at mid-pregnancy on fetal outcomes

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ABSTRACT

Objectives To compare the rates of fetal loss, low birth weight, and preterm birth between pregnancies undergoing cordocentesis at mid-pregnancy with placenta penetration and those without it.

Methods Consecutive cases of cordocenteses were prospectively recorded. The inclusion criteria for analysis were: (1) singleton pregnancies, (2) no fetal abnormalities, (3) gestational age of 18–22 weeks, and (4) procedures performed by experienced operators. The primary outcome was fetal loss rate, and the secondary outcomes were rates of failed procedures, low birth weight, and preterm birth.

Results Of 6147 cordocenteses recorded, 2829 met the inclusion criteria with complete data for analysis. Of these, 654 procedures were further excluded because the puncture site was at cord insertion. The remaining 2175 cases, consisting of 615 procedures with placenta penetration and 1560 cases with no penetration, were analyzed. Cordocenteses with placenta penetration had a significantly higher rate of fetal loss (3.6% vs 1.3%, $p = 0.01$) as well as of low birth weight and preterm birth.

Conclusion Cordocentesis with placenta penetration carries a higher risk for fetal loss, preterm birth, and low birth weight. This information may be helpful in prenatal diagnosis counseling, and it may encourage performers to avoid placenta penetration, if possible. © 2012 John Wiley & Sons, Ltd.

Funding sources: The authors wish to thank Thai Research Fund and the National Research University Project under Thailand's Office of the Higher Education Commission for financial support

Conflicts of interest: None declared

INTRODUCTION

Cordocentesis or percutaneous umbilical blood sampling is a prenatal diagnostic procedure that has been performed since 1983.¹ Moreover, cordocentesis is required prior to fetal treatment, such as blood transfusion. Therefore, it is one of the most important procedures in prenatal diagnosis and therapy. Although the procedure is relatively safe in experienced hands, it can be associated with a significant fetal risk, such as fetal loss (procedure-related fetal loss) of about 0.2–3.2%.^{2–5} The factors affecting the safety of cordocentesis are yet to be investigated. For example, it is unknown whether placenta penetration during cordocentesis carries a higher risk for fetal loss. To answer such question, a large number of cordocenteses in a homogeneous study population is needed, controlling for several confounders, especially gestational age at procedure, experience of the operators, umbilical cord puncture site, and indications for the procedure.

The objectives of the study were to compare fetal loss rate, failed procedure rate as well as rates of low birth weight, and

preterm birth between pregnancies undergoing cordocentesis at mid-pregnancy with placenta penetration and those without placenta penetration.

MATERIALS AND METHODS

From September 1989 to December 2010, 6147 consecutive cordocenteses were performed at our institution. The details of each procedure, such as puncture site, the presence of umbilical cord bleeding or bradycardia, duration of the procedure, and placenta penetration, were prospectively recorded and stored for subsequent analysis. The inclusion criteria for the current study were: (1) singleton pregnancies, (2) no fetal anomalies or fetal growth restriction, (3) gestational age of 18–22 weeks, based on ultrasound examination in the first half of pregnancy, and (4) procedures performed by experienced operators. The exclusion criteria consisted of (1) major anomalies or chromosomal abnormalities diagnosed in utero or at delivery, (2) loss to follow-up or final pregnancy

outcomes are unobtainable, (3) the initial 50 procedures of practice of any operator, and (4) previous chorionic villous sampling, amniocentesis or cordocentesis in the current pregnancy to avoid their confounding effects on pregnancy outcomes. The cordocentesis was performed with freehand technique under transabdominal ultrasound guidance, using the convex transducer of 3.5 MHz (model SSD 620, 680EX, SSD 1700, SSD 5000, alpha 10 Aloka, Tokyo, Japan, and E-8 GE Medical System, Wisconsin, USA). Cordocentesis techniques have been described in details elsewhere.⁶ The procedure was performed in an outpatient setting following ultrasound examination to establish number of fetuses, viability, gestational age, anatomy survey, location of placenta, and an approximate area for needle insertion. Typically, the puncture site, either near the cord insertion or a free-floating loop of cord, was chosen based on accessibility and quality of visualization with an attempt to avoid placental penetration, if possible. No more than two attempts were performed at each cordocentesis. Immediately after the needle removal, the puncture site was observed for bleeding, and the fetal heart rate was recorded. The patients were asked to rest for at least 30 min and then allowed to go home. Prophylactic antibiotics or tocolysis were not administered. Pregnancies with normal results were followed with standard obstetric care, whereas pregnancies with abnormal results were excluded from analysis. Definitions used in this study were as follows: (1) *difficult procedure* was a cordocentesis defined as subjectively difficult by the operator and documented as such immediately after the procedure, (2) *successful procedure* was a procedure yielding an adequate amount of fetal blood for analysis, (3) *fetal bradycardia*: fetal heart rate below 100 beats per minute (bpm) lasting more than 1 min, (4) *umbilical and placental bleeding*: any amount of significant bleeding according to the subjective impression of the operator (usually lasting more than 5 s), (5) *mid-pregnancy*: a pregnancy with a gestational age of 18–22 weeks, (6) *experienced operator*: a clinician who had performed at least 50 cordocenteses, (7) *fetal loss*: pregnancy ending in spontaneous abortion (at 20 weeks or earlier) or perinatal death (after 20 weeks of gestation), including fetal death or neonatal death, (8) *preterm infant*: a neonate born after 20 weeks but before 37 completed weeks of gestation, (9) *perinatal mortality*: a death of infant (born after 20 weeks) within 28 complete days after birth. Perinatal mortality rate was the number of stillbirths plus neonatal deaths per 1000 total births, and (10) *low birth weight*: a newborn whose weight was <2500 g. The primary outcome was a fetal loss rate, and the secondary outcomes included rates of successful procedures, of newborns with low birth weight (less than 2500 g), and preterm birth (less than 37 complete weeks of gestation). In addition, duration of the procedure, birth weight, and gestational age were also analyzed. This research was approved by the research ethics committee of our institution.

Statistical analysis

Analyses were done using the statistical package for the social sciences (SPSS) version 17.0 (Chicago, IL). Student's *t*-test was used for comparison of continuous variables, whereas Pearson's chi-square test was used for categorical variables. The sample size was determined by the assumption that fetal loss associated with placenta penetration was approximately increased to 2%, when compared to that in nonpenetration group. The sample size of each group should be more than 600 cases to achieve significance at the 5% level with a power of 80%.

RESULTS

During the study period, 6147 cordocenteses were performed. After excluding twin pregnancies, fetal abnormalities, cases lost to follow-up or with incomplete data, procedures performed during the operators' learning curves, prior amniocentesis, chorionic villous sampling or cordocentesis, 2829 had complete data for analysis (Figure 1). Of these, 654 procedures involving puncture site at cord insertion were further excluded because of a potential confounder. Finally, 2175 procedures were suitable for analysis. Indications for mid-pregnancy cordocentesis are presented in Table 1. Fetal risk for severe thalassemia and abnormal chromosomes were the most common indications.

Of the procedures, 615 required placenta penetration (group 1), and the remaining 1560 did not (Group 2). Nearly all cases of placenta penetration (group 1) had an anterior placental location, which was significantly more frequent than in group 2 (Table 2, $p < 0.001$). The mean maternal age between the two groups were not significantly different (30.8 ± 7.8 vs 31.2 ± 8.2 years, Student's *t*-test, $p = 0.26$). The pregnancy outcomes and procedure parameters are presented in Table 3. Cordocenteses involving placenta penetration were associated with a significantly higher rate of fetal loss (3.6% vs 1.3%, $p = 0.01$) and low birth weight (14.5% vs 11.0%, $p < 0.05$). However, the preterm birth rate in group 1 was not significantly different from that in group 2 (13.8% vs 13.1%, $p > 0.05$). Additionally, the mean gestational age and mean birth weight were slightly but significantly lower in group 1 than group 2 (38.0 weeks vs 38.6 weeks, $p < 0.05$, and 2870 g vs 2993 g, $p < 0.05$, respectively).

As for procedure complications, cordocenteses with placenta penetration were associated with a significant increase in the rate of umbilical cord bleeding (32.0% vs 28.4%, $p < 0.05$). Additionally, about one-third of cases with placenta penetration had placental bleeding at the penetration site, whereas this complication was not noted in the procedures without placental penetration. There were no significant differences in the duration of the procedure, success rates, and rates of fetal bradycardia. Regarding technical aspect, the mean time used to complete the procedure in group 1 was nonsignificantly longer than in group 2 (12.19 vs 8.63, $p > 0.05$).

All procedures 18-22 weeks	6147	
Singleton pregnancies	6092	55: Excluded 55 sets of twins
Complete data	4820	1272: Loss to follow-up and incomplete data
Experienced performers	3697	1123: Fellow training & learning curve cases
Primary cordocenteses	3631	66: Prior amniocentesis or CVS or cordocentesis
Normal pregnancy	2829	802: Fetal abnormalities
Available for analysis	2175	654: cord insertion site puncture
	615	placenta penetration
	1560	placenta non-penetration

Figure 1 Cascade in recruitment of the study population

Table 1 Indications for cordocentesis

	Group 1	Group 2	Total
Risk of fetal Hb Bart's disease	132	295	427
Risk of fetal beta-thalassemia major	78	188	266
Risk of fetal beta-thalassemia/HbE	169	412	581
Mixed risk of fetal severe thalassemia	5	15	20
Rapid karyotyping	119	375	494
Risk of fetal severe thalassemia + chromosome	106	266	372
Others	6	9	15
	615	1560	2175

Hb, hemoglobin; HbE, hemoglobin E.

Table 2 Placental location

	Group 1	Group 2	Total
Anterior	595	384	979
Anteroposterior	3	22	25
Posterior	14	1031	1045
Posterior lateral	1	19	20
Lateral	2	23	24
Posterior fundus	0	6	6
Fundus	0	75	75
Unknown	0	1	1
Total	615	1560	2175

DISCUSSION

We have found that cordocenteses involving placental penetration are associated with greater rates of bleeding from the

umbilical cord or placental puncture sites, greater risk of fetal loss, lower gestational age at delivery, and lower birth weight. Cordocentesis has been done in our hospital for 20 years, and several aspects about this procedure have been studied. However, many questions about safety have yet to be answered. Needle penetration of the placenta can plausibly injure both maternal and chorionic vessels, resulting in fetomaternal hemorrhage or fetal bleeding from the placenta or chorionic vessels into the amniotic fluid. This may lead to fetal anemia or thrombosis and can subsequently cause abortion or preterm labor. These complications may occur immediately or may be delayed as a consequence of fetal anemia or placental insufficiency. Although several articles have been published on cordocentesis-related complications^{5,7-12} none has examined the effect of placenta penetration on pregnancy outcomes with adequate statistical power, especially as it relates to fetal loss associated with the procedure, which rarely occurs. The previous studies on the subject could not elucidate this issue because of too small sample size and lack of control for several confounders, especially gestational age and experience level of the operators. To determine the effect of placenta penetration on adverse outcomes, we thus excluded all known confounders as much as possible so as to ensure that both the study and control groups had similar baseline characteristics. These possible confounders included twin pregnancies, fetal chromosomal or structural abnormalities, puncture site at cord insertion, gestational age of less than 18 weeks or more than 22 weeks of gestation, as well as cases with prior invasive procedures. Gestational age is an important determinant. Unlike procedures in several previous studies in which cordocenteses were performed in the late second trimester or third trimester, all the procedures in our study were confined to mid-pregnancy at 18-22 weeks of gestation. It has been suggested that the first 50 cases for any operator represent early learning curve.⁶ Level of experience

Table 3 Comparisons of the outcomes between placenta penetration group (group 1) and nonpenetration group (group 2)

	Group 1 (n=615)		Group 2 (n=1560)		p-value
	Mean	SD	Mean	SD	
Procedure time (min)	12.2	67.8	8.6	50.4	0.199
Birth weight (g)	2870	462	2993	532	<0.001
GA at delivery (weeks)	38.0	4.3	38.6	5.5	0.028
	Group 1 (n=615)		Group 2 (n=1560)		p-value
	n	%	n	%	
Fetal loss	22/615	3.6	20/1560	1.3	0.001
Difficult procedure	48/614	7.8	162/1560	10.4	0.106
Success rate	613/615	99.7	1539/1560	98.9	0.141
Umbilical cord bleeding	197/615	32.0	443/1560	28.4	0.032
Placental bleeding	208/615	38.2	0/1560	0	< 0.001
Bradycardia	35/615	5.7	73/1560	4.7	0.328
Low birth weight	89/615	14.5	172/1560	11.0	0.026
Preterm birth	85/615	13.8	204/1560	13.1	0.645

GA, gestational age.

can possibly affect the procedure and pregnancy outcomes because this procedure needs considerable skills. Moreover, the indications for cases assigned to new practitioners during their learning curve may be different than for experienced operators. To avoid this possible confounder, we excluded procedures of the first 50 cases of experience.

In our practice, the puncture site is usually based on accessibility and cord insertion. Although cord insertion site is usually the ideal puncture site in cases with anterior placental location because the cord remains anchored, the situation is quite different in cases of posterior placental location. In such cases, the thrust, direction, and weight of the needle insertion is less well controlled, resulting possibly in a lower success rate and a higher risk of injury to the cord vessels or bleeding. To avoid this potential confounder, we therefore excluded all cordocenteses performed at the cord insertion site and included only procedures performed at a free loop.

We have found that the rate of umbilical cord bleeding after the procedure is significantly higher in cases with placenta penetration (32.0% vs 28.4%, $p < 0.05$). This suggests that cordocenteses with placenta penetration may be technically more difficult than those without penetration, thus leading to greater risk of injury to the punctured vessels. Indeed, immediate complications such as fetal bradycardia were also more frequent (though not significantly so) with placenta penetration. We hypothesize that the findings may be related to difficulty in needle control because the needle must pass greater tissue thickness (abdominal wall, uterine wall, and placental thickness) to reach the target site. The needle is thus less freely steered. Significant placental bleeding was observed in about one-third of the cases with placenta

penetration. This is a unique complication of cordocentesis specific to placenta penetration, as it does not occur in cases with no placenta penetration. It is reasonable to hypothesize that the increased rate of adverse outcomes associated with placenta penetration was likely related to placental bleeding, either overt or within the placental tissue. The number of procedures with placenta penetration was far lower than that without penetration because physicians usually tried to avoid placenta penetration, except when it was the most accessible part and no other areas were suitable for easy puncture.

Some pitfalls in our study which should be mentioned are: we could not evaluate some possible confounders, especially maternal BMI or abdominal wall thickness. Different ultrasound machines were used in different time periods, which often directly affect the ease of the procedure. However, this may be only a minimal effect on the result because these factors did not influence the decision on choosing whether to penetrate the placenta. Another limitation of our study is that the procedure-related fetal losses could not be accurately evaluated, as the background fetal losses in both groups (i.e., unrelated to the procedure) were also included in the analysis. Some authors define the procedure-related fetal loss as one occurring within 2 weeks of the procedure.^{6,10,12} Unfortunately, we could not reliably analyze the procedure-related losses because the number of fetal losses was too low for adequate statistical power. Finally, several obstetric complications such as placental abruption or preeclampsia were not thoroughly evaluated. Therefore, we could not establish whether placenta penetration has any effect on these complications.

The strength of our study is the large sample size that allowed enough statistical power to assess rare outcomes

such as fetal loss rate. Another strength is that known confounders were excluded as much as possible. The included cases were homogeneous in terms of singleton pregnancy, gestational age, puncture site, experience levels of the operators and being performed at only one single center, reflecting similar techniques, assistance team, and environmental factors.

In conclusion, our findings suggests that cordocentesis with placenta penetration carries a higher risk for adverse pregnancy outcomes. In addition to fetal loss rate, rates of preterm birth rate, and low birth weight were also higher in the group with placenta penetration, leading to lower birth weight and gestational age at delivery. We suggest

that operators should avoid placenta penetration if at all possible.

WHAT'S ALREADY KNOWN ABOUT THIS TOPIC?

- Cordocentesis carries a higher risk of fetal loss.
- The cordocentesis-related fetal loss may be related to cord bleeding or fetal bradycardia.

WHAT DOES THIS STUDY ADD?

- Cordocentesis with placenta penetration carries a higher risk for fetal loss, preterm birth and low birth weight than that with no penetration.

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ORIGINAL ARTICLE

Predisposing factors and effects of fetal bradycardia following cordocentesis at mid-pregnancy

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Objectives: To identify predisposing factors of fetal bradycardia following cordocentesis at mid-pregnancy and to compare the pregnancy outcomes to those without bradycardia. **Methods:** All cordocenteses performed at 18–22 weeks of gestation were prospectively enrolled. The inclusion criteria consisted of: (i) singleton pregnancies; (ii) no fetal structural or chromosomal abnormalities; (iii) the procedures done by experienced operators. They were divided into two groups; procedures with fetal bradycardia (Group 1) and those without bradycardia (Group 2). Factors related to bradycardia were identified and pregnancy outcomes between the two groups were also compared. **Results:** Of 6147 cordocenteses recruited, 2829 met the inclusion criteria. Of these, 152 had fetal bradycardia whereas the remaining 2677 did not. The procedures involving placenta penetration, and umbilical cord bleeding were significantly related to a higher rate of fetal bradycardia. On the other hand, cordocenteses with fetal bradycardia had a significantly higher rate of fetal loss (11.8 vs. 1.9%, respectively, $p = 0.001$) as well as a higher rate of low birth weight and preterm birth. **Conclusions:** Cordocentesis with placenta penetration and umbilical cord bleeding carries a higher risk for fetal bradycardia and fetal bradycardia was an independent factor for a higher rate of fetal loss, preterm birth and low birth weight.

Keywords: cordocentesis, fetal bradycardia, fetal outcome, prenatal diagnosis

Introduction

Cordocentesis or funipuncture is a prenatal diagnostic procedure for fetal blood sampling which has been first described since 1983 [1]. It has become one of the most important procedures in maternal-fetal medicine. Cordocentesis is usually performed during mid and late pregnancy for assessment of various fetal disorders such as fetal growth restriction, hydrops fetalis, intra-uterine infections, and abnormal hemoglobin in fetuses at genetic risk for hemoglobinopathies [2]. The procedure is relatively simple and safe but needs well-trained practice as well as high experience. However, it carries a significant fetal risk such as fetal loss (procedure-related fetal loss) about 0.2–3.2% [3–6]. However, the risk may be higher in some specific conditions, especially when it is performed by clinicians in early learning curve, or procedures with placenta penetration or difficult procedures secondary to poor assessment or poor visualization. It is believed that fetal bradycardia following the procedure can be a prognostic factor

for adverse outcomes. Transient fetal bradycardia is reported in 1.5–12% [3,7–11]. The occurrence of sustained fetal bradycardia following needle insertion into the umbilical vessel, or even after the procedure, has been mentioned in several studies. However, despite several reports on adverse outcomes secondary to fetal bradycardia after cordocentesis, its clinical significance is unknown. Whether fetal bradycardia during or following cordocentesis carries a higher risk for fetal loss or not has remained unclear. To answer this question, the great number of sample size with homogeneity of study population is required and many confounders should be controlled; for example, gestational age, expertise levels of operators, fetal abnormalities or fetal serious disorders etc.

The primary objectives of the study were to determine whether fetal bradycardia following cordocentesis at 18–22 weeks of gestation had adverse effects on pregnancy outcomes or not, and to identify its predisposing factors.

Materials and methods

All consecutive cases of cordocenteses at mid-pregnancy were enrolled to the study. The procedures were performed during September 1989–January 2010, from a single tertiary center, Maharaj Nakorn Chiang Mai Hospital, Chiang Mai University Thailand. The most common indications were fetal risk for severe thalassemia syndrome and rapid karyotyping. The technical details such as procedural time, the presence and duration of puncture site bleeding as well as fetal bradycardia, placenta penetration/non-penetration, etc. were immediately recorded and stored in computers for subsequent analysis. In addition, the pregnancies were prospectively followed-up to obtain the final outcomes. The inclusion criteria for the study were as follows: (i) singleton pregnancies; (ii) gestational age of 18–22 weeks, based on ultrasound in the first half of pregnancy; (iii) no fetal structural and chromosomal abnormalities; (iv) the procedures done by experienced performers, defined by experience of more than 50 cases of real practice. The exclusion criteria were: (i) the final pregnancy outcomes could not be obtained or loss to follow-up; (ii) fetuses which were subsequently proven to be serious anomalies, or chromosomal abnormalities, or disorders required pregnancy termination; (iii) pregnancies with previous history of cordocentesis or chorionic villous sampling or amniocentesis in the current pregnancy. The cordocentesis was performed under ultrasound guidance, using the convex transducer of 3.5 MHz (model SSD 620, 680EX, SSD 1700; SSD 5000, alpha 10 Aloka,

Tokyo, Japan and E8 GE medical system, Wisconsin, USA). Cordocentesis was performed using freehand technique, at an out-patient setting with standard ultrasound examination for fetal biometry, gestational age, growth evaluation, anatomical survey, location of placenta and an approximate point for needle puncture. In our practice, the puncture site was selected based on good accessibility and quality of visualization of the puncture point, either cord insertion site or free umbilical loop, with an attempt to avoid penetrating the placenta, if possible. Just after the needle removal, bleeding from the puncture site and fetal heart rate was quantified and recorded immediately. After completion of the procedures, the women were asked to have a rest for at 30–45 min before permitted to go home. Pregnancies with normal results of prenatal diagnosis were followed and taken care as low-risk pregnancy, while pregnancies with abnormal fetuses were excluded from analysis. More details of cordocentesis techniques in our practice are described elsewhere [12]. Some definitions used in this study were as follows: (i) *fetal bradycardia*: fetal heart rate of less than 100 bpm lasting more than 1 min; (ii) *mid-pregnancy*: pregnancy at gestational age between 18 and 22 weeks; (iii) *experienced operator*: a performer who had experience in cordocentesis of more than 50 cases; (iv) *fetal loss*: pregnancy ending up with spontaneous abortion or perinatal death, including dead fetus in utero or stillbirth and neonatal death; (v) *preterm birth*: delivery after 20 weeks and before 37 completed weeks of gestation; (vi) *low birth weight*: birth weight of less than 2500 g; (vii) *perinatal mortality*: a death of infant born after 20 weeks and ending at 28 days after birth. The primary outcomes of measures included the fetal loss rate, success rate as well as rates of low birth weight and preterm birth. In addition, predisposing factors of fetal bradycardia were also assessed, including time used to complete the procedure, difficulty of the procedure defined by the operators' impression, placenta penetration, and the presence of umbilical cord bleeding during and after the procedures.

Statistical analysis

Analyses were performed using the statistical package for the social sciences (SPSS) software version 17.0 (Chicago, IL, USA). The data were categorized into two groups based on the presence or absence of fetal bradycardia. Student's *t* test was used to compare the time used for the procedures, birth weight, or gestational age at delivery between both groups while Pearson χ^2 test and logistic regression analysis was used to compare a rate of fetal loss, preterm birth, and low birth weight between both groups.

Results

During the study period, 6147 cordocenteses at 18–22 weeks of gestation were prospectively enrolled and followed-up for final pregnancy outcomes. After excluding the cases of twin

pregnancies (55), no availability of final outcomes (1272), procedures performed during learning curve of practices (1123), prior cordocentesis or amniocentesis or chorionic villous samplings (66), and fetal abnormalities (802), the remaining 2829 were suitable for analysis. Of these, 152 procedures had fetal bradycardia (Group 1) and the remaining 2677 had no bradycardia (Group 2). The means of maternal age ($\pm$ SD) at the time of cordocentesis were 30.7 ± 7.1 and 30.9 ± 7.9 years for Group 1 and Group 2, respectively, not significantly different (Student's *t* test; $p = 0.767$). Indications for cordocentesis were comparable (χ^2 , $p = 0.362$). The most common indication was fetal risk for severe thalassemia syndrome, followed by rapid karyotyping as presented in Table I.

For technical aspects as shown in Table II, puncture site either free loop of umbilical cord or placental cord insertion was not significantly associated with the prevalence of fetal bradycardia ($p = 0.0176$) whereas procedures with placenta penetration had a significantly higher rate of fetal bradycardia ($p = 0.010$). In addition, the procedures with difficulty based on performers' impression were significantly associated with a higher rate of fetal bradycardia ($p = 0.005$). Likewise, duration of the procedures, reflecting levels of difficulty, was also significantly related to the prevalence of fetal bradycardia (Student's *t* test; $p < 0.001$). Of note, percentage of umbilical bleeding significantly increased the rate of fetal bradycardia ($p = 0.032$). However, the success rates of cordocentesis between the two groups were similar ($p = 0.822$).

Regarding to complications secondary to fetal bradycardia, pregnancy outcomes could be summarized as follows: the rate of fetal loss was significantly higher in Group 1, 11.8 versus 1.9%, respectively (Pearson χ^2 ; $p = 0.001$). In multivariate analysis using logistic regression, fetal bradycardia was an independent factor for fetal loss after control other potential confounders including placenta penetration, puncture site, umbilical cord bleeding and duration of procedures (odds ratio 6.092; 95% CI: 3.379–10.983). In addition, the rate of low birth weight was significantly higher in the group of fetal bradycardia (odds ratio 2.340; 95% CI: 1.583–3.459). Likewise, the rate of preterm birth was also significantly higher in Group 1 (odds ratio 1.691; 95% CI: 1.115–2.564). The pregnancies outcomes are presented in Table III.

Discussion

Cordocentesis has been performed in our hospital for more than 20 years, and several aspects about this procedure have been studied [12–16]. However many questions about safety are yet to be answered. Previous small series suggest that fetal bradycardia following cordocentesis may be a predictor of adverse outcomes. However, only inconclusive evidence is available on the clinical significance of fetal bradycardia following cordocentesis. Though several studies report its prevalence, ranging from 1.5 to 12%

Table I. Indications for cordocentesis.

Indications	Bradycardia <i>n</i> (%)		No bradycardia <i>n</i> (%)		Total <i>n</i> (%)	
Risk of fetal Hb Bart's disease	41	27.0	518	19.4	559	19.8
Risk of fetal beta-thalassemia major	17	11.2	355	13.3	372	13.1
Risk of fetal beta-thalassemia/HbE	39	25.7	727	27.2	766	27.1
Mixed Risk of fetal severe thalassemia	2	1.3	22	0.8	24	0.8
Rapid karyotyping	27	17.8	588	22.0	615	21.7
Risk of fetal severe thalassemia + chromosome	26	17.1	448	16.7	474	16.8
Others	0	0.0	19	0.7	19	0.7
	152	100.0	2677	100.0	2829	100.0

Table II. Comparisons of the outcomes between the group of procedures with fetal bradycardia (group 1) and the group of those no fetal bradycardia (group 2).

	Group 1 (n = 152)		Group 2 (2677)		p value
	Mean	SD	Mean	SD	
Procedure time	8.92	12.95	5.97	7.93	<0.001
Birth weight	2703.45	787.89	2978.74	514.96	<0.001
GA at delivery	36.80	4.47	38.53	5.41	<0.001

	Group 1 (n = 152)		Group 2 (2677)		p value
	n	Percent	n	Percent	
Fetal loss	18/152	11.8	50/2677	1.9	<0.001
Difficult procedure	24/152	15.8	223/2487	9.0	0.005
Success rate	151/152	99.3	2640/2662	99.2	0.822
Cord insertion puncture	30/152	19.7	658/2677	24.6	0.176
Umbilical cord bleeding	197/615	32.0	443/1560	28.4	0.032
Placenta penetration	54/149	36.2	710/2670	26.6	0.010
Low birth weight	40/152	26.3	326/2677	12.2	<0.001
Preterm birth	33/152	21.7	336/2677	13.1	0.001

Table III. Logistic regression analysis in assessment of the relationships between procedural factors and pregnancies outcomes.

	Fetal loss odds ratio (95% CI)	Low birth weight odds ratio (95% CI)	Preterm birth odds ratio (95% CI)
Bradycardia	6.092 (3.379–10.983) p < 0.001	2.340 (1.583–3.459) p < 0.001	1.691 (1.115–2.564) p = 0.013
Placenta penetration	2.535 (1.526–4.210) p < 0.001	1.268 (0.982–1.636) p = 0.069	1.275 (1.002–1.624) p = 0.049
Puncture site	1.578 (0.879–2.832) p = 0.127	0.971 (0.754–1.249) p = 0.817	1.188 (0.917–1.538) p = 0.191
Cord bleeding	2.609 (1.791–3.801) P < 0.001	1.499 (1.227–1.830) p < 0.001	1.462 (1.198–1.785) p < 0.001

[3,7–11], only two reports have linked this complication to pregnancy outcomes [17,18]. Han and Nava-Ocampo [17] studied on FHR response to cordocentesis on 117 pregnancies. They found the incidence of bradycardia was 10.3% and all returned to normal in less than 5 min. They concluded that transient fetal tachycardia and bradycardia were uncommon and were not associated with adverse fetal or pregnancy outcomes. Ulm et al. [18] found that fetal loss rate per procedure was 61.5% (8/13) in cases with bradycardia and 3.1% (10/326) in those without bradycardia ($p < 0.001$). However, several fetuses with bradycardia in their report had anomalies or hydrops fetalis, which were usually associated with poor fetal outcomes. Thus, the real effects of fetal bradycardia were inclusive. None of previous studies compared effects of bradycardia on pregnancy outcomes between procedures with and without fetal bradycardia. Moreover, those studies contained too small sample size to answer such a low rate like fetal loss. In addition to large sample size, to answer this question needs control of several confounders, especially gestational age and experience level of performers, which are usually heterogeneous in several studies. Therefore, to be more suitable for comparison, we excluded the known confounders as much as possible and used multivariate analysis to estimate the outcomes. The potential confounders included twin pregnancies, fetal chromosomal or structural abnormalities, and procedures in early learning curve. Furthermore, gestational age of less than 18 weeks or more than 22 weeks of gestation as well as cases with prior invasive diagnosis in early gestation were also excluded. Unlike in several previous studies in which the baseline data were heterogeneous, all cordocenteses in this study were confined to gestational age of 18–22 weeks whereas, most cordocenteses in the western countries are usually varied in late second trimester or third trimester.

Though the primary causes of bradycardia following cordocentesis remains unclear, this study demonstrates that the placenta penetration, puncture site bleeding and difficulty of the procedures can increase a chance of fetal bradycardia following cordocentesis. However, several cases had no known predisposing factors. Bell and Weiner [19] showed that fetal bradycardia during puncture umbilical artery may be caused by a spasm of the vascular wall induced by the insertion of the needle and it may occasionally be caused by a vasovagal response due to local vasospasm [4] or a reflex secondary to compression of the cord from thrombosis. Cord hematoma can occasionally be associated with a transient or prolonged sudden fetal bradycardia [20,21]. On examination of the umbilical cords after delivery, hematomas following cordocentesis could be noted in as many as 17% of cases [21]. Additionally, endothelial adaptation to hypoxia may partly explain why hypoxemia is a risk factor for bradycardia [4]. Rizzo demonstrated that umbilical vein puncture released a potent vasoconstrictor, endothelin, in growth restricted but not appropriately grown fetuses [22]. Fetuses with bradycardia release more endothelin, possibly causing focal vasoconstriction at or near the puncture site.

Based on our results, fetal bradycardia was strongly related to adverse fetal outcomes, especially fetal loss. The mechanism of these adverse effects remains inconclusive. However, it is possible that a direct cause of bradycardia may also play a role in fetal loss such as anemia secondary to umbilical cord bleeding, or some degree of subtle circulatory obstruction due to thrombosis or fetomaternal hemorrhage resulting in poor fetal circulation and abortion or preterm labor. Though controlling other confounding factors as much as possible by either excluding procedure in early learning curve or fetal abnormalities or using multivariate analysis, we have found that fetal bradycardia was an independent factor placing the pregnancy to be a higher risk of fetal loss.

The strength of this study includes large sample size from a single center with high homogeneity of the baseline characteristics including: singleton pregnancies, normal fetuses, the same gestational age, and the same experience levels of the operators, providing a more suitable comparison. One limitation should be mentioned is that a significant number of losses to follow-up in this study may be associated with an overestimated overall rate of adverse outcomes since the cases with bad outcomes are more likely to be reported.

In conclusion, this study suggests that fetal bradycardia following cordocentesis was strongly associated with a higher risk of fetal loss. Additionally, rates of preterm birth and low birth weight were also slightly higher in cases of fetal bradycardia. Fetal bradycardia was related to the procedures with placenta penetration, difficulty, and umbilical cord bleeding. Thus, the performers should try as much as possible to avoid placenta penetration if it is possible, or puncture site bleeding, for example; not perform during active fetal movement which is often prone to vessel injury.

Acknowledgments

The authors wish to thank Thai Research Fund and the National Research University Project under Thailand's Office of the Higher Education Commission for financial support.

Declaration of Interest: The authors report no conflicts of interest.

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RESEARCH ARTICLE

A comparison of cordocentesis outcomes between early and conventional procedures

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Objective: To compare procedural outcomes between early cordocentesis (16–17 weeks) and those at 18–22 weeks.

Materials and Methods: A prospective database of cordocentesis was assessed. The eligible criteria included are (1) singleton pregnancy; (2) no fetal abnormalities; (3) gestational age of 16–22 weeks and (4) procedures performed by experienced operators. The procedures performed during 16–17 weeks were defined as early cordocentesis (Study group). The Control group (conventional cordocentesis; 18–22 weeks) were selected by matching maternal age and years of procedures, with a ratio of 1:3. **Results:** Of 2942 eligible procedures, 103 procedures were undertaken during 16–17 weeks and 309 matched controls were selected. Baseline characteristics of both groups were comparable. Time used to complete the procedure was significantly longer in Study group (8.45 ± 9.91 vs. 6.07 ± 6.63 min; p value 0.007). Similarly, rate of difficult procedures was significantly higher in Study group (7.8 vs. 2.3%; p value 0.011). However, rates of fetal loss, low birth weight infants and preterm births were not significantly different, but there was a tendency to be higher in Study group. **Conclusion:** Early cordocentesis is more difficult but relatively safe when performed by experienced hands, although it has a tendency to increase rates of adverse outcomes. This information may be helpful in counseling the couples.

Keywords: Cordocentesis, early pregnancy, prenatal diagnosis

Introduction

Cordocentesis or fetal blood sampling from the umbilical cord is one of the important invasive prenatal diagnostic techniques. It is performed primarily for fetal blood analysis with various indications such as rapid karyotype or fetal risk of anemia. In our practice, it is typically performed at 18–22 weeks of pregnancy. How early in gestation, cordocentesis could be safely done is of concern. Some studies on cordocentesis as early as 12–14 weeks of gestation [1–4], though mixed gestational age and small sample size, suggest that such a very early cordocentesis is associated with a higher rate of fetal loss, probably as high as 8% [2]. Therefore we do not perform cordocentesis at very early gestation (12–15 weeks) except in some specific situations [5]. The safety of conventional cordocentesis at 18–22 weeks is well established in our settings [6,7]. The safety of early cordocentesis at 16–17 weeks or grey periods which is focused in this study has never been evaluated.

Though we have usually not performed cordocentesis before 18 weeks of gestation, in few situations we may have to do at 16–17 weeks of gestation with approval of the couple, especially in cases of strong indication for fetal blood analysis, who are referred from far distance and the patients are not convenient to revisit. No clear definition of early procedure has been well accepted. It varies from 12 to 21 weeks of gestation. To avoid confusion, we define early cordocentesis specifically for this article to be a procedure performed during 16–17 weeks of gestation. For a long period of practice, we have a significant number of the procedures performed at 16–17 weeks for analysis. We therefore conducted this study to assess the safety of such procedures. The objective of this study was to compare the immediate procedural complications and pregnancy outcomes between patients undergoing diagnostic cordocentesis at 16–17 weeks of pregnancy and those undergoing diagnostic cordocentesis at 18–22 weeks of gestation.

Materials and methods

The computerized prospective database of cordocentesis at Maternal Fetal Medicine Unit, Maharaj Nakorn Chiang Mai Hospital, Chiang Mai University was assessed. The database contained details of patient characteristics, indications for cordocentesis, technical aspects and immediate complications which were recorded during each procedure and pregnancy outcomes which were added once available. The study design was a retrospective cohort which was conducted with the approval of the institutional ethical committee.

In assessment, all consecutive cases of cordocentesis with various indications between September 1989 and December 2010 were reviewed. The eligible criteria for analysis included were (1) singleton pregnancy; (2) no fetal structural or chromosomal abnormalities; (3) gestational age of 16–22 weeks, at the time of cordocentesis, based on ultrasound in the first half of pregnancy and (4) the procedures performed by experienced operators, defined by actual practice on patients of more than 50 procedures. Twin pregnancies, pregnancies with previous chorionic villous sampling or amniocentesis, or cordocentesis in the current pregnancy, pregnancies with serious fetal disease requiring termination of pregnancy, procedures during the learning curve (first 50 cases of practice) and cases with amount of fetal blood sample more than 2 ml were excluded. Of pregnancies meeting the eligible criteria, the procedures performed during 16–17 weeks of gestation (early cordocentesis) were considered as Study

group. Each study case was matched for maternal age and years of the procedures to identify the controls in which cordocentesis was done between 18 and 22 weeks of gestation (conventional cordocentesis), in the ratio of 1:3.

All procedures were performed with free-hand technique under real time ultrasound guidance using transabdominal 3.5-MHz curvilinear transducers equipped with machines either model voluson E8 GE medical system, Wisconsin, USA or Aloka model SSD 620, 680EX, SSD 1700, SSD 5000, and alpha-10, Tokyo, Japan. In each procedure, standard ultrasound examination was firstly done to determine the fetal number, fetal viability, fetal biometry with growth and gestational age assessment, and anatomical survey. Additionally placental location and umbilical cord insertions were also visualized. Cordocentesis was performed under aseptic technique in outpatient setting under local anesthesia at the maternal abdominal wall. A 22-gauge spinal needle was usually used for 18–22 weeks of gestation, and 23-gauge in most cases of 16–17 weeks of gestation. The puncture site, either near placental cord insertion or free-floating loop, was chosen based on accessibility and quality of visualization, with an attempt to avoid placenta puncture. Usually, 1 ml of fetal blood was aspirated into a disposable syringe, and was sent to the laboratories relevant to the indication. During the needle puncture, aspiration and removal, bleeding from the puncture site and fetal heart rate were observed and recorded. In cases of fetal bradycardia and bleeding at the puncture site, the duration was assessed and immediately recorded. The duration of bleeding from puncture site lasting less than 1 min was considered as transient and the duration of ≥ 1 min was defined as prolonged bleeding. Difficulty of performance was also graded by subjective impression of the performers. After cordocentesis, the patients were usually asked to take a bed rest for 20–30 min. Neither tocolysis nor antibiotic was given. Non-sensitized Rh-negative women were given an isoimmunization prophylaxis. The continuing pregnancies were taken care as a standard antenatal protocol. The pregnancies outcomes were subsequently assessed and recorded in the database. In technical aspects, the outcomes including rates of successful samplings, puncture site bleeding, fetal bradycardia, difficult procedures, and duration of the procedure were compared between both the

groups. The pregnancy outcomes including rates of fetal loss, low birth weight infants (less than 2500 g), and preterm births (less than 37 complete weeks of gestation) were also compared.

Statistical analysis

To compare the qualitative data such as rates of umbilical cord bleeding, fetal bradycardia, or fetal loss, etc. between the two groups, McNemar Chi square test was used whereas paired *T* test was used to compare means of the procedural duration, birth weight and gestational age at delivery. The analysis was carried out using the statistical software for the social sciences (SPSS) version 17.0 (Chicago, IL, USA).

Results

During the study period, a total of 6377 pregnancies underwent cordocentesis between 16 and 22 weeks of gestations. After excluding twin pregnancies (57), loss to follow-up or unavailable final outcomes (1302), procedures performed during learning curves (1164), prior amniocentesis or CVS (70), fetal chromosomal or structural abnormalities (842), 2942 procedures were eligible for the study. Of them, 103 procedures were undertaken during 16–17 weeks of gestation and were considered as “Study Group”. The Control Group consisted of 309 procedures selected from the remaining 2839 cases by matching maternal age and years of procedures on basis of 3:1 ratio. The means ($\pm$ SD) of maternal age between both the groups were comparable, 29.83 ± 6.5 vs. 29.93 ± 6.8 years ($p = 0.899$). Baseline characteristics and outcomes of procedures of both groups are presented in Table I. In the aspects of procedural techniques, success rate of sampling in Control and Study group were not significantly different (99.4 vs. 98.1%; p value = 0.246). Nevertheless, time used to complete the procedure was significantly longer in Study group (8.45 ± 9.91 min vs. 6.07 ± 6.63 min; $p = 0.007$). Similarly, a rate of difficult procedures impressed by the performers was significantly higher in Study group (7.8 vs. 2.3%; $p = 0.011$). Notably, the percentages of placental penetration (22.2 vs. 20.4%; $p = 0.696$), puncture site at cord insertion (16.8 vs. 13.6%; $p = 0.438$) and bleeding as well as prolonged bradycardia were not significantly different between

Table I. A comparison of technical aspects of cordocentesis and immediate complications between singleton and twin groups.

Technical aspects and immediate complications	Control Group (309 fetuses)	Study Group (103 fetuses)	<i>p</i> value
Indications for cordocentesis			
Risk of severe thalassemia disease	242 (78.3%)	100 (97.1%)	<0.005
Karyotyping	38 (12.3%)	–	
Karyotype and severe thalassemia	21 (6.8%)	1 (1.0%)	
others	8 (2.6%)	2 (1.9%)	
Anterior placenta (fetuses, %)	130 (42.1%)	42 (40.8%)	>0.05
Placental penetration (%)	68 (22.2%)	21 (20.4)	0.696
Puncture site (fetuses, %)			
free-floating loop	257 (83.2%)	89 (86.4%)	0.438
cord insertion	52 (16.8%)	14 (13.6%)	
Time used; min (mean $\pm$ SD)	6.07 ± 6.63	8.45 ± 9.91	0.007
Difficult procedure (%)	7 (2.3%)	8 (7.8%)	0.011
Success rate of sampling (fetuses, %)	307 (99.4%)	101 (98.1%)	0.246
Fetal bradycardia (fetuses, %)	16 (5.2%)	5 (4.9%)	0.897
Bleeding from puncture site (fetuses, %)			
Prolonged bleeding	4 (1.7%)	3 (3.8%)	0.472
Transient bleeding	59 (24.9%)	17 (21.3%)	
No bleeding	174 (73.4%)	75 (75.0%)	
Missing data	72	23	

Table II. Pregnancy outcomes, compared between Control group and Study group.

Outcomes	18–22 weeks (309 fetuses)	16–17 weeks (103 fetuses)	<i>p</i> value
Fetal loss in 2 weeks after cordocentesis (fetuses, %)	7 (2.3%)	4 (3.9%)	0.378
Birth weight, g (mean $\pm$ SD)	2963 $\pm$ 502	2874 $\pm$ 638	0.149
Low birth weight infant (fetuses, %)	44 (14.2%)	17 (16.5%)	0.575
Gestational age at delivery, weeks (mean $\pm$ SD)	38.54 $\pm$ 5.35	37.53 $\pm$ 2.71	0.066
Preterm birth (fetuses, %)	33 (10.7%)	16 (15.5%)	0.187

both groups. In the aspects of pregnancy outcomes as shown in Table II, mean birth weight and gestational age at delivery were not significantly different between both groups, though there was a tendency to be lower in Study group. Likewise, rates of fetal loss within 2 weeks of procedures (3.9 vs. 2.3%, $p = 0.378$), low birth weight infants (16.5 vs. 14.2%, $p = 0.575$) and preterm births (15.5 vs. 10.7%, $p = 0.187$) tended to be increased in Study group but the differences were not reaching statistical significance.

Discussion

Cordocentesis before 17 weeks of gestation is theoretically associated with high rate of fetal loss because of its difficulty secondary to small cord and the operation when amnion and chorion or uterine wall is not yet completely fused, vulnerable to membrane injury causing amniotic fluid leakage. Therefore it should be avoided as much as possible. To our best knowledge, cordocentesis before 15 weeks of gestation carries a higher risk of fetal loss as mentioned above, and conventional cordocentesis at 18–22 weeks is associated with fetal loss approximately 1.5% [7,8], which is usually acceptable for proper indications of some specific fetal serious diseases such as severe thalassemia. However, the safety of such a procedure at 16–17 weeks is not known and never been reported. Therefore we conducted this study to evaluate the outcomes of such procedures. Our findings support that early cordocentesis at 16–17 weeks was relatively more difficult when compared to mid-pregnancy procedure, but it was relatively safe in term of pregnancy outcomes.

Most fetuses at 16–17 weeks of gestation had a smaller cord diameter than those in later gestation. Certainly, this is directly associated with more difficulty in operation. In addition to taking more time, the difficulty can cause more injury to the umbilical cord, bleeding, fetal bradycardia or thrombosis. These complications can be associated with fetal anemia or circulatory obstruction leading to fetal loss or compromise or growth restriction. Though an increase in these complications could not be demonstrated in this study, possibly due to a small sample size, the more difficulty in early gestation indicates that the procedure should be performed only by experienced hands with highest precaution.

Notably, in Study group, the rates of fetal loss, preterm births and low birth weight infants have a tendency to be higher in the group of early cordocentesis but not reaching a statistical significance. It is possible that the sample size was too small to gain power to demonstrate such a small increase in those rates. Of note, all parameters of pregnancy outcomes are consistent either fetal loss rate, low birth weight rate, preterm birth rate, mean birth weight or mean gestational age. These findings signify that early cordocentesis at 16–17 weeks may possibly increase these adverse outcomes but could not be demonstrated by this study because of too small sample size.

Because of a long study period with several changes in experience, ultrasound machines, and some techniques may affect on outcomes of the procedures, we preferred to select the control cases by matching the years of the procedures to make more suitable for comparison. Likewise, maternal age may directly effect on pregnancy outcomes and should be controlled by matching. Difference in technical parameters between the two groups could be a confounding factor on the outcomes of measurement. For examples, placental location, placenta penetration, and puncture site at cord insertion may affect on the difficulty of the procedures. Although these parameters were not matched for control, the distributions of such parameters were comparable between the two groups as presented in Table I. Therefore, it is unlikely for these factors to confound the outcomes.

In addition to the same baseline characteristics between both groups, other strengths of this study may include homogeneity of gestational age in each group, unlike a wide range of gestation in other previous reports. Furthermore, experience of the performers could not greatly impact on the comparison since all were experienced performers beyond learning curve of practice. Finally, the sample size was rather large for comparisons of technical aspects, though a fetal loss rate, a rare outcome, required a larger sample size. Moreover, the potential confounders included twin pregnancies, fetal chromosomal or structural abnormalities, or fetuses with diseases requiring termination of pregnancy were excluded.

The limitations of this study included the following: fetal loss rate which was the main outcome measurements, though it is a clear-cut endpoint, included both natural loss and procedure-related loss, not implying the real effect of cordocentesis on fetal loss rate. As in several previous studies, we defined the procedure-related fetal loss to be a loss occurring within 2 weeks of gestation [6,9,10], but it could not be a true effect of cordocentesis. Nevertheless, no significant difference between the two groups signifying that cordocentesis at 16–17 weeks had no or only minimal effect on an increased loss rate when compared to cordocentesis at 18–22 weeks. Additionally, some outcomes were subjectively impressed by the performers, especially the difficulty of the procedures. Nevertheless the rate of difficulty was consistent with duration of the procedure which was more objective. Furthermore, some other effects could not be evaluated especially abdominal wall thickness which was not assessed in this study. The effect of needle size on the outcomes was also not accessed. Our performers preferred with smaller size needle (23-gauge in early cases), while 22-gauge needle was usually preferred in conventional cordocentesis. Use of small needle should be considered for early procedure. It is reported that a 25-gauge needle, may be safer for less blood loss [11].

In conclusion, early cordocentesis at 16–17 weeks is relatively more difficult when compared to the procedure at 18–22 weeks. However, our results suggest it is a relatively safe procedure when performed by experienced hands though it has a tendency to increase rates of adverse outcomes, but not statistically significant. This information may be helpful in counseling the couples who need fetal blood diagnosis when early procedures are needed.

Acknowledgement

The authors wish to thank Thailand Research Fund and the National Research University Project under Thailand's Office of the Higher Education Commission for financial support.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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Prenatal Hemodynamic Assessment of Dicephalus Twins: Sonographic-Pathologic Correlation

Dicephalus twins are very rare, occurring in 10% of all conjoined twins.¹ The most well-known dicephalus twins are Abigail and Brittany Hensel, born in the American Midwest in 1990.¹ They are two persons in one body but developed surprisingly well, both physically and emotionally. Only a few cases of dicephalus twins were prenatally diagnosed,^{2–6} However, in such studies, no details of prenatal hemodynamic evaluation of the shared vascular complex and correlated abnormalities were described, despite the fact that early delineation of the shared organs and hemodynamic assessment are essential for prenatal management. Prenatal diagnosis of dicephalus twins is rather simple, but delineation of the shared structures, especially the cardiovascular system, is challenging and very informative. In the case presented here, we thoroughly studied the hemodynamics of remarkably well-developed dicephalus dibrachius dipus (two heads, two arms, and two legs) in which one was acardiac and completely parasitic. The blood supply was reversely perfused via the descending aorta and aortic arch. The objective of this report was to emphasize the prenatal hemodynamic assessment of conjoined twins, which could be very informative for counseling and management, especially the possibility of in utero intervention, and also to inspire sonographers to prenatally assess the fetal cardiovascular system as much as possible.

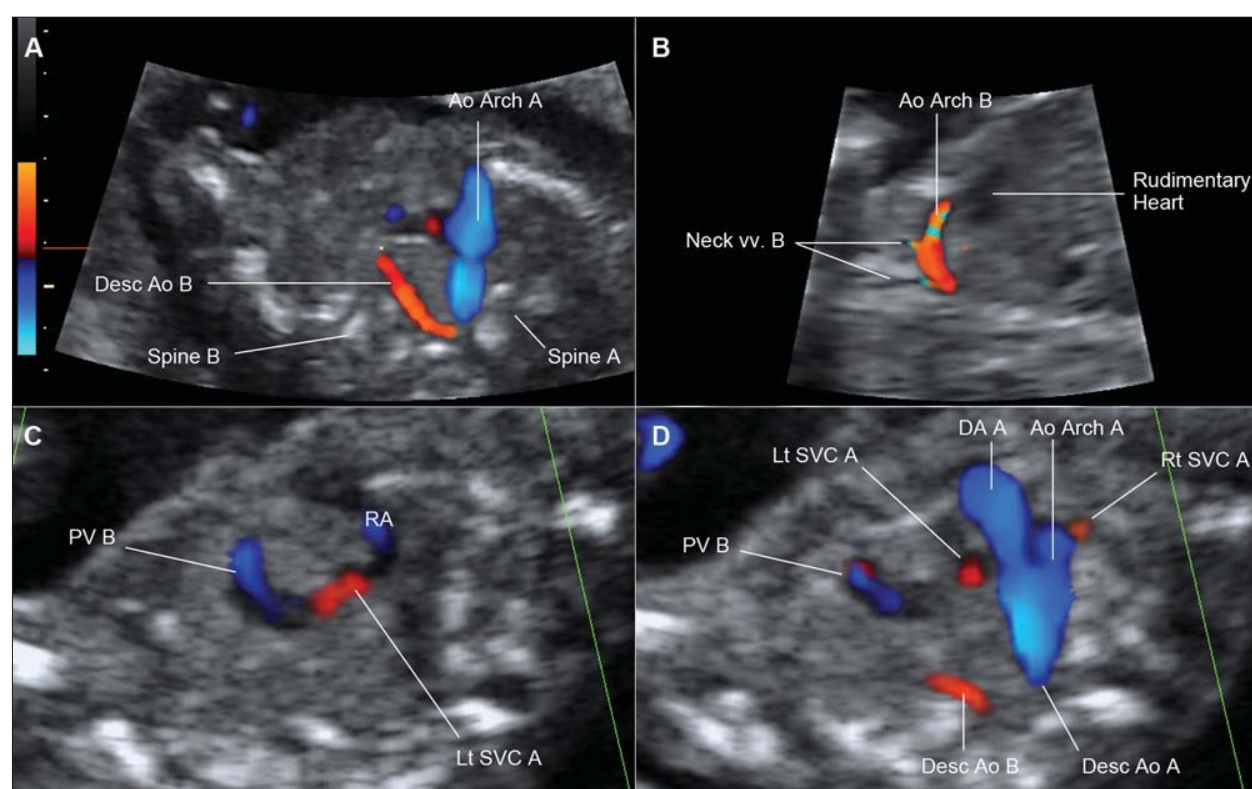
A 24-year-old woman underwent sonography at 21 weeks' gestation with a Voluson E8 system (GE Healthcare, Milwaukee, WI) and transabdominal 2- to 4-MHz curvilinear transducers with the 4-dimensional spatiotemporal image correlation function. The scan showed conjoined twins with duplicated sets of heads, spines, stomachs, and large fused livers but one set of all other structures below the stomachs, including one 3-vessel cord and placenta. Both heads were well-developed in term of size, shape, and intracranial structures such as ventricles and the thalamus. Twin A had a diaphragmatic hernia with right stomach protrusion behind the heart. On hemodynamic assessment (Figures 1 and 2), the heart of twin A had 4 normal chambers with outflow tracts and normal courses of the ascending aorta, aortic arch with neck vessels, pulmonary trunk, inferior vena cava, and right superior vena cava. On color flow mapping, the left superior vena cava collected blood from the heads of twins A and B as well as the pulmonary veins of twin B and emptied to the right atrium. The descending aorta of twin B joined with that of twin A at the upper thorax. Blood from the heart of

twin A had forward flow through the descending aorta of twin A and retrograde flow via the descending aorta and aortic arch of twin B, running through subclavian and both common carotid arteries (Figure 1, A and B). No flow could be shown in the small rudimentary nonpulsating heart of twin B. Pulsations in twin B were synchronized with those in twin A. The cerebral vessels of the head of twin B, including the circle of Willis, were well developed and clearly visualized (Figure 2, A and B). Fetal biometric measurements of the two heads were consistent with 20 weeks' gestation. The spectral Doppler pattern of the cerebral blood flow in twin B was similar to that in the umbilical artery, different from the normal cerebral blood flow as seen in twin A. The peak systolic velocity in the aorta and pulmonary artery of twin A were abnormally high for 20 weeks' gestation (>95th percentile), whereas Doppler indices of the inferior vena cava, ductus venosus, umbilical vein, and umbilical artery were within normal ranges, indicating a high-output state but normal preload without any signs of congestive heart failure. Other cardiac bio-

metric measurements and functional characteristics also suggested a hyperhemodynamic state.

Autopsy findings revealed the dicephalus dibrachius dipus twins with duplicated sets of spines, lungs, stomachs, spleens, gallbladders, and fused livers but one set of all structures below the stomachs (Figures 3 and 4). Two stomachs joined together at the midline to form one duodenum. One 3-vessel umbilical cord had a velamentous insertion. The heart of twin A had completely normal structures, including the aorta and pulmonary trunk with branches. Two superior vena cavae emptied to the right atrium. The left superior vena cava received blood from both twins A and B as well as pulmonary veins from both lungs of twin B. The heart of twin B was rudimentary (without a lumen), and the ascending aorta was atretic. The aortic arch gave rise to the left subclavian artery and left and right common carotid arteries. The descending aorta of twin B joined with that of twin A and received blood from the heart of twin with a reversed direction. No other anomaly was shown except a diaphragmatic hernia with protrusion of the stomach and spleen in twin A.

Figure 1. **A**, Cross-sectional scan at the upper thorax showing reversed flow in the descending aorta (Desc Ao) of twin B. **B**, Sagittal scan at the upper thorax of twin B showing a rudimentary heart and reversed flow in the aortic arch (Ao Arch), continuing from the descending aorta in A. **C** and **D**, Cross-sectional scans at the upper thorax showing left superior vena cava (SVC) collecting blood from the pulmonary vein (PV) of twin B before emptying to the right atrium (RA). DA indicates ductal arch.



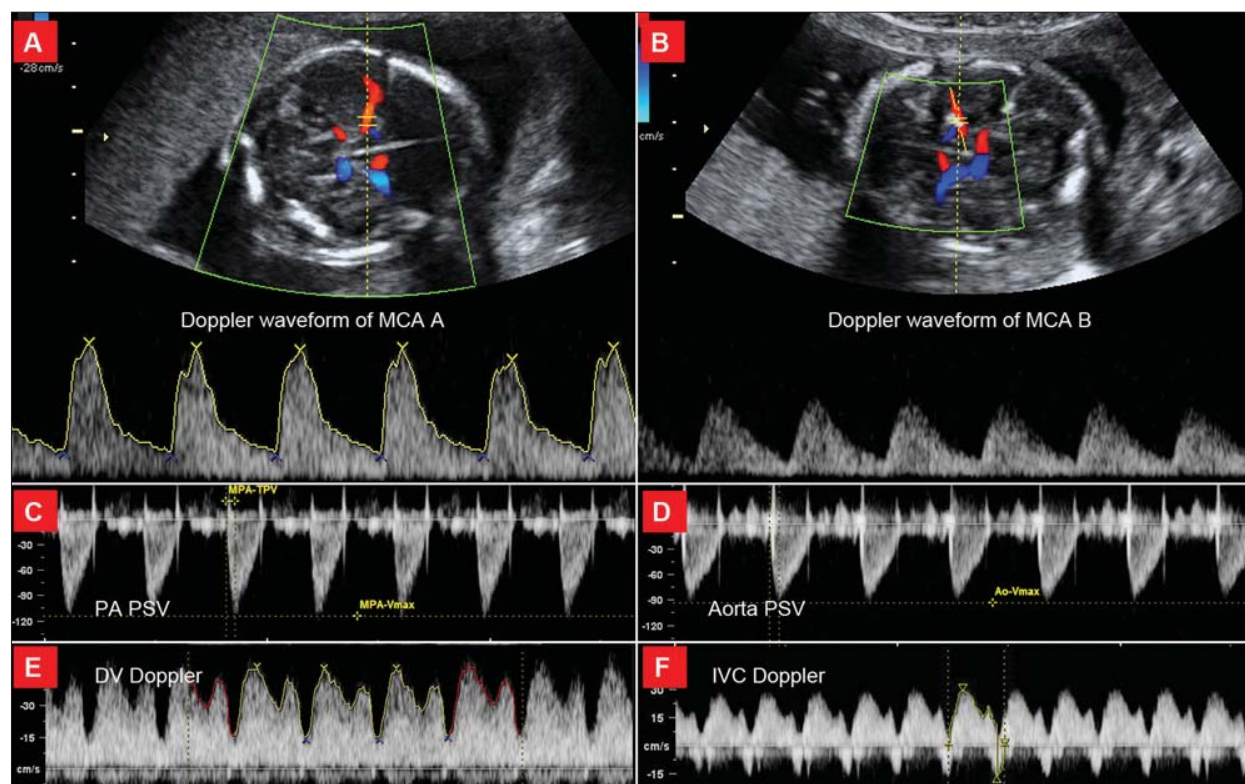
To our best knowledge, documented dicephalus twins usually have two hearts, although several sets formed a single shared complex heart with fused chambers. Uniquely, this case had one normal heart and one separate nonfunctioning heart. The functioning heart served the two fetuses without failure. With high-resolution sonography, it is not difficult to diagnose dicephalus twins prenatally, even in the first trimester,^{2,7} because of the simplicity of showing two heads and one body. However, to delineate how they share the organs, especially the cardiovascular system, is very challenging. This report emphasizes prenatal assessment of the fetal circulation, which could not be provided by postnatal autopsy.

At a glance, the healthy fetuses in our study might seem to have the same classic pattern as the famous Hensel twins, who have duplicated sets of normal heads, hearts, and circulatory systems but one body and two legs. In fact, they are totally different in terms of hemodynamics, although they might have been similar in early fetal life because of having two well-developed heads. However,

twin B in our case must have been dead sometime after the cardiocirculatory systems were established, and twin A took over the system after that. That only one heart served two lives perfectly is amazing and to our knowledge has never been described before. Theoretically, this case must have had a much poorer prognosis because of a risk of heart failure or hydrops fetalis in late pregnancy, although not occurring at the time of diagnosis.

We hypothesize that many parasitic twins with varying degrees of development and fetuses containing an acardiac twin may be a consequence of incomplete reversed perfusion to the acardiac twin after one heart becomes nonfunctional, occurring sometime in early development. Subsequently, the existing cardiovascular system is taken over by the surviving twin. This parasitic twin should be considered a variety of the twin reversed arterial perfusion sequence, but the reversal occurs in the conjoined bodies instead of the placenta. Our case is an instance of the extreme end of perfect reversed perfusion, resulting in an amazing phenomenon, a well-developed and live head on

Figure 2. Doppler study. **A** and **B**, Peak systolic velocity (PSV) in the middle cerebral artery (MCA) of twins A and B depicting normal circles of Willis and normal waveforms in twin A but aberrant waveforms in twin B, simulating umbilical artery waveforms. **C** and **D**, Abnormally high peak systolic velocity in the ascending aorta and pulmonary artery (PA). **E** and **F**, Normal venous Doppler waveforms in the ductus venosus (DV) and inferior vena cava (IVC).

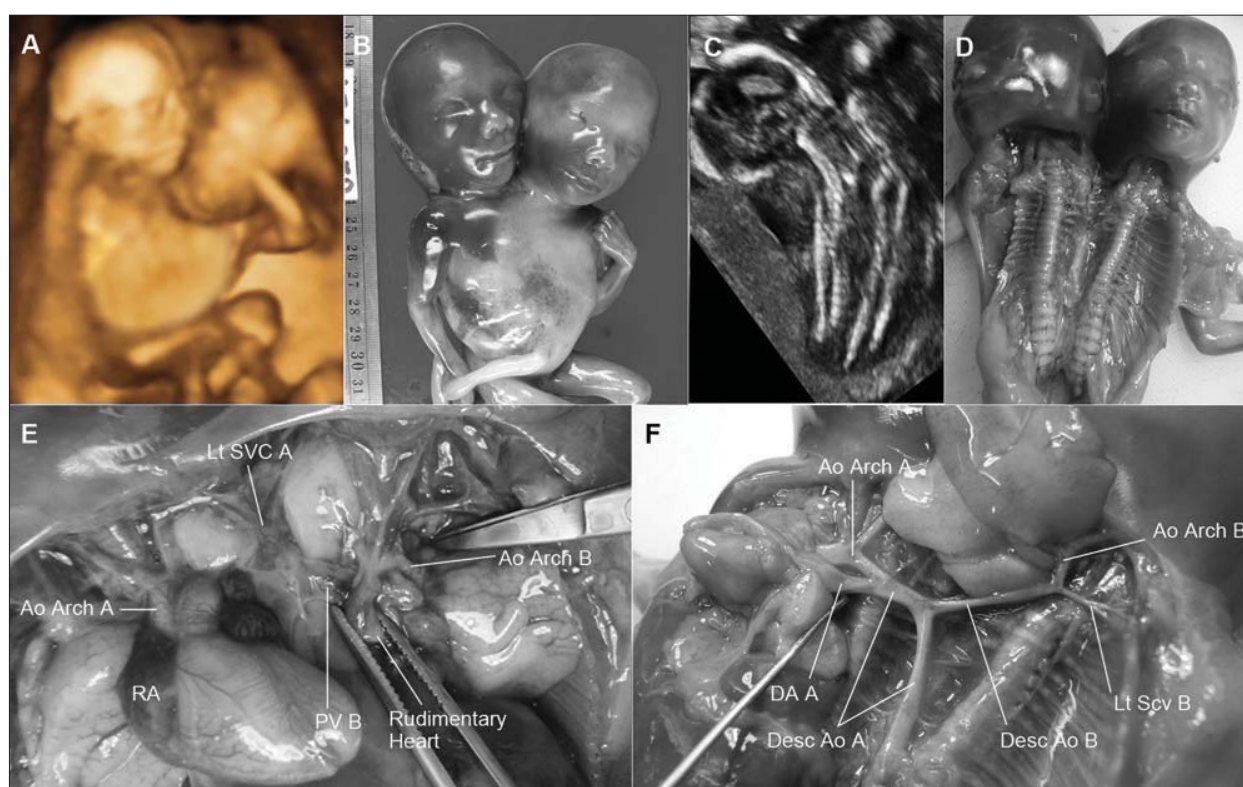


the dead fetus. The complete perfusion does usually not occur in the typical **twin reversed arterial perfusion sequence**, resulting in severely malformed fetuses or amorphous masses. Different from reversed perfusion through the umbilical artery in the typical twin reversed arterial perfusion sequence, the intriguing reversed perfusion through the descending aorta in our case may much more effectively supply the head, instead of providing mainly the lower limbs through the umbilical artery, as seen in the typical twin reversed arterial perfusion sequence.

Surprisingly, at the time of diagnosis, the cardiac function was perfectly normal, very well compensated. Therefore, this case is evidence of high adaptation of the fetal heart to cope with very high oxygen and nutritional demands of two lives. However, we expected that if termination of pregnancy had not been performed, cardiac failure or hydrops fetalis would have occurred in later pregnancy. At the time of study, the twins had a greater cardiac output and peak systolic velocity in the aorta and pulmonary artery, as indicated by the cardiac Doppler study.

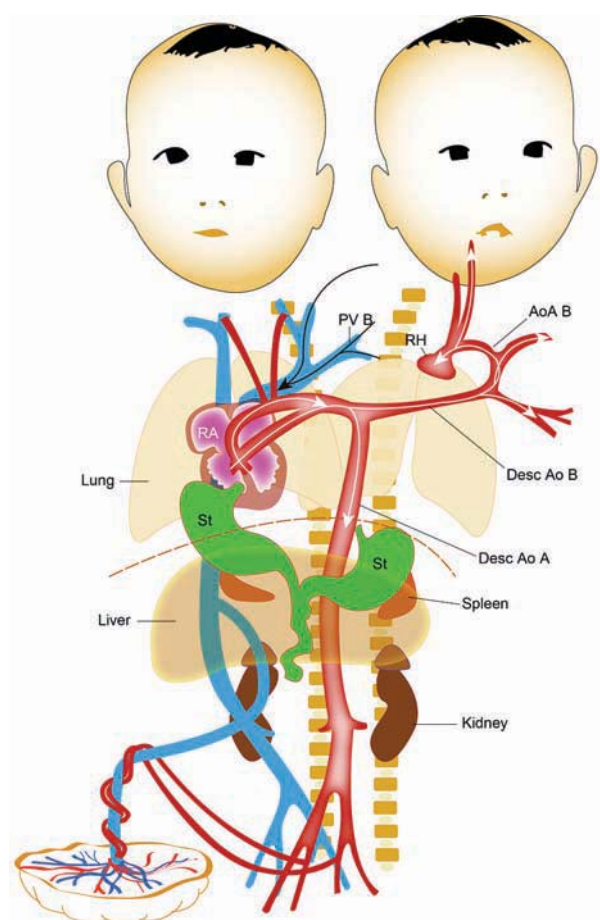
However, on the basis of the normal preload indicated by venous Doppler studies in all cardiac dimensions and a normal ventricular shortened fraction, the studies signified that the heart of twin had very high reserve potentials to serve the development of the twins.

Several fascinating items that can be learned from this case include the following: (1) It was one of the extremely rare cases of prenatal diagnosis of well-developed dicephalus dibrachius dipus, with sonographic-pathologic correlation that is informative for prenatal management. (2) In the prenatal hemodynamic study of conjoined twins, evaluation of the shared cardiovascular system was relatively complete, not only anatomically but also physiologically, which was impossible to evaluate with postnatal autopsy, providing insights for both the anatomy and function of fetal development. (3) The unique case of twin reversed arterial perfusion sequence with perfect development of human head growth signified perfect perfusion through the aorta, which to our knowledge has not been described before. (4) Aberrant cerebral Doppler waveforms were clearly



documented. (5) A hypothetical mechanism for an acardiac twin secondary to incomplete reversed perfusion from the surviving cotwin is suggested: intracorporeal anastomosis has already existed, and once one twin dies, reversed flow from the surviving twin will take over the existing circulatory system immediately and maintain at various degrees of development. (6) Strong evidence of the twin reversed arterial perfusion sequence can occur in conjoined twins and may be a basis for future studies of conjoined twin development. The mechanism of development of conjoined twins is incompletely understood, but our twins, having two complete vertebral columns, two hearts, and two sets of lungs, seemed to be caused by an initial fission followed by subsequent fusion of the embryos, as suggested by Spencer.⁸

Figure 4. Illustration of the hemodynamics and main structures of twins A and B. AoA indicates aortic arch; Desc Ao, descending aorta; PV, pulmonary vein; RA, right atrium; RH, rudimentary heart; and St, stomach.



This case might inspire sonographers to try their best to prenatally assess hemodynamics not only for diagnosis of dicephalus twins but also for determining how they join to each other. This process is essential for management planning and counseling, including potential in utero surgery, expectant management, and even termination of pregnancy. In cases of surgery, especially for a malformed head of twin B, it is theoretically possible to obliterate all blood supply to twin B by in utero laser ablation of the descending aorta with reversed perfusion.

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We thank the Thailand Research Fund and the Commission on Higher Education of Thailand for financial support.

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Effect of Umbilical Cord Bleeding following Mid-Pregnancy Cordocentesis on Pregnancy Outcomes

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Key Words

Fetal outcomes • Prenatal diagnosis • Cordocentesis

Abstract

Objectives: To compare the adverse pregnancy outcomes among the three groups of umbilical cord bleeding following cordocentesis; a group of no bleeding (group 1), transient bleeding (group 2), and persistent bleeding (>60 s: group 3). **Methods:** Consecutive cases of cordocentesis were prospectively recorded and followed up. The inclusion criteria for analysis consisted of (1) singleton pregnancies; (2) gestational age of 18–22 weeks; (3) no fetal anomalies; (4) no previous invasive procedures; (5) performed by experienced operators, and (6) known pregnancy outcomes. The main outcomes were rates of fetal loss, low birth weight and preterm birth. **Results:** Of 2,174 procedures, 1,614 were in group 1, 509 were in group 2, and 51 were in group 3. The mean birth weight and gestational age at delivery were significantly lower in group 3 than those in groups 1 and 2. The fetal loss rate was significantly higher in group 3. Rates of low birth weight and preterm birth were significantly higher in group 3 whereas the rates in group 2 had a tendency to be higher than those in group 1. **Conclusion:** This study suggests that cord bleeding during cordocentesis carries a higher risk of fetal loss, low birth weight and preterm birth.

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Introduction

Cordocentesis or funipuncture is a prenatal diagnostic procedure for fetal blood sampling which was first performed under ultrasound guidance by Daffos et al. [1] in 1980. This prenatal procedure has been well accepted as an efficient and relatively safe procedure and has permitted new insight into fetal medicine. A variety of indications for cordocentesis have been reported, especially rapid karyotype, fetal hemolytic disease and severe, early-onset growth restriction as well as fetal infection [2–6]. Unlike in the Western countries, cordocentesis in our practice for more than 20 years is most commonly indicated for fetal diagnosis of severe thalassemia syndrome, followed by rapid karyotype [7, 8]. However, this procedure carries a significant risk of pregnancy complications [8–13]. Though the rate of complications following cordocentesis has been ardently discussed in the literature, only a few studies have addressed this question comprehensively. Cordocentesis-related fetal loss is presumably associated with several factors, such as prolonged bleeding from the puncture site, formation of umbilical cord hematoma, premature rupture of membranes, etc. [9, 10, 14, 15]. Umbilical cord bleeding which is commonly observed following cordocentesis is often linked to subsequent serious complications even fetal loss. However, only inconclusive data are available on the clinical sig-

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0378–7346/12/0000–0000\$38.00/0

Accessible online at:
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nificance of umbilical cord bleeding following cordocentesis. No study has specifically focused on such a relationship. The data in previous studies are heterogeneous and those studies are flawed by several uncontrolled confounders such as mixed gestational age, various levels of expertise of the performers, and the presence of placenta penetration, etc. Thus, this study aimed to compare the adverse pregnancy outcomes among the three groups of umbilical cord bleeding secondary to cordocentesis – no bleeding, transient bleeding and prolonged bleeding – after excluding or controlling for potential confounding factors on adverse pregnancy outcomes.

Materials and Methods

A prospective study to compare adverse pregnancy outcomes between cordocentesis with cord bleeding and without bleeding was undertaken from a single center, Maharaj Nakorn Chiang Mai Hospital, Chiang Mai, Thailand, between September 1989 and December 2010. During the study period all cases of cordocentesis were prospectively recorded for procedure details as well as baseline characteristics of the patients, such as gestational age, indications for cordocentesis. The procedure profiles were also immediately recorded during the procedure such as the puncture cord site, puncture with/without placenta penetration, the presence of umbilical cord bleeding as well as its duration, bradycardia and duration of the procedure time, etc. All pregnancies were followed up for final pregnancy outcomes. These data were stored in a computer for subsequent analysis. The inclusion criteria for analysis consisted of (1) singleton pregnancies; (2) gestational age of 18–22 weeks at the time of performing cordocentesis; (3) no fetal congenital anomalies; (4) pregnancies with previous cordocentesis (usually from other hospitals or previous failure), or amniocentesis or chorionic villous sampling in the current pregnancy; (5) the procedures performed by experienced operators, referred to those experienced of more than 50 procedures, and (6) known pregnancy outcomes. The exclusion criteria were (1) the procedures involving placenta penetration; (2) fetuses subsequently proven to have chromosomal or structural abnormalities or any serious disorders required termination of pregnancy, and (3) failed or difficult procedures (procedures with duration time >30 min.) All procedures were performed under transabdominal-ultrasound guidance, using the convex transducer of 3.5 MHz (model E-8; GE Medical Systems, Milwaukee, Wisc., USA, and model SSD 620, 680EX, SSD 1700; SSD 5000, alpha-10; Aloka, Tokyo, Japan).

We used the same techniques in all cordocenteses, freehand technique, using local anesthesia without prophylactic antibiotics and without fetal paralysis to eliminate fetal movement. Puncture site selection was based on accessibility and quality of visualization with an attempt to avoid placenta penetration, if possible. The details of techniques are described elsewhere [7]. The failed procedures were based on operators' decisions. There was no definite number of punctures or exact duration of procedure, though a procedure lasting >30 min was considered a failure in most cases. The pregnancies with normal results of fetal blood analysis were usually taken care of as routine antenatal care.

Cases meeting the inclusion criteria were divided into three groups as follows: group 1: no bleeding; group 2: transient bleeding defined by bleeding from the umbilical cord puncture <1 min, and group 3: cordocentesis with prolonged bleeding, defined by bleeding from the umbilical cord puncture >1 min.

The primary outcomes of this study consisted of rates of fetal loss, low birth weight and preterm birth, as well as rate fetal bradycardia. Fetal loss was defined by spontaneous abortion (before 20 weeks of gestation) or a dead fetus in utero of any gestational age, and neonatal death. Low birth weight was defined by infants with a birth weight <2,500 g. Preterm birth referred to delivery before 37 complete weeks of gestation. This research was approved by the research ethics committee of our institute.

Statistical Analysis

Pearson's χ^2 test was used to compare the qualitative data or rates of adverse outcomes such as fetal loss rates among the three groups, whereas the ANOVA test with the post-hoc test were used for comparison of the quantitative data like means of gestational age at delivery or birth weight. The test results were considered statistically significant at $p < 0.05$. Statistical analyses were performed using the software for the social sciences (SPSS-PC+) software package version 17.0 (SPSS, Inc., Chicago, Ill., USA).

Results

During the study period, a total of 6,147 cordocenteses were performed at 18–22 weeks of gestation. To reduce as many potential confounding factors as possible and make the available data homogeneous, 3,973 cases were excluded due to the following reasons: twin pregnancies (55), loss to follow-up/incomplete data, especially final outcomes of pregnancies (1,272), procedures undergoing during early learning curve of experience (1,123), cases with prior cordocentesis/amniocentesis or chorionic villous samplings (866), failed or difficult procedures (40), fetuses later proven to have structural or chromosomal abnormalities or serious disorders required for termination of pregnancy (802), and procedures with placenta penetration (615). Finally, 2,174 procedures were suitable for analysis. Of these, 1,614 (74.2%) procedures had no cord bleeding (group 1), 509 (23.4%) had transient cord bleeding (≤ 60 s) (group 2) and the remaining 51 (2.4%) had prolonged cord bleeding (> 60 s) (group 3). Indications for cordocentesis are presented in table 1. Distribution of percentages of the indications was comparable among the three groups. Fetal risk for severe thalassemia and higher risk for chromosomal abnormalities was the most common indication. Distribution of percentages of placental location was also comparable in the three groups, as shown in table 2. The means of maternal age among the three groups were not significantly different

Table 1. Indications for cordocentesis

	Group 1		Group 2		Group 3	
	n	%	n	%	n	%
Risk of fetal Hb Bart's disease	312	19.3	94	18.5	13	25.5
Risk of fetal β -thalassemia major	210	13.0	66	13.0	9	17.6
Risk of fetal β -thalassemia/HbE	435	27.0	142	27.9	8	15.7
Mixed risk of fetal severe thalassemia	14	0.9	5	1.0	0	0.0
Rapid karyotyping	372	23.0	103	20.2	11	21.6
Risk of fetal severe thalassemia + chromosome	262	16.2	97	19.1	8	15.7
Others	9	0.6	2	0.4	2	3.9
Total	1,614	100.0	509	100.0	51	100.0

Table 2. Placental location

	Group 1		Group 2		Group 3	
	n	%	n	%	n	%
Anterior	452	28.0	146	28.7	12	23.5
Anterior lateral	125	7.7	23	4.5	3	5.9
Anteroposterior	17	1.1	7	1.4	1	2.0
Posterior	915	56.7	288	56.6	32	62.7
Posterior lateral	25	1.5	3	0.6	0	0.0
Lateral	19	1.2	3	0.6	1	2.0
Posterior fundus	4	0.2	5	1.0	0	0.0
Fundus	56	3.5	34	6.7	2	3.9
Unspecified	1	0.1	0	0.0	0	0.0
Total	1,614	100.0	509	100.0	51	100.0

χ^2 test: there were no significant differences between the three groups.

(30.78 $\pm$ 7.51 vs. 30.52 $\pm$ 8.1 vs. 30.44 $\pm$ 6.9 years; F test; $p = 0.62$). The procedure-related complications and outcomes of pregnancy outcomes were presented in table 3.

Considering technical aspects, the mean duration to complete the procedure in group 3 was not significantly different (ANOVA test, $p < 0.001$). Likewise, the rates of the difficult procedure and successful rates were not significantly different among the three groups (χ^2 , $p > 0.05$). However, the rates of fetal bradycardia were significantly higher in the prolonged bleeding group (3.3 vs. 4.1 and 15.7% in groups 1–3 respectively, $p < 0.001$). Regarding adverse outcomes of pregnancy, the mean birth weight and gestational age at delivery were also significantly lower in group 3 than those in groups 1 and 2, respectively, as shown in table 3. The fetal loss rate in group 3 was significantly higher when compared to that in groups

1 and 2, and the rate in group 2 was also significantly higher than that in group 1 (1.6 vs. 4.1 and 11.8% in group 1–3 respectively, $p < 0.001$). Likewise, low birth weight rates and preterm birth rates were significantly higher in group 3 ($p < 0.001$), whereas the rates in group 2 had a tendency to be higher than those in group 1, but were not statistically significant.

Discussion

Transient bleeding from the needle puncture site is a common finding often lasting no more than 1 min. The prevalence in this study was approximately 25% which was nearly the same as the occurrence we observed in our previous report, 20% for transient bleeding and 5% for

Table 3. Comparisons of procedure and pregnancy outcomes among the three groups (prolonged bleeding, transient bleeding, no bleeding)

	Group 1		Group 2		Group 3		p value
	mean	SD	mean	SD	mean	SD	
Procedure time	5.48	5.89	5.37	5.43	7.08	8.22	0.219*
Birth weight	3,003	503	2,998	592	2,541	870	<0.001**
GA at delivery	38.2	2.2	37.9	2.7	36.2	4.7	<0.001**

	Group 1		Group 2		Group 3		p value
	n	%	n	%	n	%	
Success rate	1,598/1,612	99.1	500/507	98.6	51/51	100.0	0.457
Bradycardia	53/1,614	3.3	21/509	4.1	8/51	15.7	<0.001
Fetal loss	26/1,614	1.6	21/509	4.1	6/51	11.8	<0.001 [#]
Low birth weight	185/1,614	11.5	62/509	12.2	14/51	27.5	<0.001 ^{##}
Preterm birth	195/1,614	12.1	70/509	13.8	14/51	27.5	<0.001 ^{##}

* ANOVA test shows no significant mean differences among the three groups.

** Post hoc test shows that the pairs of significant mean difference is group 3-group 2 and group 1-group 3.

[#] χ^2 test: significant difference between group 3-groups 1 & 2 and group 1-group 2.

^{##} Significant difference between group 3-groups 1 & 2 (note that there are unsuccessful cases including contamination of maternal blood or laboratory failure in diagnosis, etc.)

prolonged bleeding [7]. This is also comparable with that reported by Liao et al. [10], who found a prevalence of 19.8%. Our results indicate that transient bleeding has a tendency to increase the rate of adverse outcomes while prolonged bleeding is strongly associated with such outcomes. Bleeding from the cord puncture site is visible and is usually monitored to cessation and recorded for bleeding duration. Several factors may be associated with umbilical cord bleeding, especially difficult procedures, thick maternal abdominal wall, not the easiest accessible site to target, poor visualization, and active fetal movement. Active fetal movement while the needle tip is in lumen of the umbilical vessels is likely to increase risk of injury to those vessels, leading to bleeding.

Several authors believe that umbilical cord bleeding during the procedures carries a higher risk of adverse pregnancy outcomes, but no strong evidence has confirmed this hypothesis before, although several cases of fetal compromise have been reported to be associated with cord bleeding. For example, Maeda et al. [13] reported that 1 case in their series of 43 cases had continuous bleeding for 11 min and ended up undergoing cesarean section due to fetal distress. Many studies [9, 12–14, 16–19] have been published regarding cordocentesis-related complications, but most did not have enough statistical

power to show the real effect of umbilical cord bleeding on pregnancy adverse outcomes of low prevalence, especially fetal loss rates. Additionally, previous studies are flawed by heterogeneous baseline characteristics leading to be unsuitable for comparison.

To be more perfectly compared between those groups, we tried to eliminate all known or potential confounding factors which might have affected adverse outcomes of the procedures to achieve high homogeneity of all baseline characteristics in the three groups. The potential confounders needed to be controlled or excluded, included placenta traversed, difficult procedures, twin pregnancies, gestational age, and levels of operators' experience. Placenta traversed has been associated with adverse outcomes [20]. Gestational age certainly has an influence on the outcomes of procedures and pregnancy. Therefore, all cordocenteses included in this study were only confined to mid-pregnancy, i.e. a gestational age of 18–22 weeks. Our previous studies suggest that the procedures during the early learning curve usually take more time to complete, implying an increase in adverse outcomes of pregnancies [7]. Thereby, all of the procedures in the first 50 cases of practice were excluded from this study to reduce the expertise effect on pregnancy outcomes. Moreover, all difficult cases defined by the procedure duration

time of >30 min were also excluded. It makes sense to assume that difficult procedures or long operations are likely to increase fetal risk either with or without umbilical cord bleeding, thus confounding the results. Theoretically, the procedures involving penetration of the placenta are likely to increase the risk of trauma to placental vasculatures, leading to fetal or maternal hemorrhage, either bleeding into amniotic fluid or subtle bleeding in the placenta. This certainly can cause fetal anemia or thrombosis or placental ischemia, or even result in abortion or subsequent preterm labor. Thus, procedures with placenta penetration were excluded from our analysis.

Fetal bradycardia, immediate complications during the procedure, was significantly increased with duration of umbilical cord bleeding. It is reasonable to assume that umbilical cord bleeding can cause fetal bradycardia, implying fetal compromise and possibly leading to fetal loss. In addition to the fetal loss rate, rates of preterm birth rate and low birth weight were also slightly, but significantly higher in the group of cord bleeding, consistent with lower mean birth weight and mean gestational age. These complications may be associated with fetal anemia or some thrombosis with possibly impeding fetal circulation. Note that the rate of procedure time was comparable in all groups since the difficult cases were excluded.

It is theoretically reasonable to assume that more bleeding time is likely to be associated with a higher rate of adverse pregnancy outcomes. Whether such a transient bleeding, which is one of the most common findings during operation, can have any true effects on adverse outcomes or not has not been determined before. This study strongly suggests that any umbilical cord bleeding should not be ignored and the performers encouraged to avoid any condition leading to umbilical cord bleeding, e.g. not making an attempt to puncture during fetal active movement, temporary discontinuation of the procedures during bleeding, and no puncture with poor visualization. We recommend that in case of excessive fetal movement compromising access to the ideal cord site or placing an increased risk to the fetus, performers should wait for a while or even postpone the procedure until several hours later. Using a smaller needle, a 25-gauge needle, may be helpful for less blood loss [21].

Some pitfalls in this study may exist. The procedure-related fetal loss could not be accurately evaluated. The fetal loss rate in this study included a background risk. Some authors define the procedure-related fetal loss to be that it occurs within 2 weeks of gestation. Unfortunately, we could not reliably analyze the effect on such a specific fetal loss since the number of fetal losses was too low to

gain adequate power for such a purpose. Additionally, some confounding factors such as types of punctured vessels or the number of needle insertions could not be exactly provided for analysis in this study. Finally, the long period of study associated with considerable changes in operators, loss to follow-up rates as well as advancement in ultrasound equipment may affect the outcomes of the procedure. However, such changes were unlikely to alter the results since they should be the same among the groups of bleeding.

The strength of this study was the large sample size from a single center which was highly homogeneous and gained enough power to assess the rare outcomes like fetal loss rate. The effects of known confounders were controlled, especially the expertise levels of performers and similar gestational age. Besides, some highly potential confounders to outcomes of measurement were excluded as often as possible, such as the procedures with placenta penetration, difficult cases, and twin pregnancy, etc. Therefore the interpretation of the results may probably be highly reliable.

In conclusion, this study suggests that umbilical cord bleeding during cordocentesis carries a higher risk of fetal loss, low birth weight and preterm birth, and it seems to be related to the duration of bleeding. This information may encourage the performers to avoid any condition leading to umbilical cord bleeding.

Acknowledgements

The authors wish to thank Thai Research Fund and the National Research University Project under Thailand's Office of the Higher Education Commission for financial support.

Disclosure Statement

The authors have no conflicts of interest to disclose.

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ORIGINAL ARTICLE

Second trimester maternal serum markers and a predictive model for predicting fetal hemoglobin Bart's disease

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Objective: To compare the levels of maternal serum α -fetoprotein (AFP), unconjugated estriol (uE3) and free β -human chorionic gonadotropin (free β -hCG) between pregnancies with fetal Hb Bart's disease and unaffected pregnancies. **Methods:** 148 pregnancies at risk of fetal Hb Bart's disease scheduled for cordocentesis at 18 to 22 weeks were prospectively recruited into the study. AFP, uE3 and free β -hCG concentrations were measured before cordocentesis and the final fetal diagnosis of Hb Bart's disease was based on fetal Hb typing using high-performance liquid chromatography. **Results:** AFP and free β -hCG were significantly higher whereas uE3 was lower in women with fetal Hb Bart's disease than those with unaffected fetuses (1.94 MoM, 1.38 MoM and 0.81 MoM respectively). Hb Bart's predictive model; probability = $1/1 + e^{-[2.876 + 1.333(\text{AFP}) - 6.310(\text{uE3})]}$, effectively predicted fetal Hb Bart's disease (AUC ROC 0.91, 95% CI 0.84–0.97) with 61.5% sensitivity and 98.1% specificity using a cut-off probability at greater than 0.5. **Conclusions:** In triple test, serum AFP and hCG levels are significantly higher while serum uE3 is significantly lower in pregnancies with fetal Hb Bart's disease. Hb Bart's predictive model included AFP and uE3 is relatively effective and may be helpful in Hb Bart's prenatal screening.

Keywords: homozygous α -thalassemia-1 disease, triple test, maternal serum α -fetoprotein, unconjugated estriol and free β -human chorionic gonadotropin

Introduction

Fetal homozygous α -thalassemia-1 or hemoglobin (Hb) Bart's disease is the most common cause of hydrops fetalis in South East Asia. The hydropic changes manifested by cardiomegaly, hepatomegaly, skin edema, ascites and placentomegaly finally occur as a consequence of fetal anemia. Prenatal diagnosis is usually made using invasive techniques such as chorionic villous sampling, amniocentesis or cordocentesis which can be associated with fetal loss. Additionally, ultrasonographic markers such as cardiothoracic ratio, placental thickness and peak systolic velocity of middle cerebral artery have been shown to be helpful in diagnosis among pregnancies at risk [1–3]. Though highly effective, these methods require ultrasound machines and experienced sonographers leading to limitations for wide application. Thus, simple noninvasive prenatal diagnosis techniques are yet to be sought for. Maternal serum α -fetoprotein (AFP), free β -human chorionic gonadotropin (free β -hCG) and unconjugated estriol (uE3),

which are widely used for Down syndrome screening, have been reported to be associated with fetal hydrops [4–6] and enlarged placenta which are characteristics of pregnancies with fetal Hb Bart's disease. These serum markers have never been thoroughly studied in pregnancies with fetal anemia secondary to Hb Bart's disease but their levels may theoretically be increased in these fetuses, which are related to anemia, enlarged placenta and hepatomegaly. We hypothesize that these serum markers may probably be effective in predicting of Hb Bart's disease, especially among pregnancies at risk. The aim of this study was firstly to compare the levels of AFP, free β -hCG and uE3 between pregnancies with fetal Hb Bart's disease and unaffected pregnancies and secondly to develop a predictive model based on these serum markers in predicting fetal Hb Bart's disease.

Methods

A prospective study was conducted at Maharaj Nakorn Chiang Mai Hospital, Department of Obstetrics and Gynecology, Faculty of Medicine, Chiang Mai University with approval of the Research Ethical Committee. Pregnant women at risk of fetal Hb Bart's disease, scheduled for cordocentesis at 18–22 weeks between October 2009 and September 2011 were enrolled to the study. Pregnancy at risk was confirmed when both of the couple were α -thalassemia-1 carriers, diagnosed by PCR (SEA-type) technique. Gestational age was based on a certain last menstrual period and early fetal biometry either by crown-rump length in the first trimester or biparietal diameter in the second trimester. Exclusion criteria included: (i) multiple pregnancies; (ii) fetal structural or chromosomal abnormalities; (iii) fetal anemia secondary to any causes other than Hb Bart's disease; (iv) known maternal medical diseases and (v) failure of fetal blood sampling or contamination with maternal blood.

After informed and written consent was obtained, the demographic characteristics and medical history were recorded. Maternal blood sample was collected by venipuncture and was allowed to clot. The serum was immediately separated by centrifugation and stored at -20°C . AFP, free β -hCG and uE3 concentrations were measured using the 6000 DELFIA® Xpress clinical random access screening platform (PerkinElmer Life and Analytical Sciences, Turku, Finland). A transabdominal sonographic examination was performed for fetal biometry and fetal anatomical survey. Cordocentesis was then carried out under transabdominal real-time ultrasound guidance, using techniques as previously described [7]. Fetal blood sample was collected

and tested for maternal blood contamination using a simple acid elution test. Hemoglobin typing using high-performance liquid chromatography and DNA analysis, if needed, was performed for diagnosis of fetal thalassemia status.

Statistical analysis

All statistical analyses were performed using SPSS 17.0 (SPSS Inc, Chicago, IL). The demographic characteristics were described and compared between the affected group (the pregnancies with fetal Hb Bart's disease) and the unaffected group (the pregnancies with normal fetal Hb typing or α -thalassemia-1 carrier) by the independent samples T-test. AFP, free β -hCG and uE3 (expressed as concentrations and a multiple of median for each gestational week; MoM) were compared between both groups using the Mann-Whitney U test. The serum markers that were significantly associated with Hb Bart's disease were selected to enter in a logistic regression model. Backward stepwise technique was used with p value > 0.05 setting for removal from the model. The model's ability to discriminate between the pregnancies with Hb Bart's disease from the unaffected pregnancies was then evaluated by the area under the receiver operating characteristic (ROC) curve. The performance of each serum marker and the model in identifying affected fetuses were determined by calculating sensitivity and specificity using a cut-off probability at greater than 0.5. $p < 0.05$ was considered statistically significant.

Results

One hundred and forty-eight pregnant women were recruited to the study and maternal serum samples were collected and analyzed. The fetuses affected by Hb Bart's disease and unaffected fetuses (α -thalassemia-1 carriers and normal hemoglobin typing) were identified in 28 cases (18.9%) and 117 cases (79.1%), respectively. Three fetuses (2.0%) were diagnosed for hemoglobin H/Constant spring disease and were excluded from analysis. The remaining 145 pregnancies were available for analysis and their baseline characteristics are shown in Table I. No statistically significant difference in maternal age, gestational age at sampling and maternal body weight was found between the two groups ($p > 0.05$).

Table II shows AFP, free β -hCG and uE3 levels by mean of concentration and MoM in the affected and unaffected group. AFP and free β -hCG was significantly higher in affected pregnancies than in those with unaffected fetuses, whereas maternal serum uE3 was significantly lower in the affected group as illustrated in Figure 1.

In the logistic regression analysis using backward stepwise reduction, AFP and uE3 remained associated with Hb Bart's fetuses. Test of multicollinearity suggested the three biochemical

markers be independent to each other. The best fit Hb Bart's predictive model was as follows: probability of Hb Bart's fetus is $1/1+e^{-[2.876 + 1.333(\text{AFP}_\text{MoM}) - 6.310(\text{uE3}_\text{MoM})]}$. The ROC curves of maternal serum markers and Hb Bart's predictive model for predicting fetal Hb Bart's disease are demonstrated in Figure 2. ROC curve of Hb Bart's predictive model in identifying affected fetuses gave the greatest area under curve of 0.91 (95% CI = 0.84–0.97). Using a cut-off probability at greater than 0.5, the performance of each maternal serum marker and Hb Bart's predictive model in predicting fetal Hb Bart's disease are shown in Table III. Hb Bart's predictive model gave the best sensitivity and specificity of 61.5% and 98.1%, respectively.

Discussion

Hb Bart's or homozygous α -thalassemia-1 disease has a defect in a globin chain production resulting in gamma-4 tetramers (Hb Bart) formation. Hb Bart binds oxygen with high affinity causing fetal tissue hypoxia. Consequently, the compensatory extramedullary erythropoiesis in fetal liver or spleen and increased cardiac output extensively develop to overcome fetal tissue hypoxia, resulting in hydropic changes, such as subcutaneous edema, cardiomegaly, hepatomegaly, ascites and placentalomegaly. This pathophysiology may be associated with abnormal production of placental hormones or alteration in feto-maternal biochemical exchange. As a result, clinical interpretation of these biochemical markers in maternal serum may be unreliable, and they may be useful in identifying pregnancies affected by Hb Bart's disease. In this study, we found a significant difference of the levels of three maternal serum markers commonly used in screening Down syndrome (AFP, free β -hCG and uE3, known as triple test) among pregnancies with fetal Hb Bart's disease. To the best of our knowledge, this is the first study investigating whether or not triple test could be helpful in prenatal screening of Hb Bart's disease.

Free β -hCG

A higher level of free β -hCG has been reported to be associated with fetal chromosomal abnormalities such as Down syndrome, Turner syndrome and triploidy, especially in hydropic fetuses [4,6,8,9]. During the first trimester, maternal serum free β -hCG has been shown to be significantly higher in pregnancies affected by Hb Bart's disease than those in the control group (1.41 MoM in affected group vs 0.92 MoM in the control [10]. The hCG elevation associated with fetal Hb Bart's disease seems to be more obvious with advancing gestational age. Tseng et al. [11], reported that about 78% (14/18) of affected pregnancies had hCG levels >2.5 MoM between 14 and 23 weeks, though the conclusion might be flawed by a small sample size and no control.

This study demonstrated that, during 18–22 weeks of gestation, levels of free β -hCG in pregnancies with affected fetuses were significantly higher than those in the unaffected group (1.38 MoM vs 0.88 MoM). However, the clinical use to differentiate pregnancies with fetal Hb Bart's disease from unaffected pregnancies may be limited since a wide range of overlapping between its levels in both groups. When used as a solely marker, free β -hCG had low detection rate of only 11.5% but nearly all unaffected fetus had normal free β -hCG levels. Thus among fetuses at risk, the low probability indicated by this serum marker may be helpful in reassuring of no disease. However, in the triple test, β -hCG did not add sensitivity in predicting fetal Hb Bart's disease to the other two markers.

Table I. Baseline characteristics of the pregnancies affected and unaffected by fetal Hb Bart's disease.

	Affected group	Unaffected group	p value
Maternal age (year)			
• Mean $\pm$ SD	29.58 $\pm$ 6.68	27.82 $\pm$ 6.01	0.19
• Range	17–41	16–45	
Gestational age at sampling (week)			
• Mean $\pm$ SD	18.43 $\pm$ 0.96	18.66 $\pm$ 0.91	0.24
• Range	17–20	17–21	
Maternal body weight (kg)			
• Mean $\pm$ SD	57.76 $\pm$ 11.73	56.73 $\pm$ 9.49	0.63
• Range	39.1–97.5	38.2–101.1	

Table II. Maternal serum α -fetoprotein (AFP), free β -human chorionic gonadotropin (free β -hCG) and unconjugated estriol (uE3) concentrations and multiple of medians (MoM) in pregnancies affected and unaffected by fetal Hb Bart's disease.

Maternal serum markers	Unaffected group (<i>n</i> = 108)			Affected group (<i>n</i> = 26)			<i>p</i> value
	Percentile			Percentile			
	5th	50th	95th	5th	50th	95th	
hCG (ng/mL)	2.19	8.20	26.87	3.06	11.55	62.17	0.018
hCG (MoM)	0.21	0.88	3.00	0.26	1.38	7.32	0.020
AFP (mU/L)	30.35	54.00	127.38	40.31	93.00	205.62	<0.001
AFP (MoM)	0.68	1.14	2.36	0.91	1.94	3.80	<0.001
uE3 (nmol/L)	5.39	8.30	13.31	1.68	5.60	8.53	<0.001
uE3 (MoM)	0.78	1.20	1.82	0.22	0.81	1.34	<0.001

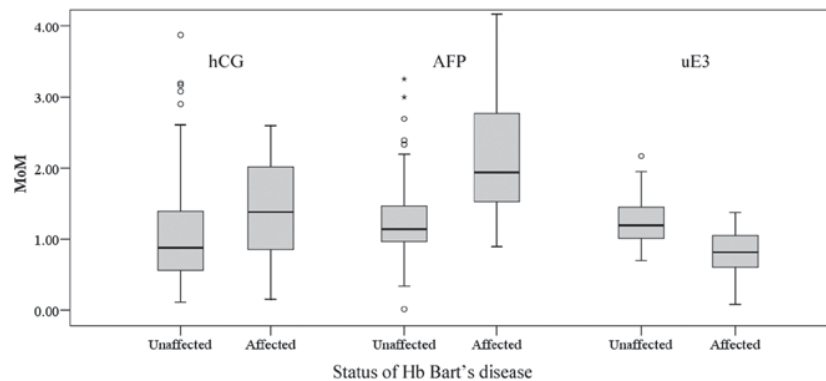


Figure 1. Boxplots of maternal serum α -fetoprotein (AFP), free β -human chorionic gonadotropin (free β -hCG) and unconjugated estriol (uE3) multiple of medians (MoM) in pregnancies affected and unaffected by fetal Hb Bart's disease.

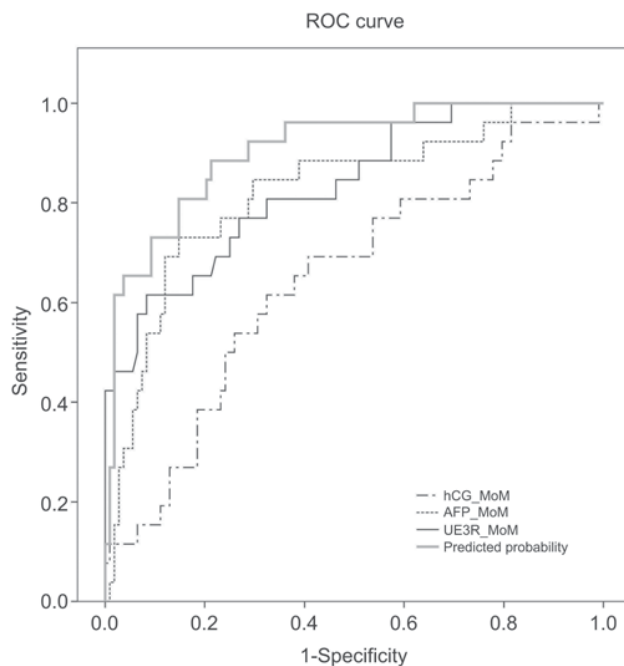


Figure 2. ROC curves of maternal serum markers and Hb Bart's predictive model in predicting fetal Hb Bart's disease.

Table III. Diagnostic performance of each maternal serum marker and model (derived from multivariate analysis: logistic regression) in predicting fetal Hb Bart's disease, using a cut-off probability at greater than 0.5.

Markers	Observed	Predicted (cases)		Correct prediction (%)
		Unaffected fetuses	Hb Bart's fetuses	
hCG	Unaffected fetuses	107	1	99.1
	Hb Bart's fetuses	23	3	11.5
AFP	Unaffected fetuses	105	3	97.2
	Hb Bart's fetuses	19	7	26.9
uE3	Unaffected fetuses	104	4	96.3
	Hb Bart's fetuses	14	12	46.2
Model	Unaffected fetuses	106	2	98.1
	Hb Bart's fetuses	10	16	61.5

AFP

Maternal serum AFP has been found to be elevated in pregnant women with fetal anemia such as parvovirus B19 infection and rhesus alloimmunization [12,13]. Bartha JL et al. reported that the levels of AFP in pregnancies with fetal anemia were significantly higher than those in pregnancies with normal fetuses (1.7 MoM vs 0.8 MoM) [14]. In 1990, there was a case report of 28 weeks of gestation Hb Bart's fetus with rising 3 MoM of AFP level. In our preliminary study, we also demonstrated the statistically higher levels of AFP in 11 Hb Bart's fetuses when compared to 42 unaffected fetuses [15]. The findings in this study as well as previous reports indicate that fetal anemia secondary to any causes can probably cause an elevated maternal serum AFP, either parvovirus infection, Rh isoimmunization or fetal Hb Bart's disease. It is unclear whether enlarged placenta permits a larger amount of AFP transferring from the fetus to mother or the fetus

increases the production of AFP in the liver in anemic environment. Similarly, this study with a larger sample size confirmed that AFP levels in pregnancies with fetal Hb Bart's disease were significantly higher than those in normal pregnancies (1.94 MoM vs 1.14 MoM). Nevertheless, though such increase in AFP levels seems to be obvious, maternal serum AFP as a single marker had a relatively low detection rate of less than 30%.

uE3

Of pregnant women carrying hydropic Down syndrome fetuses, serum uE3 levels have been shown to be lower than those in non-hydropic Down syndrome fetuses (0.33 MoM vs 0.63 MoM, $p < 0.001$) [4]. Similarly, pregnancies with hydropic Turner syndrome have been found to have the lower uE3 levels than non-hydropic Turner syndrome pregnancies (0.52 MoM vs 0.65 MoM, but no statistically significance) [5]. These previous reports suggest that hydropic placenta may be associated with poor placental function in producing estriol. Based on our new findings, this may also be true in hydropic placenta in fetal Hb Bart's disease, in spite of subtle hydriops without obvious signs of placentomegaly. This is the first report demonstrating that fetal Hb Bart's disease, in spite of no hydropic changes, is associated with lower maternal serum uE3. It is unclear how fetal Hb Bart's disease plays a role in estriol production. It may possibly be associated with placental dysfunction secondary to subtle hydropic changes or defect in precursor production from fetal adrenal glands.

Hb Bart's predictive model

In fact, this is the original study to establish Hb Bart's predictive model using maternal serum markers. We preferred maternal serum markers because the aim of this study was to find out a screening test that the skilled sonographers or perinatologists are not necessary. Although each serum marker could be predictive of Hb Bart's disease, the predictive model derived from logistic regression analysis including AFP and uE3 provided the best performance. The ability to predict Hb Bart's disease is satisfactory high shown as AUC ROC 0.91, 95% CI = 0.84–0.97. A cut-off point at probability of greater than 0.5 offered the acceptable sensitivity (61.5%) and high specificity (98.1%). However, serum markers screening was not perfect in screening Hb Bart's since it failed to detect 12 affected fetuses (42.8% of all Hb Bart's fetuses). Therefore, it could not be effectively used as a single screening test. Nevertheless, triple test may be helpful when used as a combination with sonomarkers, such as cardiothoracic ratio, placental thickness or peak systolic velocity of middle cerebral artery (MCA-PSV). The effectiveness of sonomarkers combined with maternal serum markers in predicting fetal Hb Bart's disease among pregnancies at risk is yet to be explored by further studies.

Importantly, the calculated risk of triple test for Down syndrome screening should be interpreted with high precaution in pregnant women at risk of fetal Hb Bart's disease, since all of the three serum markers are significantly different in pregnancies with fetal Hb Bart's disease. Therefore, abnormal triple tests among pregnancies at risk of fetal Hb Bart's disease should warrant the possibility of fetal Hb Bart's disease.

In conclusion, serum AFP and hCG levels are significantly higher in pregnancies with fetal Hb Bart's disease while serum uE3 is significantly lower in affected pregnancies. Hb Bart's predictive model included AFP and uE3 is relatively effective and may be helpful in Hb Bart's prenatal screening, especially when combined with ultrasonographic markers. Finally, since all of the three markers are significantly different in pregnancies with fetal Hb Bart's disease, in the area of high prevalence of Hb Bart's disease, it should be kept in mind that interpretation of the triple test may be unreliable in pregnancies with fetal Hb Bart's disease.

Declaration of Interest: The authors wish to thank the Thailand Research Fund (TRF) for financial support in the conduct of this research. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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ORIGINAL ARTICLE

Hemoglobin levels and red blood cell indices in mid-gestational fetuses with beta-thalassemia/HbE, beta-thalassemia trait or Hb E trait and normal fetuses

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ABSTRACT

Objective To compare red blood cell indices among normal, beta-thalassemia trait or hemoglobin (Hb) E trait, and beta-thalassemia/Hb E diseases mid-gestational fetuses.

Methods One hundred seventy-five fetuses at risk of beta-thalassemia/Hb E disease undergoing cordocentesis with determination of Hb levels and red blood cell indices at mid-pregnancy were recruited. The fetal diagnoses were based on fetal Hb typing and DNA analysis. The fetuses were divided into three groups: normal, beta-thalassemia trait or Hb E *trait*, and beta-thalassemia/Hb E *disease*.

Results The prevalence of beta-thalassemia/Hb E disease, beta-thalassemia trait or Hb E trait, and normal fetuses was 32.6% (57 cases), 28.6% (50 cases) and 36.6% (64 cases), respectively. Mean Hb levels and red blood cell indices were not significantly different among the three groups of fetuses. However, almost 10% of beta-thalassemia/Hb E fetuses had some degree of anemia. The most anemic fetuses had a β^0 mutation.

Conclusion There is no significant difference in Hb level and red blood cell indices among fetuses at risk of beta-thalassemia/Hb E disease. © 2013 John Wiley & Sons, Ltd.

Funding sources: The Thailand Research Fund and National Research University Project under Thailand's Office of the Higher Education Commission for financial support.
Conflicts of interest: None declared

INTRODUCTION

Thalassemia is a major inheritable healthcare problem in areas with a high prevalence such as Southeast Asia. Patients with severe thalassemia diseases such as homozygous beta-thalassemia, and beta-thalassemia/hemoglobin (Hb) E have a poor quality of life secondary to severe anemia and transfusion-associated complications. Thus, prenatal prevention and control of thalassemia is very important. Our center has extensive experience in the prenatal management of severe thalassemia disease;^{1–13} however, we currently have no knowledge on what hematologic parameters we can expect in these fetuses. Although several studies on hematology of normal fetuses have been published,^{14–22} no similar studies have been performed in fetuses with beta-thalassemia/Hb E trait or disease. Within the framework of a prenatal program of prevention and control of severe thalassemia, cordocentesis is commonly used. This creates a unique opportunity for good data collection on fetal hematologic parameters in the population at risk.

MATERIALS AND METHODS

A total of 175 singleton pregnancies who were at risk of having a fetus with beta-thalassemia/Hb E disease were invited and consented in written to the study. Inclusion criteria were the following: (1) singleton pregnancy, (2) gestational age of 18–22 weeks, (3) no other known hematologic disorders in the parents, and (4) being at risk of beta-thalassemia/Hb E disease, indicated by carrier status in both the couples as identified within a screening system established in our center and described elsewhere.¹¹ Exclusion criteria were the following: (1) subsequent diagnosis of other fetal hematologic disorders such as abnormal Hb, and (2) failed cordocentesis or no available final outcome. Cordocentesis was performed at 18–22 weeks of gestation at Maharaj Nakorn Chiang Mai hospital, between December 2010 and November 2011. Fetal blood samples of 0.5–1.0 mL were collected for analysis of hematological parameters, including Hb level, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), and

the degree of anemia in each fetus. All cordocenteses were performed by seven experienced perinatologists under real time ultrasound guidance using Aloka SSD Prosound α -10 or Aloka Prosound α -6 model (Tokyo, Japan) with transabdominal 3.5 MHz curvilinear transducers. All hematological parameters were measured within 24 hours after cordocentesis using an automated complete blood count machine (Coulter STKS analyzer; Beckman, USA). Severity of anemia in each fetus was defined by a fetal Hb of less than the 5th percentile for each gestational week, on the basis of reference ranges reported by Mari and *et al.*²³

Before high performance liquid chromatography (HPLC) was performed for Hb typing analysis, the acid elution test was used to differentiate maternal cell contamination in suspected cases. The final diagnosis of beta-thalassemia/Hb E status was based on fetal hemoglobin typing analysis using HPLC together with DNA analysis to determine gene mutation. All fetuses were post hoc categorized into three groups according to the final diagnosis: (1) normal, (2) beta-thalassemia trait or Hb E trait, and (3) beta-thalassemia/Hb E disease (affected fetuses). This study was approved by the Institutional Ethics Committee and financially supported by the Thailand Research Fund (TRF) and National Research University Project under Thailand's Office of the Higher Education Commission.

Statistical analysis

The data were analyzed using the statistical package for the social science (Chicago, USA), version 15.0. Descriptive data were presented as percentage, means, and standard deviation. Comparison among the three groups was performed using the analysis of variance (ANOVA) test for continuous variable and Pearson chi-square test for categorical data. As the focus of the primary outcome variables was the hemoglobin level, we calculated that we needed a sample size of at least 44 affected fetuses to show a significant difference at maximum allowable error of 10%.

RESULTS

One hundred and seventy-five fetuses were recruited into the study with a mean gestational age of 19.1 ± 1.03 weeks. Two fetuses had homozygous Hb E disease, one had Hb H Constant Spring disease, and one had an abnormal Hb Q. Because these were incidentally detected, they were excluded from the study.

These left 171 fetuses for analysis. The prevalence of beta-thalassemia/Hb E disease, beta-thalassemia trait or Hb E trait, and normal fetuses were 32.6% (57 cases), 28.6% (50 cases), and 36.6% (64 cases), respectively. Hemoglobin typing of the 57 fetuses with beta-thalassemia/Hb E diseases showed that Hb F was the main hemoglobin with a small proportion of Hb E with an average of 1.4% (range 0.6–2.7). Although there were some affected fetuses who had the β^+ mutation, but most of the fetuses in this group had the β^0 mutation; therefore, the proportion of Hb A was undetectable or less than 1% (average 0.16). However, two fetuses with the β^+ mutation had a relatively high proportion of Hb A, when compared with other fetuses (1.1 and 3.5%, respectively).

There were no cases of significant maternal cell contamination, as shown by the red blood cell distribution width (RDW) in Table 1 (no differences between the three groups). Red blood cell hematology (Hb level, MCV, MCH, and MCHC) for each of the groups is also presented in Table 1. Again, no significant differences were identified. However, their gestational ages were significantly different, and further tests with ANOVA for post hoc multiple comparisons showed that this was between normal fetuses and those with beta-thalassemia trait or Hb E trait fetuses ($p < 0.001$) and between beta-thalassemia trait or Hb E trait and beta-thalassemia/Hb E ($p = 0.006$). The relevance of this difference of less than 1 week is unlikely to be clinically relevant.

Eight out of the 171 fetuses (4.7%) were anemic. Of them, six had mild anemia and two had moderate anemia. There were no differences in number of anemic fetuses among the different groups (Pearson chi-square, $p = 0.10$). However, 5 out of 57 fetuses (8.7%) in the affected group had anemia, including one with moderate severity and the other four with mild anemia. One affected fetus with moderate anemia had codon 41/42 and codon 26 mutations. For the remaining four fetuses with mild anemia, two had codon 17 and codon 26 mutation, the other one had a 3.4 kb deletion with codon 26 mutation, and the last one had nucleotide-28 and codon 26 mutation together with Hb constant spring. All these pregnancies were offered termination of pregnancy because of the diagnosis of severe thalassemia. On the other hand, 3 out of the 114 unaffected fetuses (2.6%) also had anemia, that is, two with mild and one with moderate anemia. These three fetuses were born

Table 1 Hematology of red blood cell indices in mid-pregnancy fetuses (ANOVA test)

Parameters (mean $\pm$ SD)	Fetuses ($n = 171$)			P-value
	Normal ($n = 64$)	Beta or Hb E trait ($n = 50$)	Beta-thalassemia/Hb E disease ($n = 57$)	
GA (weeks)	18.84 ± 0.91	19.56 ± 1.07	19.04 ± 0.96	0.001
RDW (%)	7.72 ± 1.30	8.03 ± 1.42	7.80 ± 1.24	0.45
Hb level (gm/dl)	10.87 ± 1.04	10.86 ± 0.79	10.77 ± 1.27	0.86
MCV (fl)	122.34 ± 8.77	120.7 ± 9.24	121.19 ± 9.37	0.6
MCH (pg)	39.95 ± 3.17	39.59 ± 3.54	39.63 ± 3.56	0.81
MCHC (g/l)	32.66 ± 0.89	32.75 ± 0.88	32.66 ± 0.96	0.83

GA, gestational age; RDW, red blood cell distribution width; Hb, hemoglobin; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean cell hemoglobin concentration.

at term with no perinatal complications except that one fetus was moderately anemic with later neonatal jaundice caused by ABO incompatibility. All three babies were eventually alive and well without anemia or hematological disorders at 6 months of age.

DISCUSSION

Thalassemia is the most common hematologic genetic disease in Southeast Asia. The prevalence of beta-thalassemia and the Hb E gene in the Thai population are 3–9% and 13%, respectively. Beta-thalassemia/Hb E disease is considered as severe thalassemia, especially with β^0 mutation, because most patients have a low quality of life due to severe anemia and transfusion-dependency with an estimated life expectancy of 30 years.¹³ Therefore, prenatal diagnostic programmes with preventive measures seem warranted in the endemic area.

Beta-thalassemia is caused by reduced (β^+) or absent (β^0) of beta globin chain synthesis. The beta globin gene maps in the short arm of chromosome 11 and more than 200 disease-causing mutations have been identified so far. The majority of mutations are single nucleotide substitutions, deletions, or insertion of nucleotides and leading to frame shift mutation.^{24,25} Clinical severity is related to the extent of imbalance between the alpha and non-alpha globin chain. Beta-thalassemia/HbE disease has a variable clinical pattern, ranking from mild to severe transfusion-dependent diseases. Overall, pregnancies with fetuses that have beta-thalassemia/Hb E are not at higher risk for adverse outcome, and the fetuses have no clinical symptoms until postnatally. Therefore, it is reasonable to assume that the hematologic parameters in beta-thalassemia/Hb E fetuses would be normal. However, to date no strong data on this were available. This is the first study providing reliable prenatal data and useful information for clinician on this point, which may be helpful to differentiate affected fetuses among couples at risk.

As expectedly, the red blood cell indices and Hb levels were not significantly different among the three groups. These findings are consistent with the theory that beta-globin-dependent hemoglobin production does not occur until after birth.²⁶ As a result, our findings confirm that Hb level or red blood cell indices during mid-pregnancy period cannot be used to differentiate affected fetuses from unaffected fetuses.

Of interest, eight out of 171 fetuses had anemia. (Hb level under the 5th percentile for that particular gestational week)²³ Five of them were fetuses with beta-thalassemia/Hb E disease (8.7%), and the remaining three fetuses were normal fetuses (2.6%). Of the five affected fetuses with anemia, four had mild anemia and one was moderately anemic. Most of them had β^0 mutation, but one fetus with mild anemia had β^+ (nucleotide-28) mutation combined with an abnormal Hb (Hb constant spring). Thus, the defect in alpha globin gene in this fetus may potentially explain the anemia in this fetus because alpha globin is essential during *in-utero* period. However, because of incomplete

investigation of other causes of fetal anemia in our study, this explanation remains a hypothesis. Also, 10% of affected fetuses had some degree of anemia during mid-pregnancy. This is in contrast to what one expects because the beta globin gene is only expressed in adult life.²⁷ Although this observation suggested a trend of higher rate of anemia among affected fetuses, no statistical difference was found in number of anemic fetuses between the affected and unaffected groups. Therefore, this study does not provide sufficient evidence that beta-thalassemia/Hb E disease is the cause of anemia.

From this study, almost 3% of unaffected fetuses were discovered to have anemia as well. None had any sonographic signs of fetal anemia neither did they have significant hematologic disease after birth. Therefore, the reason for anemia during mid-pregnancy in these normal fetuses remains unclear, possibly normal variation or subtle causes, which were not completely investigated. Unfortunately, we do not have data about the iron status of the mother, but clinically, they were non-anemic and healthy. However, there are two women who are beta-thalassemia/Hb E disease and were recruited into this study. Both of them were referred from other hospital for cordocentesis at our center. Unfortunately, data about hemoglobin level or severity of maternal anemia of these two cases are not available, anyhow both fetuses did not have anemia at all.

On the basis of this study, it seems that a fetus who is a carrier of or has beta-thalassemia/HbE disease is unlikely to have anemic traits, which might confound interpretation of anemic signs in such fetuses. Additionally, we have obtained reference ranges of fetal Hb and red blood cell indices of normal fetuses in our geographic area, and our data are in line with what is known. The weakness of this study is that we have no true explanation for the significant difference in gestational age among the three groups of fetuses. Again, we think that this small (<1 week) difference is not relevant. The strength of this study is that it has a large sample size, including 57 fetuses with the disease all within the range of 18 to 22 weeks.

CONCLUSION

There is no significant difference in Hb level and red blood cell indices among fetuses at risk of beta-thalassemia/Hb E disease. Almost 10% of affected fetuses have some degree of anemia at mid-pregnancy.

WHAT'S ALREADY KNOWN ABOUT THIS TOPIC?

- There is a nomogram of fetal hematology in mid-pregnancy.

WHAT DOES THIS STUDY ADD?

- We provide novel information about fetal hematologic parameters (Hb level and red blood cell indices), in mid-gestational fetuses with beta-thalassemia/Hb E disease and fetuses with beta-thalassemia or Hb E trait.

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Case Report

Hemodynamic Assessment of Indomethacin-Induced Fetal Heart Failure in High-Output State

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Received 3 August 2012; accepted 27 March 2013

ABSTRACT: Hemodynamic study was performed on a 32-year-old woman presenting at 27 weeks' gestation. Ultrasound revealed a single normal fetus with mild cardiomegaly, polyhydramnios, and placental chorioangioma. Doppler study showed increased middle cerebral artery peak systolic velocity, normal Tei index, and forward flow of "a" wave with normal preload in the ductus venosus. Twelve hours after initiation of indomethacin for tocolysis, frank hydrops fetalis developed rapidly. The right ventricle showed poor contractility and performance. Markedly increased preload in ductus venosus with reversed "a" wave and pulsations in the umbilical vein were demonstrated. This study suggests that indomethacin treatment in fetal high-cardiac output state should be used with extreme caution. © 2013 Wiley Periodicals, Inc. *J Clin Ultrasound* 00:000–000, 2013; Published online in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/jcu.22055

Keywords: indomethacin; chorioangioma; hydrops fetalis; heart failure; prenatal diagnosis; Doppler; sonography

Indomethacin, one of the first-line drugs for tocolysis, is the most common medication causing constriction of the ductus arteriosus, leading to restriction of blood flow through the ductus, an increase in right ventricular afterload, tricuspid regurgitation, right atrial dilatation, and increased cardiac preload. Although such effects are temporary and reversible in most cases, they can result in hydrops fetalis or

even fetal death in some rare circumstances. Significant constriction of the fetal ductus arteriosus caused by indomethacin or spontaneous occurrence has been reported as a rare cause of serious hemodynamic deterioration associated with hydrops fetalis or fetal death.^{1–4}

Fetuses with ductus-dependent heart defects, for example, critical aortic stenosis and interrupted aortic arch, carry a higher risk of indomethacin-induced hydrops fetalis.^{5,6} Different from other previous reports, the fetus in our case had a structurally normal heart with a high-cardiac output caused by chorioangioma and indomethacin-precipitated closure of the ductus and rapid development of hydrops fetalis. To the best of our knowledge, demonstration of rapid hemodynamic deterioration from high-output compensatory heart to cardiac failure, which was accelerated by indomethacin use, has never been reported. The objectives of this report were to describe the acceleration of hydropic changes in a fetus with high-output state and to demonstrate rapid hemodynamic deterioration following indomethacin administration.

CASE REPORT

A 32-year-old woman, G3 P2002, was diagnosed for chorioangioma with polyhydramnios at 27 weeks' gestation. Her previous medical and familial history as well as course of current pregnancy was unremarkable. On admission, ultrasound examination (Voluson E8; GE Medical Systems, Milwaukee, WI) revealed a single live fetus, fetal biometry consistent with date,

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no fetal structural anomaly except mild cardiomegaly (cardiothoracic ratio of 0.65), with good contractility (shortening fraction; SF of 32%) in both ventricles (Video Clip 1) and minimal scalp edema without any other signs of hydrops fetalis, polyhydramnios with amniotic fluid index of 30, and placental tumor (chorioangioma) of 8 cm in diameter on the chorionic side. The tumor was highly vascularized. Doppler study showed normal waveforms of the umbilical arteries [systolic/diastolic (S/D) ratio of 2.63], increased middle cerebral artery peak systolic velocity of 65 cm/s, and Tei index of 0.27 for both ventricles. Venous Doppler showed forward flow of “a” wave with normal preload index (0.445) in the ductus venosus and relatively steady flow with minimal pulsations in the umbilical vein. The spectral Doppler studies are shown in Figure 1. The patient was scheduled for alcoholization of the tumor on the next day to obliterate the feeding vessels.⁷ However, preterm labor occurred on the admission night and the doctors in charge prescribed tocolysis with indomethacin. Twelve hours after initiation of indomethacin, the patient was sonographically re-evaluated. Frank hydrops fetalis was clearly demonstrated, including ascites, pleural effusion, pericardial effusion, and subcutaneous edema. The right ventricle with poor contractility (SF less than 5% and Tei index 0.55) was larger than the left ventricle, which maintained normal contractility and performance (SF of 32% and Tei index of 0.27) (Video Clip 2). Venous Doppler study showed markedly increased preload index in the ductus venosus (0.688) with reversed flow of “a” wave. The diameter of ductus arteriosus and peak velocity could not be obtained. Indomethacin was stopped and preterm labor was well controlled with nifedipine. The feeding vessels to the tumor were sclerosed with 2 ml 95% ethanol. However, although the flow in the tumor was decreased, the clinical outcomes did not improve. The patient underwent cesarean section on the following day for postnatal treatment of hydrops fetalis. The female neonate, 1420 g, was severely depressed but gradually improved and survived.

DISCUSSION

Like other blood sequestration pathologies such as sacrococcygeal teratoma or aneurysm of the vein of Galen, chorioangioma was likely to contribute to a high-output state, which may eventually lead to cardiac decompensation or failure. Nevertheless, the cardiac function in our case

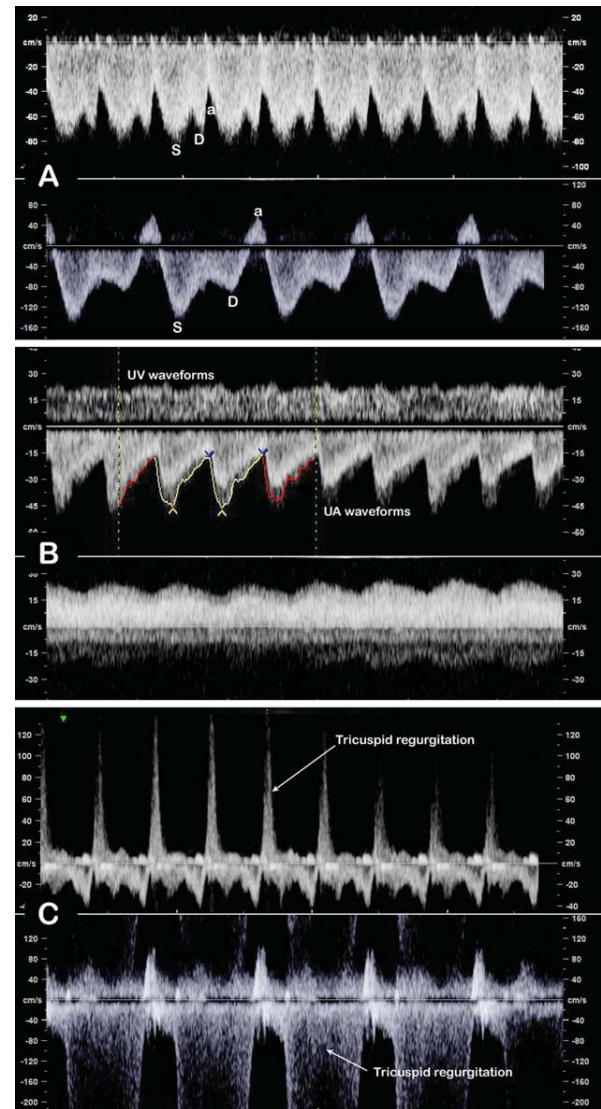


FIGURE 1. Spectral Doppler study before (upper panel) and 12 hours after indomethacin administration (lower panel): (A) waveforms in the ductus venosus changed from forward “a” wave to reversed “a” wave; (B) waveforms in the umbilical vein changed from relatively steady to venous pulsations; and (C) waveforms at the tricuspid valves showing short periods of regurgitation to holosystolic regurgitation. Abbreviations: a, atrial contraction wave; D, diastolic wave; S, systolic wave; UA, umbilical artery; UV, umbilical vein.

was well compensated at the time of admission as indicated by normal preload and normal cardiac function based on both normal Tei index and shortening fraction, whereas the middle cerebral artery peak systolic velocity was markedly increased. The baby adapted to blood sequestration in the tumor by increasing cardiac output, although cardiomegaly and polyhydramnios existed. Cardiomegaly was a mechanism to increase fetal cardiac output without any other signs of hydrops fetalis. However, 12 hours after indomethacin administration, fetal

cardiac failure rapidly developed. The high-output state changed to a low-output state with decreasing cardiac output in the right side of the heart. We hypothesize that the heart was well adapted to the high-output state; however, it was vulnerable to decompensation, when exposed to high afterload following ductal constriction, evidenced by the reduction in the shortening fraction and ductus venosus flow reversal.

Although several studies indicate that fetal ductal constriction following indomethacin treatment is usually reversible after cessation of the treatment,^{6,8,9} we hypothesize that in our case the heart had been overworked from the high-output state and was too vulnerable to tolerate the extra afterload caused by indomethacin-induced ductal closure, resulting in rapid development of cardiac failure and frank hydrops fetalis.

However, contrary to our findings, Kriplani et al¹⁰ reported a good response with indomethacin in the treatment of polyhydramnios due to chorioangioma at 30 weeks' gestation with preterm labor with the pregnancy prolonged for 3 weeks. However, the case presented here may have been more compromised before treatment than Kriplani's case, as their case showed no sign of cardiac decompensation, cardiomegaly, or any signs of hydrops fetalis. Therefore, based on available evidence, if indomethacin has to be used in this setting, and possibly in other fetal high-output states, it should be used only with very close fetal monitoring for ductal closure and cardiac decompensation, as well as progressive changes of hydrops fetalis.

In conclusion, this case provides new insight that indomethacin can hasten the progression of high-cardiac output heart failure to low-output failure if ductal constriction occurs. The development of hydropic changes may occur more rapidly than in normal fetuses. Although indomethacin may be effective and relatively safe when used as the tocolysis of choice in cases of polyhydramnios and normal heart, it may be contraindicated or used only with extreme caution in a fetus with high-cardiac output. Comprehensive fetal hemodynamic assessment should be performed before tocolysis

or treatment of polyhydramnios with indomethacin, and if used frequently, reassessment is recommended.

ACKNOWLEDGMENTS

The authors wish to thank the Thailand Research Fund (TRF) and the National Research University (NRU) for financially support.

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Reference Ranges of Ductus Arteriosus Derived by Cardio-Spatiotemporal Image Correlation from 14 to 40 Weeks of Gestation

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Key Words

Fetus · Ductus arteriosus · Prenatal diagnosis · Reference range · Spatiotemporal image correlation · 4D ultrasound · Z-scores

Abstract

Objective: To construct reference ranges of fetal ductus arteriosus (DA) derived by volume datasets of cardio-spatiotemporal image correlation (cardio-STIC). **Methods:** Cardio-STIC volume datasets were acquired from low-risk singleton pregnancies with a reliable gestational age from 14 to 40 weeks. In offline analysis with 4D View version 9, fetal DA was measured in the transverse ductal arch view with orthogonal control in the multiplanar view. The reference ranges of the DA and Z-score equation were constructed against gestational weeks and biparietal diameter (BPD) as independent variables. **Results:** A total of satisfactory 606 volumes were analyzed. The reference ranges for predicting means and SDs of fetal DA were constructed based on the best-fit regression model. Mean DA (mm) was best predicted by linear model as a function of GA (weeks) and BPD (cm) as follows: Predicted DA diameter (cm) = $-0.051 + 0.014 \times \text{GA (weeks)}$ ($r = 0.84$) and Predicted DA diameter (cm) = $-0.015 + 0.053 \times \text{BPD (cm)}$ ($r = 0.83$). Models for Z-score calculation and centile charts for predicting fetal DA were also provided.

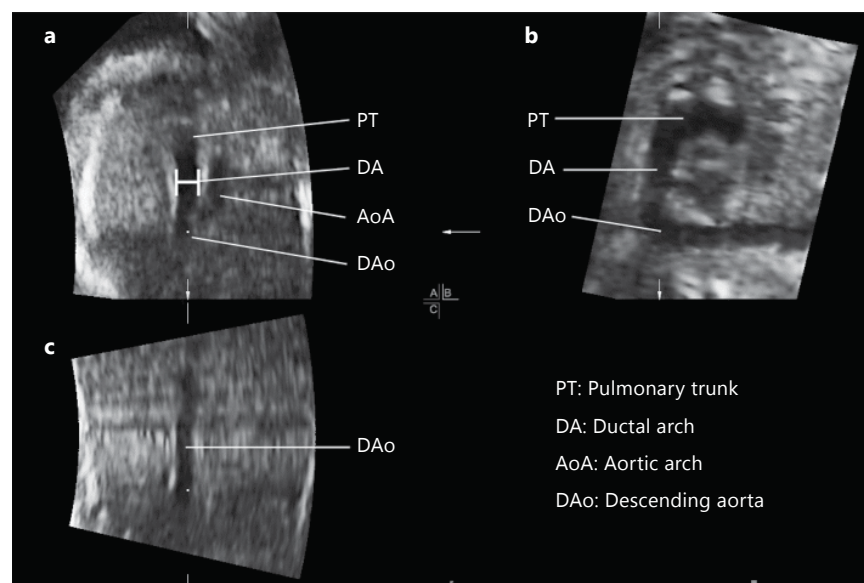
Conclusion: Reference ranges of the fetal DA and Z-score model are provided. These may serve as a useful tool in the assessment of fetal DA, especially in fetal cardiac anomalies or in monitoring fetuses exposed to maternal indomethacin.

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Introduction

The ductus arteriosus (DA) is a direct continuation of the main pulmonary trunk, connecting the aorta distal to the left subclavian artery [1, 2]. In normal cardiovascular development it is a persistent distal portion of the left sixth aortic arch [2], which diverts the right ventricular output bypassing the pulmonary to umbilical-placental circulation [2, 3]. In prenatal diagnosis of various cardiac conditions, evaluation of DA including size, shape, and color flow mapping can increase the detection rate of malformation. Determination of the ductal diameter may be helpful in the diagnosis of intrauterine ductal closure that occurs spontaneously or after exposure to prostaglandin synthetase inhibitor, aneurysm of DA and cardiac malformation such as obstruction of the outflow tract, transposition of the great arteries, tetralogy of Fallot, and truncus arteriosus [1–4]. In fact, several studies have published nomograms of DA diameter using 2D ultrasound [1, 4, 5]

Fig. 1. a–c Examples of fetal ductal arch diameter measurement displayed in multiplanar views of cardio-STIC volume at four-chamber view. Note the measurement performed in figure 1a at the portion of greatest dimension.



or morphology study in abortus and stillbirth fetuses [3]. However, all previous studies were based on 2D ultrasound with which it is often difficult to obtain the accurate plane for measurement. Furthermore, the sample sizes were relatively small and did not include fetuses in the early second trimester. In addition, Z-score, a useful parameter in the interpretation of measurement that quantifies the degree to which an individual value lies above or below the mean for a given population, was not included in those studies except that of Pasquini et al. [5].

With cardio-spatiotemporal image correlation (cardio-STIC) and compound imaging capabilities, which allows for off-axis beam steering, a volume dataset of the fetal heart can easily be maneuvered to obtain the correct cardiac plane in the three orthogonal planes. Therefore, we could measure the DA dimension more accurately than that done in the past. The objective of this study was to establish normal reference ranges and to develop a Z-score for fetal DA diameter at 14–40 weeks' gestation derived from cardio-STIC volume datasets, since we can rotate the cardiac volume datasets to control the axis and to establish exact dimension of the DA.

Materials and Methods

The 4D-STIC datasets were obtained between September 1, 2007 and October 31, 2010 at Maharaj Nakorn Chiang Mai Hospital, Chiang Mai University, with approval from the institute's ethics committee. Inclusion criteria included: (a) correct gestational age (GA) based on sonographic fetal biometry before 20 weeks' gestation and

regular menstruation prior to pregnancy with known date of the last menstrual period; (b) GA between 14 and 40 weeks, and (c) low-risk pregnancy without known obstetric and medical complications. Exclusion criteria included: (a) multiple pregnancies; (b) fetal anomalies; (c) abnormal fetal growth, either small for GA (<10th percentile for each gestational week) or macrosomia (>90th centile), and (d) 4D-STIC volume datasets could not be obtained satisfactorily.

All ultrasound examinations were performed by the authors using a real-time scanner with transabdominal 2–4 MHz curvilinear transducers equipped with 4D-STIC (Voluson E8; GE Healthcare, USA). During the examination, a routine standard ultrasound scan including fetal biometry and anomaly screening was first performed. The acquired volume datasets were acquired with transverse sweeps through the fetal chest and stored for subsequent analysis.

Offline analysis of the volume datasets was performed using 4D View version 9.0 (GE Medical Systems, Zipf, Austria) as follows: the volume datasets were displayed on multiplanar view based on the four-chamber view acquisition. Dynamic images of the fetal heart were simultaneously visualized in the three orthogonal planes (axial, coronal and sagittal). From the based images of the four-chamber view in figure 1a–c, the volumes were scrolled up to the transverse ductal arch view or three-vessel trachea view to display the ductal arch in the greatest dimension. The procedures usually needed minimal rotation of the fetal heart around the three orthogonal axes (x, y and z) to obtain exactly the greatest dimensions. With proper images of the transverse arch (fig. 1a) the longitudinal view of the ductal arch would be displayed (fig. 1b) and the en face view of the descending aorta displayed (fig. 1c). The internal DA diameter was then electronically measured during end-systole at the greatest dimension of the DA, usually somewhat distal to the pulmonary bifurcation. The first 40 volume datasets were used to determine intra- and interobserver variability.

Regression analysis of DA diameter was performed, using SPSS version 15.0 software, to determine the best-fit equation which was then used for construction of reference intervals according to Royston and Wright [6]. The regression models were

Table 1. Fetal DA diameter as a function of GA (n represents the sample size for each gestational week)

GA weeks	n	DA diameter, mm						
		2.5th	5th	10th	50th	90th	95th	97.5th
14	15	1.1	1.1	1.2	1.5	1.7	1.8	1.9
15	15	1.2	1.2	1.3	1.6	1.9	2.0	2.0
16	17	1.3	1.3	1.4	1.7	2.0	2.1	2.2
17	18	1.4	1.5	1.6	1.9	2.2	2.3	2.4
18	19	1.5	1.6	1.7	2.0	2.4	2.5	2.5
19	18	1.6	1.7	1.8	2.2	2.5	2.6	2.7
20	16	1.7	1.8	1.9	2.3	2.7	2.8	2.9
21	22	1.8	1.9	2.0	2.4	2.8	3.0	3.1
22	26	1.9	2.0	2.1	2.6	3.0	3.1	3.2
23	27	2.0	2.1	2.3	2.7	3.2	3.3	3.4
24	23	2.1	2.3	2.4	2.9	3.3	3.5	3.6
25	23	2.2	2.4	2.5	3.0	3.5	3.6	3.8
26	25	2.4	2.5	2.6	3.1	3.7	3.8	3.9
27	30	2.5	2.6	2.7	3.3	3.8	4.0	4.1
28	26	2.6	2.7	2.9	3.4	4.0	4.1	4.3
29	23	2.7	2.8	3.0	3.6	4.1	4.3	4.4
30	29	2.8	2.9	3.1	3.7	4.3	4.5	4.6
31	35	2.9	3.0	3.2	3.8	4.5	4.6	4.8
32	26	3.0	3.2	3.3	4.0	4.6	4.8	5.0
33	17	3.1	3.3	3.5	4.1	4.8	5.0	5.1
34	22	3.2	3.4	3.6	4.3	4.9	5.1	5.3
35	28	3.3	3.5	3.7	4.4	5.1	5.3	5.5
36	24	3.4	3.6	3.8	4.5	5.3	5.5	5.7
37	22	3.5	3.7	3.9	4.7	5.4	5.6	5.8
38	24	3.6	3.8	4.1	4.8	5.6	5.8	6.0
39	19	3.7	3.9	4.2	5.0	5.8	6.0	6.2
40	17	3.9	4.1	4.3	5.1	5.9	6.1	6.3

fit separately to the mean and SD of the DA diameter as functions of GA and biparietal diameter (BPD) to generate the best-fit regression equations. The assumption of normality and the goodness of fit of each regression model were checked by examination of the scatter patterns of points relative to fitted means and SDs, expressed as Z-scores. Z-scores were assessed for normality by Q-Q plots and the Shapiro-Wilk test. Intra- and interobserver variability was identified with an intraclass correlation coefficient technique.

Results

Of 672 acquired volume datasets, 66 (9.8%) were excluded from analysis secondary to poor quality. Of the 66 excluded, 20 were acquired before 16 weeks and 26 acquired after 32 weeks and remaining 20 were confined in a period of 16–32 weeks, which were mostly related to poor fetal posture. A total of 606 volume datasets from 606 fetuses had a satisfactory image for offline analysis. The number of cases at each GA is shown in table 1.

Predicted mean and SD of DA diameter, based on gestational weeks and BPD, was best described by a linear regression model, as follows:

$$\text{Predicted mean DA diameter (cm)} = -0.051 + 0.014 \times \text{GA (weeks)} \quad (r = 0.84).$$

$$\text{Predicted SD of DA diameter (cm)} = -0.003 + 0.002 \times \text{GA (weeks)} \quad (r = 0.33).$$

$$\text{Predicted mean DA diameter (cm)} = -0.015 + 0.053 \times \text{BPD (cm)} \quad (r = 0.83).$$

$$\text{Predicted SD of DA diameter (cm)} = 0.005 + 0.006 \times \text{BPD (cm)} \quad (r = 0.30).$$

Based on the models above, the reference ranges of DA as a function of GA and BPD are constructed and shown in tables 1 and 2, as well as percentile charts in figures 2 and 3. These equations were validated by a calculated Z-score for each variable. The Z-score had a normal distribution which was as presented in figure 4. Additionally, the normality of distribution was shown by Shapiro-Wilk test.

The Z-score for DA diameter value can be calculated as follows:

$$\text{Z-score} = (\text{measured DA diameter} - \text{predicted DA diameter}) / \text{predicted SD}.$$

Example of Z-score calculation: at GA 20 weeks and a fetal ductal arch diameter of 0.12 cm, the following formula was used:

$$\text{Predicted mean DA diameter (cm)} = -0.051 + 0.014 \times \text{GA (weeks)} = 0.229.$$

$$\text{Predicted SD of DA diameter (cm)} = -0.003 + 0.002 \times \text{GA (weeks)} = 0.037.$$

$$\text{Z-score} = (0.12 - 0.229) / 0.037 = -2.95.$$

Therefore the fetus has a small ductal arch at 2.95 SD below the predicted mean fetal ductal arch for a fetus whose GA is 20 weeks, i.e. out of normal range. Intra- and interobserver variabilities of DA measurement were analyzed and found to be in good agreement with an intraclass correlation coefficient of 0.921 (95% CI 0.904–0.966, $p < 0.001$) and 0.896 (95% CI 0.886–0.924, $p < 0.001$), respectively.

Discussion

Ductal measurement is useful for fetal echocardiography. An abnormal ductal diameter can alert the physician that the fetus has some anomaly such as complex

Table 2. Fetal DA diameter (mm) as a function of BPD (cm)

BPD	2.5th	5th	10th	50th	90th	95th	97.5th	BPD	2.5th	5th	10th	50th	90th	95th	97.5th
2.5	0.8	0.9	1.0	1.2	1.4	1.5	1.5	6.1	2.3	2.4	2.6	3.1	3.6	3.7	3.9
2.6	0.9	0.9	1.0	1.2	1.5	1.5	1.6	6.2	2.3	2.5	2.6	3.1	3.6	3.8	3.9
2.7	0.9	1.0	1.0	1.3	1.5	1.6	1.6	6.3	2.4	2.5	2.7	3.2	3.7	3.9	4.0
2.8	1.0	1.0	1.1	1.3	1.6	1.6	1.7	6.4	2.4	2.6	2.7	3.2	3.8	3.9	4.0
2.9	1.0	1.1	1.1	1.4	1.6	1.7	1.8	6.5	2.5	2.6	2.8	3.3	3.8	4.0	4.1
3.0	1.0	1.1	1.2	1.4	1.7	1.8	1.8	6.6	2.5	2.6	2.8	3.3	3.9	4.0	4.2
3.1	1.1	1.1	1.2	1.5	1.8	1.8	1.9	6.7	2.5	2.7	2.8	3.4	3.9	4.1	4.2
3.2	1.1	1.2	1.3	1.5	1.8	1.9	2.0	6.8	2.6	2.7	2.9	3.4	4.0	4.2	4.3
3.3	1.2	1.2	1.3	1.6	1.9	2.0	2.0	6.9	2.6	2.8	2.9	3.5	4.1	4.2	4.4
3.4	1.2	1.3	1.4	1.7	1.9	2.0	2.1	7.0	2.7	2.8	3.0	3.6	4.1	4.3	4.4
3.5	1.2	1.3	1.4	1.7	2.0	2.1	2.2	7.1	2.7	2.9	3.0	3.6	4.2	4.4	4.5
3.6	1.3	1.4	1.5	1.8	2.1	2.2	2.2	7.2	2.8	2.9	3.1	3.7	4.3	4.4	4.6
3.7	1.3	1.4	1.5	1.8	2.1	2.2	2.3	7.3	2.8	2.9	3.1	3.7	4.3	4.5	4.6
3.8	1.4	1.4	1.5	1.9	2.2	2.3	2.4	7.4	2.8	3.0	3.2	3.8	4.4	4.5	4.7
3.9	1.4	1.5	1.6	1.9	2.2	2.3	2.4	7.5	2.9	3.0	3.2	3.8	4.4	4.6	4.8
4.0	1.4	1.5	1.6	2.0	2.3	2.4	2.5	7.6	2.9	3.1	3.2	3.9	4.5	4.7	4.8
4.1	1.5	1.6	1.7	2.0	2.4	2.5	2.6	7.7	3.0	3.1	3.3	3.9	4.6	4.7	4.9
4.2	1.5	1.6	1.7	2.1	2.4	2.5	2.6	7.8	3.0	3.2	3.3	4.0	4.6	4.8	5.0
4.3	1.6	1.7	1.8	2.1	2.5	2.6	2.7	7.9	3.0	3.2	3.4	4.0	4.7	4.9	5.0
4.4	1.6	1.7	1.8	2.2	2.6	2.7	2.7	8.0	3.1	3.2	3.4	4.1	4.7	4.9	5.1
4.5	1.7	1.7	1.9	2.2	2.6	2.7	2.8	8.1	3.1	3.3	3.5	4.1	4.8	5.0	5.2
4.6	1.7	1.8	1.9	2.3	2.7	2.8	2.9	8.2	3.2	3.3	3.5	4.2	4.9	5.1	5.2
4.7	1.7	1.8	1.9	2.3	2.7	2.8	2.9	8.3	3.2	3.4	3.6	4.2	4.9	5.1	5.3
4.8	1.8	1.9	2.0	2.4	2.8	2.9	3.0	8.4	3.2	3.4	3.6	4.3	5.0	5.2	5.4
4.9	1.8	1.9	2.0	2.4	2.9	3.0	3.1	8.5	3.3	3.5	3.7	4.3	5.0	5.2	5.4
5.0	1.9	2.0	2.1	2.5	2.9	3.0	3.1	8.6	3.3	3.5	3.7	4.4	5.1	5.3	5.5
5.1	1.9	2.0	2.1	2.5	3.0	3.1	3.2	8.7	3.4	3.5	3.7	4.5	5.2	5.4	5.5
5.2	1.9	2.0	2.2	2.6	3.0	3.2	3.3	8.8	3.4	3.6	3.8	4.5	5.2	5.4	5.6
5.3	2.0	2.1	2.2	2.7	3.1	3.2	3.3	8.9	3.4	3.6	3.8	4.6	5.3	5.5	5.7
5.4	2.0	2.1	2.3	2.7	3.2	3.3	3.4	9.0	3.5	3.7	3.9	4.6	5.3	5.6	5.7
5.5	2.1	2.2	2.3	2.8	3.2	3.4	3.5	9.1	3.5	3.7	3.9	4.7	5.4	5.6	5.8
5.6	2.1	2.2	2.3	2.8	3.3	3.4	3.5	9.2	3.6	3.8	4.0	4.7	5.5	5.7	5.9
5.7	2.1	2.3	2.4	2.9	3.3	3.5	3.6	9.3	3.6	3.8	4.0	4.8	5.5	5.7	5.9
5.8	2.2	2.3	2.4	2.9	3.4	3.5	3.7	9.4	3.6	3.8	4.1	4.8	5.6	5.8	6.0
5.9	2.2	2.3	2.5	3.0	3.5	3.6	3.7	9.5	3.7	3.9	4.1	4.9	5.7	5.9	6.1
6.0	2.3	2.4	2.5	3.0	3.5	3.7	3.8	9.6	3.7	3.9	4.1	4.9	5.7	5.9	6.1

heart disease like tetralogy of Fallot or truncus arteriosus etc., or ductal aneurysm [1, 2, 4]. Though measurement of the fetal ductal arch is not necessary during routine anomaly screening, this should be performed whenever a suspicious disproportion is noted. Values should be compared with normal ranges or the Z-score to quantify the extent of abnormalities. In high-risk pregnancy exposed to prostaglandin inhibitor used for tocolysis, a serial ductal diameter measurement should be made to detect its constriction [4].

As in previous studies, we found that the ductal arch diameter increased with gestational age as a linear function. Nevertheless, most previous studies did not provide

a Z-score and did not perform regression analysis of the SD separately. This is essential for percentile chart construction since the SDs varied with GA.

Although normal reference ranges for ductal diameter have been published, they are still limited and different techniques of measurement have been used. The diameter of DA may be measured in the transverse [1, 5] or longitudinal [7] view of the thorax. However, we prefer the transverse view since it is more reproducible, it is simple to get the greatest dimension and is easily seen in its entire course as well as its relation to bifurcation and descending aorta in dynamic images making sure of the proper point of measurement. We emphasize on the

Fig. 2. Linear function relationship between GA in weeks and fetal ductal arch in millimeters (the lines represent the 2.5th, 5th, 10th, 50th, 90th, 95th and 97.5th percentile).

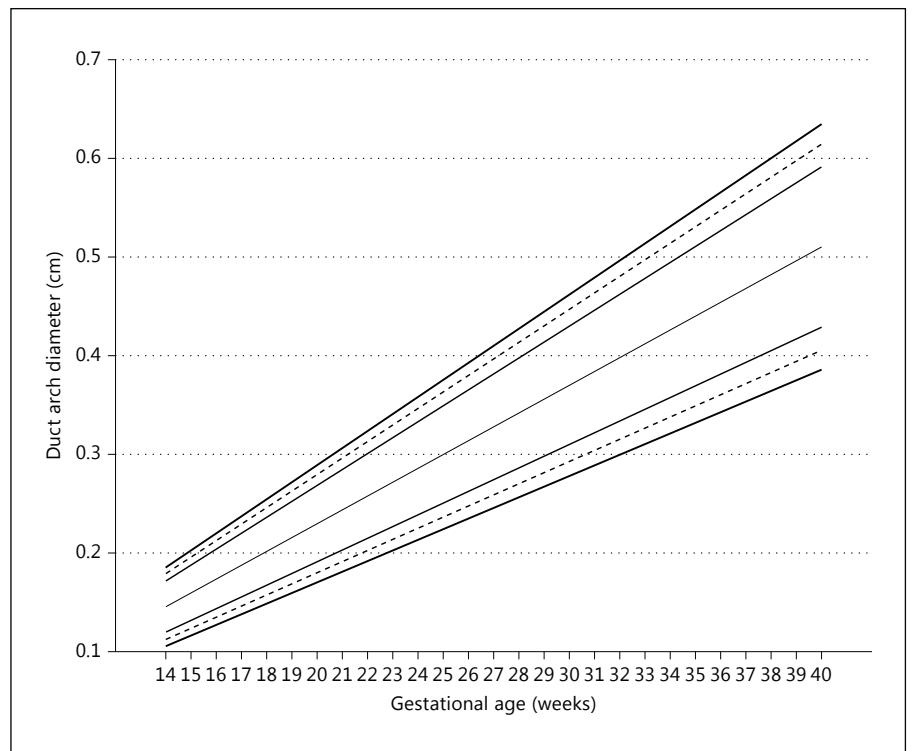
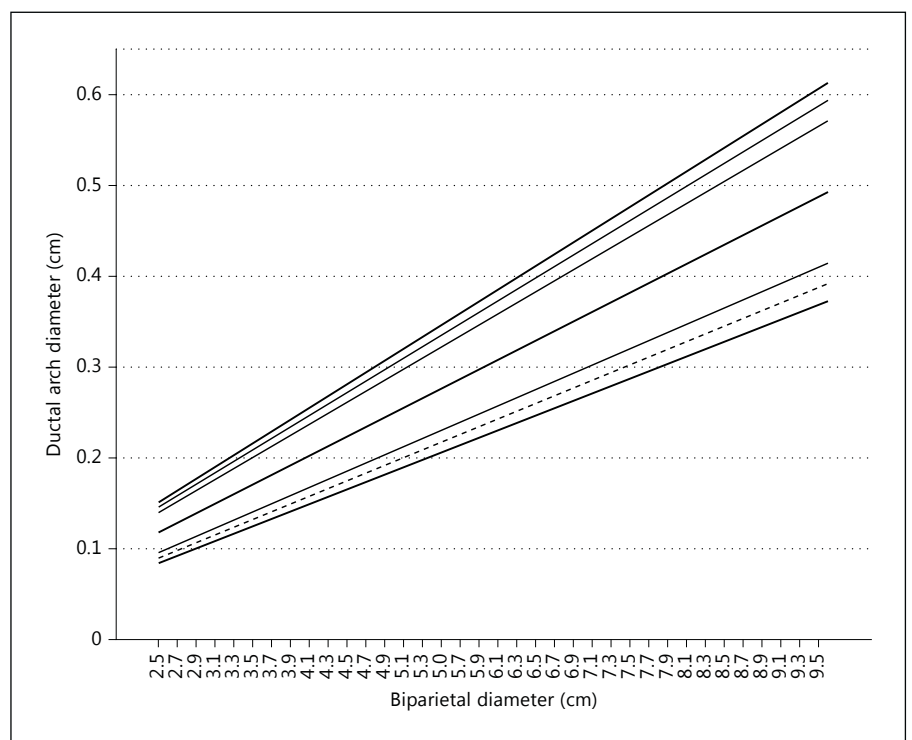


Fig. 3. Linear relationship between BPD (cm) and fetal ductal arch (mm) (the lines represent the 2.5th, 5th, 10th, 50th, 90th, 95th, and 97.5th percentile).



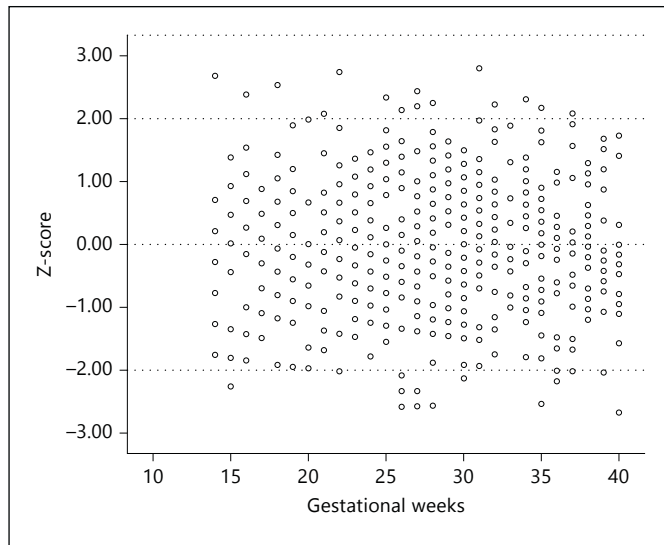


Fig. 4. Distribution of calculated Z-scores of fetal ductal arch against GA indicating adequate level of fit.

greatest dimension, usually located between the three-vessel view (3VV) described by Yoo et al. [8] and three-vessel trachea view (3VT) as described by Yagel et al. [9]. It is not necessary to clearly display the aortic arch. In our experience, the 3VV, of which bifurcation of the pulmonary is demonstrated, is not the plane of greatest dimension of the DA. Additionally, the 3VT view as suggested by some authors [1, 5] is not the perfect plane for DA measurement either, although the diameter of the two arches can be easily compared in this view and discrepancies between the two can be demonstrated. Nevertheless, due to the oblique nature of this view, absolute assessment of the size of the great arteries is not reliable. Moreover, most authors did not specify the exact point of measurement.

With 2D ultrasound, the transverse view of the ductal arch can be obtained with a slight cranial tilt from the standard 3VV. However, true transverse sections of the fetal thorax are somewhat difficult to obtain because of shadowing of the sternum and ribs. It is therefore easier to obtain a paratransverse section of the thorax from the upper abdomen with cranial tilt of the probe. With cardio-STIC offline analysis this section is much easier as it is usually obtained by simply scrolling through from the four-chamber view to 3VV and just above the 3VV with minimal adjustment of the 3D axis without attention to the aortic arch, just displaying the greatest dimension of DA.

Some limitations in this study should be mentioned, including the following. In spite of a high-resolution scanner, several volume datasets in early gestation were excluded due to poor quality because of small cardiac structures in the early second trimester resulting in difficulty in clearly distinguishing from surrounding structures. Therefore, we suggest that in cases of unsatisfactory measurement with transabdominal scan, transvaginal examination should be obtained. On the contrary, in late gestation, shadowing from the ossified bone ribs can obscure the image even if we try acquisition in the appropriate plane, resulting in exclusion of many cases in late pregnancy. Therefore, in clinical application, this measurement has a limitation in late gestation. In addition, resolution of some volume datasets may be compromised due to fetal movement and maternal breathing. Moreover, it is sometimes impossible to obtain satisfactory volumes in fetuses with very active movement for long periods, or a poorly accessible posture, especially in the prone position.

The strengths of this study included: (1) large sample size (a sample size of 606, ranging from 15 to 38 examinations per week of gestation), compared to other previous studies which consisted of approximately 200 participants. As suggested by Royston and Wright [6], in order to construct a 'normal range' for fetal variables with a $\pm 1-3$ SD curve and restricting the standard error of the limits of reference range to 10% of the SD, the study requires a sample size of about 550 fetuses (14–40 weeks); (2) measurement based on offline analysis of cardio-STIC without a time limit, allowing authors to obtain the reliable planes or exact plane of the greatest dimension of the DA; (3) our reference ranges included fetuses in the early second trimester, unlike in most previous reports, and finally (4) Z-scores for quantitative assessment of the DA are also presented and Z-score distribution against GA and BPD indicates an adequate model fit.

In conclusion, reference ranges for the DA diameter were constructed on the basis of a cardio-STIC volume dataset. These reference ranges may be useful tools for assessment of various conditions involving the ductal size.

Acknowledgement

The authors wish to thank the Thai Research Fund and the National Research University Project under Thailand's Office of the Higher Education Commission for financial support.

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ORIGINAL ARTICLE

Comparison of maternal serum PlGF and sFlt-1 between pregnancies with and without fetal hemoglobin Bart's disease

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ABSTRACT

Objective The aim of this study was to compare the levels of maternal serum placental growth factor (PlGF) and soluble fms-like tyrosine kinase-1 (sFlt-1) between pregnancies with fetal hemoglobin (Hb) Bart's disease and unaffected pregnancies.

Methods Ninety-one pregnancies at risk for fetal Hb Bart's disease scheduled for cordocentesis at 18–22 weeks were recruited into the study. Maternal serum PlGF and sFlt-1 concentrations were measured before cordocentesis. Fetal blood samples were collected for thalassemia diagnosis based on fetal Hb typing using high-performance liquid chromatography. PlGF, sFlt-1, and sFlt-1/PlGF ratio were compared between the fetal Hb Bart's group and the non-Hb Bart's group (normal Hb typing or α -thalassemia-1 carrier).

Results Maternal serum concentration of PlGF was significantly higher in women with fetal Hb Bart's disease (18 cases) than those with unaffected fetuses (71 cases) ($P=0.008$), whereas the concentration of sFlt-1 was not significantly different ($P=0.139$). However, the sFlt-1/PlGF ratio was significantly lower in women with fetal Hb Bart's disease than those with unaffected fetuses ($P=0.001$).

Conclusion Placental growth factor may help differentiate affected from unaffected fetuses among pregnancies at risk, though further studies are needed to confirm its usefulness. In addition, preeclampsia prediction using these markers may be unreliable in pregnancies with placental dysfunction secondary to severe fetal anemia. © 2013 John Wiley & Sons, Ltd.

Funding sources: The authors wish to thank the Thailand Research Fund (TRF) for financial support in the conduct of this research.

Conflicts of interest: None declared

INTRODUCTION

Placental growth factor (PlGF) is an angiogenic factor involving in trophoblast differentiation and vasculogenesis, similar to vascular endothelial growth factor (VEGF). The interaction between endothelial receptors and PlGF or VEGF is inhibited by the soluble form of the vascular endothelial growth factor receptor-1 or soluble fms-like tyrosine kinase-1 (sFlt-1), which acts as an anti-angiogenic factor. Both PlGF and sFlt-1 have been found to be associated with pathogenesis of preeclampsia due to an imbalance of circulating angiogenesis factors.¹ In preeclampsia, the pattern of PlGF was significantly lower, while sFlt-1 and sFlt-1/PlGF ratio were higher when compared with controls.¹ These factors have also been demonstrated to be altered in other diseases that are associated with a large placental mass and hydrops.^{2–5} Fetal homozygous α -thalassemia-1 or hemoglobin (Hb) Bart's disease is one of the common causes of hydrops fetalis in Southeast Asia.^{6–8} The placentomegaly and hydropic changes evidenced by cardiomegaly, hepatomegaly, skin edema, and ascites are the characteristics of this disease as a consequence of fetal

anemia.⁹ If early termination of such a pregnancy is not performed, nearly all will finally develop preeclampsia in late second or third trimester, usually severe and early-onset.¹⁰ The relationship between pregnancy with hydrops fetalis and preeclampsia is well established.¹¹ Therefore, it is reasonable to assume that placental dysfunction in pregnancies with Hb Bart's disease may occur early in gestation because nearly all these pregnancies developed preeclampsia in late pregnancy. We hypothesize that alterations in these angiogenic and anti-angiogenic factors may have already occurred by mid gestation, so that their predictive value may be questioned. If so, this might support the theory of abnormal placentation secondary to fetal anemia and hydropic changes in early gestation leading to preeclampsia in late gestation. To the best of our knowledge, this is the first study attempting to correlate placental angiogenic factors and pregnancies with fetal Hb Bart's disease. The aim of this study was to compare the levels of maternal serum PlGF and sFlt-1 between pregnancies with fetal Hb Bart's disease and unaffected pregnancies.

MATERIALS AND METHODS

This was a prospective study conducted at Maharaj Nakorn Chiang Mai Hospital, Department of Obstetrics and Gynecology, Faculty of Medicine, Chiang Mai University with approval of the Ethical Institutional Review Board. Normotensive pregnant women at risk of fetal Hb Bart's disease, scheduled for cordocentesis at 18–22 weeks of gestation between October 2010 and September 2011 were enrolled to the study. Pregnancy at risk was confirmed when both of the couple were diagnosed as α -thalassemia-1 carrier, using polymerase chain reaction (SEA-type). Gestational age was based on early fetal biometry either by crown-rump length in the first trimester or biparietal diameter in the second trimester. Exclusion criteria included are the following: (1) multiple pregnancies, (2) fetal anomalies or chromosomal abnormalities, (3) history of preeclampsia in previous pregnancy, (4) known maternal medical diseases, and (5) failure of fetal blood sampling or contamination with maternal blood.

After informed and written consent was obtained, the demographic characteristics and medical history were recorded. Maternal blood sample was collected by venipuncture and was allowed to clot. The serum was immediately separated by centrifugation and stored at -20°C . PlGF and sFlt-1 concentrations were measured using the Elecsys® PlGF and Elecsys® sFlt-1 on the automated Roche immunoanalyzers Elecsys® 2010 (Roche Diagnostics, Mannheim, Germany) using an electrochemiluminescence sandwich immunoassays. The total duration of the assays was 18 min, and the sample volume was 20 μL for sFlt-1 and 50 μL for PlGF. The Elecsys® sFlt-1 assay covers a measuring range from 10–85 000 pg/mL and the Elecsys® PlGF assay from 3–10 000 pg/mL.¹²

A transabdominal sonographic examination was performed for fetal biometry and fetal anatomical survey. Noninvasive ultrasonographic markers such as cardiothoracic ratio (CTR), placental thickness (PIT), and middle cerebral artery peak systolic velocity (MCA-PSV) were also performed using techniques as described in the previous reports.^{13–15} Cordocentesis was then carried out under transabdominal real-time ultrasound guidance, using techniques previously described.¹⁶ A fetal blood sample was collected and tested for maternal blood contamination using a simple acid elution test. Hb typing using high-performance liquid chromatography and DNA analysis (if needed) were analyzed for the fetal thalassemia status.

Statistical analysis

All statistical analyses were performed using SPSS 17.0 (SPSS Inc, Chicago, IL). The demographic characteristics were described and analyzed to compare the difference between the affected group (the pregnant women whose fetuses were affected with Hb Bart's disease) and the unaffected group (the pregnant women whose fetuses were normal Hb typing or α -thalassemia-1 carrier) by the independent samples t-test. The maternal serum PlGF concentrations, sFlt-1 concentrations, sFlt-1/PlGF ratio, CTR, PIT, and MCA-PSV were compared for medians between both groups using the Mann–Whitney U-test. $P < 0.05$ was considered statistically significant.

RESULTS

Ninety-one pregnant women were recruited to the study, and maternal serum samples were collected and analyzed. The fetuses affected by Hb Bart's disease and unaffected fetuses (α -thalassemia-1 carriers and normal hemoglobin typing) were identified in 18 cases (19.8%) and 71 cases (78.0%), respectively. Two fetuses (2.2%) were diagnosed for Hb H/Constant spring disease and were excluded from analysis. The remaining 89 pregnancies were available for analysis, and their baseline characteristics are shown in Table 1. No statistically significant difference in maternal age, gestational age at sampling, maternal body weight, and percentage of primigravida was found between the two groups ($P > 0.05$). Cardiomegaly (CTR $> 50\%$), placentomegaly (PIT $> 3\text{ cm}$), and high MCA-PSV of more than 1.5 MoM were identified in 32 cases (35.9%), 14 cases (15.7%), and 21 cases (24.1%), respectively. Of 18 Hb Bart's fetuses, all cases (100%) had cardiomegaly, 9 cases (50%) had placentomegaly, defined by thickness of more than 3 cm, and 14 cases (77.8%) had high MCA-PSV. Maternal serum concentrations of PlGF was significantly higher in pregnancies with fetuses affected by Hb Bart's disease than in those with unaffected fetuses ($P = 0.008$), whereas the concentrations of sFlt-1 was not significantly different ($P = 0.139$) as presented in Table 2 and Figure 1. However, sFlt-1/PlGF ratio was significantly lower in pregnancies with Hb Bart's disease than those with unaffected fetuses ($P = 0.001$).

DISCUSSION

Hemoglobin Bart's or homozygous α -thalassemia-1 disease is one of the common causes of severe fetal anemia, particularly

Table 1 Baseline characteristics of the pregnancies affected and unaffected by fetal hemoglobin Bart's disease

Characteristics	Unaffected group (n = 71)	Affected group (n = 18)	P-value
Maternal age, yr	27.83 $\pm$ 5.66	27.59 $\pm$ 6.98	0.25
Gestational age at sampling, wk	18.59 $\pm$ 0.75	18.56 $\pm$ 0.78	0.86
Maternal body weight, kg	55.82 $\pm$ 7.94	59.23 $\pm$ 13.49	0.19
Primigravida, n (%)	42 (60%)	9 (50%)	0.59
CTR, %	48.00 $\pm$ 3.61	57.80 $\pm$ 4.61	<0.001
PIT, cm	2.20 $\pm$ 0.50	3.06 $\pm$ 0.53	<0.001
MCA-PSV, cm/sec	24.01 $\pm$ 5.13	37.28 $\pm$ 6.91	<0.001

yr, years; wk, weeks; kg, kilograms; n, number of cases; CTR, cardiothoracic ratio; PIT, placental thickness; cm, centimeters; MCA-PSV, middle cerebral artery peak systolic velocity; cm/sec, centimeter per second.

Table 2 Maternal serum placental growth factor (PlGF) concentrations, soluble fms-like tyrosine kinase-1 (sFlt-1) concentrations and sFlt-1/PlGF ratio in pregnancies affected and unaffected by fetal hemoglobin Bart's disease

Maternal serum markers	Unaffected group (n=71)	Affected group (n=18)	P-value
PlGF (ng/mL)	263.60	373.40	0.008
sFlt-1 (ng/mL)	1440.00	1257.00	0.139
sFlt-1/PlGF ratio	5.80	3.60	0.001

in Southeast Asia. It is associated to extramedullary erythropoiesis in the fetal liver or spleen and an increased cardiac output develop resulting in hydropic changes, such as subcutaneous edema, cardiomegaly, hepatomegaly, ascites, and placentomegaly.⁹ Theoretically, such large placental mass can abnormally produce placental hormones or alter angiogenic (PlGF) and anti-angiogenic factors (sFlt-1), in particular.

The increased levels of sFlt-1 and reduced levels of PlGF in maternal circulations have been found 5 weeks before the onset of preeclampsia.¹ In women with preeclampsia, there is an exacerbation of the anti-angiogenic state results in a marked increase in sFlt-1 concentration both in the placenta and maternal serum. sFlt-1 or known as the soluble form of the vascular endothelial growth factor receptor-1 prevents the interaction between endothelial receptors and PlGF on the cell surface accompanied by a reduced amount of placental production resulting in a decrease in PlGF concentration. Additionally, the higher sFlt-1 to PlGF ratio has been shown to be a better predictor of preeclampsia than either PlGF or sFlt-1 alone.¹⁷ Maternal serum and placental anti-angiogenic factors were also increased in pregnancies with mirror syndrome associated with fetal hydrops caused from rhesus isoimmunization, fetal cytomegalovirus infection, and twin-to-twin transfusion syndrome.^{2-5,18} Mirror syndrome is characterized by the combination of fetal hydrops, placentomegaly, and maternal edema and shares similar manifestations with preeclampsia especially hypertension. Although the placental sizes are different in these two diseases (preeclampsia and mirror syndrome), it is assumed that there is placental dysfunction, coinciding with anti-angiogenic state.¹⁸

Contrary to our initial belief, a higher PlGF in Hb Bart's fetus may be associated with subtle placental functional changes secondary to the enlarged but healthy placenta developed in early state, as noted that the affected fetuses had a trend to have higher placental thickness or placentomegaly. Nevertheless, the enlarged placenta may change placental factors production and secretion. Early in gestation, placentomegaly in anemic fetuses secondary to any causes including Hb Bart's disease may not represent placental dysfunction but reflect the excessive placental function. The findings in this study are consistent with our previous observation of the early hydrops fetalis from Hb Bart's disease that the cardiomegaly was consistently found at mid-gestation. The cardiomegaly in these fetuses was not associated with heart failure but manifested of the well-compensated intensive work of the heart response to hypervolemia.^{19,20}

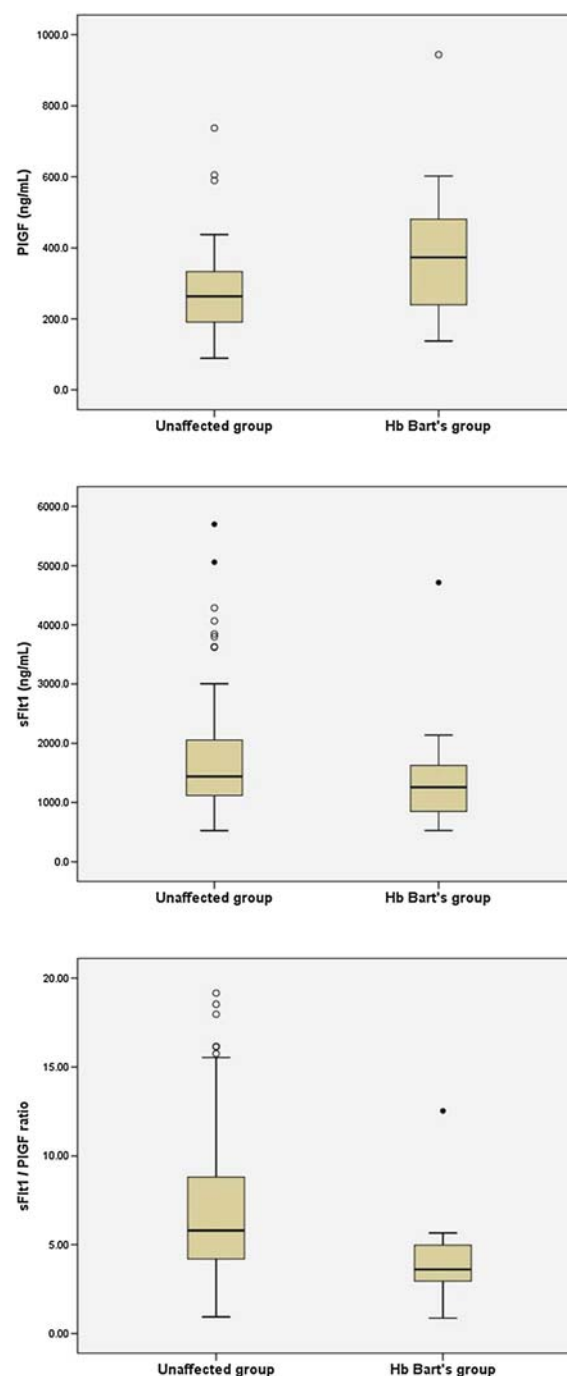


Figure 1 Boxplots of maternal serum placental growth factor (PlGF) concentrations, soluble fms-like tyrosine kinase-1 (sFlt-1) concentrations, and sFlt-1/PlGF ratio in pregnancies affected and unaffected by fetal Hb Bart's disease

Although these factors may be not much helpful in fetal diagnosis of Hb Bart's disease, this study suggests for further detailed exploration in pathogenesis of preeclampsia in case of normal placentation in early gestation, which may be different from preeclampsia in case of abnormal placentation such as in chronic hypertension. Moreover, this study may be a model for further study in other conditions associated with placentomegaly secondary to anemia from any causes other than Hb Bart's disease.

Placental growth factor and sFlt-1 are commonly proposed to use as a predictor of preeclampsia among pregnancies at a higher risk. Nevertheless, in the geographical area with high prevalence of Hb Bart's disease, one should interpret with precaution and be aware of unreliable tests if the patients are carrying fetuses with Hb Bart's disease.

CONCLUSION

At mid-pregnancy, maternal serum PlGF in pregnancies with fetal Hb Bart's disease is significantly higher, whereas sFlt-1/PlGF ratio is significantly lower than that in the unaffected ones. These placental biomarkers may clinically help differentiate affected from unaffected fetuses among fetuses at risk, although further studies are needed to confirm such usefulness. In addition, preeclampsia prediction using these

serum markers may be unreliable in pregnancies with placental dysfunction secondary to severe fetal anemia.

WHAT'S ALREADY KNOWN ABOUT THIS TOPIC?

- In preeclampsia, maternal serum sFlt-1 is increased, but PlGF is decreased.
- A higher sFlt-1/PlGF ratio is a reliable tool in the assessment of preeclampsia.
- Hb Bart's anemic fetuses complicate more often with preeclampsia in late gestation.

WHAT DOES THIS STUDY ADD?

- PlGF is higher while sFlt-1/PlGF ratio is lower in pregnancies with fetal Hb Bart's disease.
- Preeclampsia prediction using PlGF and sFlt-1 may be unreliable in pregnancies with fetal anemia.

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Fetal Myocardial Performance (Tei) Index in Fetal Hemoglobin Bart's Disease

Der fetale myokardiale Performance-Index (Tei) beim Hämoglobin-Bart's-Hydrops-fetalis-Syndrome

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Key words

- heart
- echocardiography
- ultrasound-doppler
- fetus

Zusammenfassung



Ziel: Beurteilung der Effektivität des Tei-Index, um das Hb-Bart's-Hydrops-fetalis-Syndrom bei Risikoschwangerschaften in der ersten Hälfte der Schwangerschaft abzugrenzen.

Material und Methoden: Schwangerschaften, welche die Einschlusskriterien erfüllten, wurden in Folge in die Studie aufgenommen. Die Einschlusskriterien waren im Folgenden 1) Einlingsschwangerschaften, 2) 12–20 Gestationswoche, 3) Risiko eines Hb-Bart's-Hydrops-fetalis-Syndroms, 4) bestätigte fetale Diagnose eines Hb-Bart's-Hydrops-fetalis-Syndrom. Feten mit schwerem generalisiertem Hydrops fetalis oder Feten mit Chromosomen- und strukturellen Anomalien wurden ausgeschlossen. Die Tei-Index Bestimmung wurde durchgeführt und sofort protokolliert, noch vor Durchführung einer invasiven Pränataldiagnostik.

Ergebnisse: Bei 50 der 152 Feten wurde letztlich ein Hb-Bart's-Hydrops-fetalis-Syndrom diagnostiziert, die übrigen Feten waren nicht betroffen. Die mittleren (+SD) Tei-Indizes betrugen bei den unbeeinträchtigten Feten $0,48 \pm 0,07$ und bei den betroffenen Feten $0,54 \pm 0,08$. Der mittlere Unterschied war mit 0,05 statistisch signifikant ($p < 0,001$). Ebenso unterschied sich die mittlere (+SD) isovolumetrische Kontraktionszeit der normalen Feten signifikant von derjenigen der betroffenen Feten.

Schlussfolgerung: In der ersten Schwangerschaftshälfte war der Tei-Index bei Feten mit Hb-Bart's-Hydrops-fetalis-Syndrom signifikant höher als bei normalen Feten. Dies lässt darauf schließen, dass der Tei-Index eine neue nützliche, nicht invasive Methode für die Frühdiagnose des Hb-Bart's-Hydrops-fetalis-Syndrom bei Risikoschwangeren darstellen könnte.

Abstract



Purpose: To assess the effectiveness of Tei index in differentiating fetal Hb Bart's disease in pregnancies at risk in the first half of pregnancy

Materials and Methods: Pregnancies meeting the inclusion criteria were consecutively recruited to the study. The inclusion criteria were as follows: 1) singleton pregnancies, 2) gestational age of 12–20 weeks, 3) at risk of Hb Bart's disease, 4) confirmed fetal diagnosis of Hb Bart's disease. Fetuses with frank hydrops fetalis or fetuses with chromosomal abnormalities or structural anomalies were excluded. Tei index was performed and immediately recorded before invasive prenatal diagnosis was performed.

Results: Of 152 fetuses, 50 were finally proven to be affected by Hb Bart disease, and the remainder was unaffected. Mean (+SD) Tei index were 0.48 ± 0.07 and 0.54 ± 0.08 in group of unaffected and affected fetuses, respectively. The mean difference was 0.05 which was statistically significant ($p < 0.001$). Likewise, mean (+SD) isovolumetric contraction time (ICT) in normal fetuses were also significantly different from that of affected fetuses.

Conclusion: In the first half of pregnancy, Tei index in the fetuses with Hb Bart's disease was significantly higher than that in normal fetuses implying that Tei index may be a novel useful and non-invasive tool for early detection of fetal Hb Bart's disease among pregnancies at risk.

received 6.4.2012
accepted 26.6.2012

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DOI <http://dx.doi.org/10.1055/s-0032-1313159>
Published online: 2012
Ultraschall in Med 2012; 33: 1–4
© Georg Thieme Verlag KG
Stuttgart · New York ·
ISSN 0172-4614

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Introduction

Homozygous alpha-thalassemia-1 or Hb Bart's disease is one of the common health problems in Southeast Asia [1, 2]. The affected fetuses usually die. Furthermore, serious maternal complications are often encountered in pregnancies with this fetal disorder, including severe preeclampsia, dystocia and postpartum hemorrhage associated with enlarged placenta. It is therefore essential for early diagnosis and termination of pregnancy before the presence of hydropic changes. Although invasive procedures such as chorionic villus sampling or cordocentesis are gold standard for diagnosis, sonographic diagnosis currently represents the mainstay investigation for such fetuses since it is non-invasive and highly effective [3–5]. Nevertheless, no sonomarker is perfect and a new non-invasive technique is needed. In the case of myocardial workload secondary to anemia and hypervolemia, fetal cardiac function of affected fetuses may be different from that of normal fetuses. Abnormal fetal cardiac function may probably be an early subtle sign of fetal anemia due to Hb Bart's disease before development of hydrops fetalis.

To quantify the changes that occur with ventricular systolic and/or diastolic dysfunction, cardiologists have used the Myocardial Performance Index, also known as the Tei index that is named after the investigator who first reported these relationships. This index is computed by adding the isovolumetric contraction time (ICT) and the isovolumetric relaxation time (IRT) and dividing the result by the left ventricular ejection time (ET). Several reports support the usefulness of this index in evaluation of a variety of fetal disorders [6–10]. However only a study by Chao et al. [7] was involved in evaluation of fetal Hb Bart's disease. In fetal anemia, the fetus increase cardiac output to maintain tissue perfusion, leading to greater cardiac workload. In early phase of fetal anemia, when no sonographic sign is apparent, an increase in cardiac workload may be subtly present. Change in cardiac function may indicate fetal anemia before development of other obvious hydropic signs. Chao and et al. [7] measured Tei index in 30 fetuses with Hb Bart's disease in the second half of pregnancies (20–42 weeks) and compared them with age-matched normal controls. They found that fetuses with Hb Bart's disease developed an abnormal Tei index earlier than overt cavity dilatation and impairment of systolic function suggesting the potential role of Tei index in early detection of fetal Hb Bart's disease.

However, Tei index measurement for prediction of fetuses with Hb Bart's disease in the second half of pregnancies is less clinically helpful since the other sonographic signs of hydropic changes usually become obvious. To the best of our knowledge, the effectiveness of Tei index to assess myocardial performance in differentiating affected from non-affected fetuses among fetuses at risk before hydropic changes has never been studied. Therefore, we conducted this study to assess the effectiveness of Tei index in predicting fetal Hb Bart's disease in pregnancies at risk in the first half of pregnancy.

Materials and Methods

This study was conducted at Tertiary Center for Maternal-fetal medicine. The study was undertaken with an approval of the research ethical committee. Pregnancies meeting the inclusion criteria were consecutively recruited to the study with written informed consent. The inclusion criteria were as follows: 1) single-ton pregnancies, 2) gestational age of 12–20 weeks, based on so-

nographic biometry in the first half of pregnancy, and 3) at risk of Hb Bart's disease, both of the couples were a carrier of alpha-thalassemia-1, based on PCR for Southeast Asian type. Final fetal diagnosis of Hb Bart's disease was based on either DNA analysis of chorionic villous samples or fetal cord blood analysis with high-performance liquid chromatography. Exclusion criteria included 1) fetuses with obvious sonographic signs of hydropic changes such as ascites, pleural effusion or subcutaneous edema, and 2) fetuses with chromosome abnormalities or structural anomalies other than Hb Bart's disease. Before chorionic villous sampling or cordocentesis, standard ultrasound examinations including fetal biometry, anomaly screening and Tei index measurements were performed and immediately recorded before invasive prenatal diagnosis was performed. All examinations were performed by one author (S.L.), just before prenatal diagnostic procedures, using a real-time scanner with GE Voluson E8 (GE Medical Systems, Zipf, Austria), equipped with a transabdominal curvilinear transducer of frequency 2 to 5 MHz. The Doppler tracings were recorded during fetal quiescence over 4–5 cardiac cycles. Measurements of Tei index were obtained by placing the sample volume at the outflow tract of the left ventricle to obtain the aortic and mitral flow simultaneously. Valve clicks were used to identify the closure and opening of the atrioventricular and semilunar valves during the measurement of the time intervals. At least three consecutive waveforms with high quality were measured. Tei index is defined as the sum of the isovolumetric contraction time (ICT) and the isovolumetric relaxation time (IRT), divided by the ventricular ejection time (ET): $(ICT+IRT)/ET$ (Fig. 1).

Baseline demographic and obstetric data of the pregnant women were also recorded digitally at the same time. The stored data were analyzed for a comparison of Tei index between fetuses with Hb Bart's diseases and normal fetuses, using the statistical package for the social sciences (SPSS) version 17.0 (Chicago, IL).

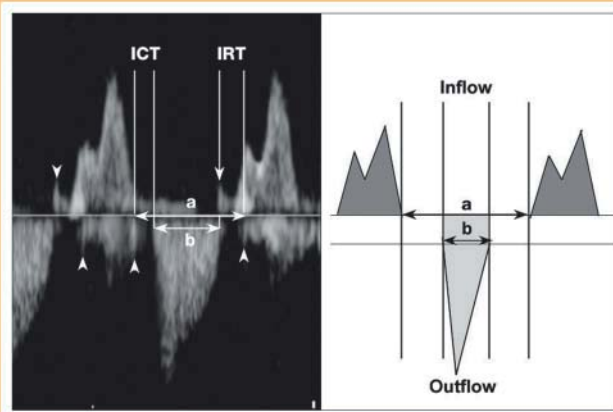


Fig. 1 Image and diagram of Doppler waveform representing the calculation of the left ventricular Tei index; Interval **a** extends from the cessation to the onset of mitral inflow and includes the isovolumetric contraction time (ICT), **b** or the ejection time (ET, the duration of left ventricular outflow) and the isovolumetric relaxation time (IRT), Note: arrowhead representing valve clicks.

Abb. 1 Bild und Diagramm des Dopplerprofils stellt die Berechnung des linken ventrikulären Tei-Index dar; das Intervall **a** erstreckt sich vom Aussetzen bis zum Beginn des Mitraleinflusses und schließt die isovolumetrische Kontraktionszeit (ICT), oder **b** die Ejektionszeit (ET, die Dauer des linksventrikulären Auswurfs) und die isovolumetrische Relaxationszeit (IRT) ein. Beachte: Die Pfeilspitzen stellen die Klappenklicks dar.

Quantitative analyses were mean and SD when the distribution was normal and median and range when it was not. The Mann-Whitney U-test was used for quantitative analyses. $P < 0.05$ was considered to be statistically significant.

Results

Over the study period, January 2010 and March 2011, 152 fetuses at risk of Hb Bart's disease meeting the inclusion criteria were assessed for Tei index. Of 152 fetuses, 50 were finally proven to be affected by Hb Bart disease, and the remainders were unaffected. Mean maternal age ($\pm$ SD) in the group of unaffected and affected fetuses was 28.35 ± 5.94 and 29.50 ± 5.30 years, respectively (Student's T test, $p > 0.05$). Mean gestational age ($\pm$ SD) at ultrasound scan was 17.16 ± 2.78 and 16.14 ± 3.11 weeks, respectively. (Student's T test, $p > 0.05$) In the group of fetuses with Hb Bart disease, mean ($\pm$ SD) Tei index were 0.48 ± 0.07 compared to 0.54 ± 0.08 in group of unaffected fetuses. The mean difference was 0.05 which was statistically significant ($p < 0.001$), as presented in **Table 1** and **Fig. 2**. Likewise, mean ($\pm$ SD) ICT was also significantly different between the two groups as shown in **Table 1**. However, IRT and ET had a tendency to be increased in the affected fetuses, no statistical significance was observed.

Discussion

Fetal cardiac function can be assessed by various Doppler indices of atrioventricular valves and semilunar valves for systolic and diastolic function. Tei index is one of the most common Doppler techniques used for such a purpose. In 1995, Tei et al. [11] presented a simple and useful tool to quantify the changes that occur with ventricular systolic and/or diastolic function. Tei index values are well correlated with those of invasive measures of systolic and diastolic function and can be used to determine prognosis in patients with various cardiac disorders, which is not dependent on cardiac geometry, heart rate, and blood pressure [11–15]. Recently, Ichizuka et al. [9] reported that Tei index was a useful tool for the assessment of fetal cardiac function in a variety of sick fetuses such as TTS, or hydrops fetalis. Likewise, Falkensammer et al. [8] demonstrated an increase in Tei index in fetus with congestive heart failure or hydrops fetalis. Chao et al. [7] first reported abnormal Tei index in Hb Bart's hydrops fetalis in the second half of pregnancy, 20–42 weeks of gestation. Conversely, this study focused on fetuses at risk of Hb Bart's disease before development of hydrops fetalis, during 12–20 weeks of gestation. Our rationale was based on the fact that diagnosis of Hb Bart's disease after mid-pregnancy is not clinically problematic since the several sonographic signs of hydropic changes are usually obvious and readily diagnosed whereas our concern is early detection of subtle changes in earlier gestation, when no other signs of hydrops have occurred. Therefore we focused on the first half of pregnancies to determine whether Tei index could differentiate affected from unaffected, among fetuses at risk.

parameters	normal fetuses (n = 102)	Hb Bart's disease (n = 50)	mean difference	p value
ICT	6.097 ± 3.023	8.850 ± 4.585	-2.753	0.000
IRT	7.709 ± 3.221	8.521 ± 3.907	-0.8116	0.176
ET	28.617 ± 11.823	32.036 ± 14.358	-3.419	0.121
Tei	0.486 ± 0.074	0.537 ± 0.084	-0.0508	0.000

Table 1 Comparison of Tei index and its components between the group of affected and unaffected fetuses.

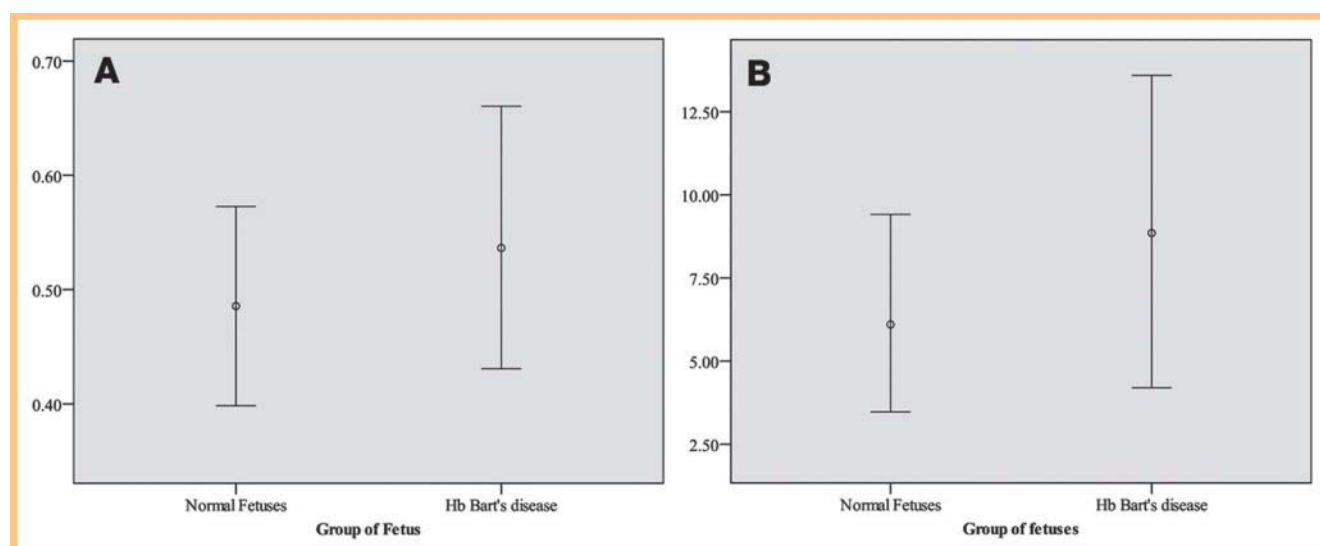


Fig. 2 Error bar chart of Tei index (A) and isovolumetric contraction time; ICT (B) values showing mean and 95 % CIs of normal fetuses and fetuses with Hb Bart's disease.

Abb. 2 Fehlerbalken-Diagramm von Tei-Index (A) und isovolumetrischer Kontraktionszeit; die ICT(B)-Werte zeigen den Mittelwert und die 95 % CIs bei normalen Feten und Feten mit Hb-Bart's-Hydrops-fetalis-Syndrom.

This study strongly suggests that abnormal myocardial performance (Tei index) in response to anemia and increased cardiac output as well as hypervolemia occurs very early in gestation before development of hydropic changes, signifying that myocardial function is very sensitive to fetal oxygenation. We hypothesize that functional myocardial response to anemia has developed long before morphological change that could be visualized by ultrasound. Presumably, Tei index may be applied to assessment of fetal ventricular myocardial performance in fetal anemia or hypervolemia due to various causes other than Hb Bart's disease or in any condition associated with an increase in the cardiac afterload, myocardial hypertrophy, and diastolic dysfunction due to heart compression.

Previous studies have established that the ICT is an important index for the assessment of systolic function [16], and IRT is an important index for the assessment of diastolic function [17]. Interestingly, ICT was significantly longer in affected fetuses while the IRT was not, although it tended to increase as well. This is our new insight signifying that fetal anemia and hypervolemia may predominantly affect systolic function. ICT may be mainly responsible for an increase in Tei Index in early stage and this may be an independent indicator for fetal anemia.

We previously reported that cardiac function in fetal Hb Bart's disease in early gestation is not associated with increased preload in spite of hypervolemia but rather with increase in cardiac output without deterioration of myocardial contractility or congestive heart failure different from that in cardiac disorders [18, 19]. However, based on this study in early fetal anemia while the fetuses maintain good cardiac function in spite of increased cardiac output, myocardium show some degree of increased workload by increase ICT and Tei index. Therefore, we speculate that this may be fetal physiologic adaptation to cope with anemic state or increased cardiac output rather than pathologic ventricular dysfunction as mostly suggested in the literature. An increase in Tei index in early stage may be not a sign of cardiac dysfunction, just a good response to hemodynamic overload.

Based on our findings and previous studies which imply ability in differentiating affected and unaffected fetuses, Tei index may probably play a role in clinical application in early detection of fetal anemia in pregnancies at risk, especially when combined with other parameters such as middle cerebral artery peak systolic velocity. Additionally, Tei index measurement is relatively simple, and angle-independent. More importantly it is gestational age-independent in most studies [8]

The strength of this study includes 1) the large sample size of pregnancy at risk and the number of affected fetuses, and 2) a prospective nature in which the examiners did not know the diagnosis at the time of ultrasound examination. The weakness of this study may include bias in the measurement of Tei index. The measurement was not completely blinded since the examiners, who were highly experienced in sonographic diagnosis of hydrops fetalis, could visualize the morphology of the fetuses, especially mild cardiomegaly on ultrasound imaging, though cases with frank hydrops had been excluded. Morphologically, the diagnosis of Hb Bart's disease in such fetuses might have been anticipated.

In summary, the Tei index and ICT of fetuses with Hb Bart's disease was increased from early gestation before hydropic change

occurred, suggesting that they may effectively differentiate affected and unaffected fetus with Hb Bart's disease among fetuses at risk in early gestation, and this might be a new clinical diagnostic tool for such a purpose in early pregnancy. However, the effectiveness in clinical application is yet to be elucidated.

Acknowledgment



The authors wish to thank the Thailand Research Fund (TRF) for financial support.

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Fetal Cardiac Circumference Derived by Spatiotemporal Image Correlation as a Predictor of Fetal Hemoglobin Bart Disease at Midpregnancy

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Received December 6, 2012, from the Maternal-Fetal Medicine Unit, Department of Obstetrics and Gynecology, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand. Revision requested December 28, 2012. Revised manuscript accepted for publication January 7, 2013.

We thank the Thailand Research Fund and the National Research University Project under Thailand's Office of Higher Education Commission for financial support.

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Abbreviations

CI, confidence interval; Hb, hemoglobin; MoM, multiples of the median; NPV, negative predictive value; PPV, positive predictive value; STIC, spatiotemporal image correlation

Objectives—The purpose of this study was to evaluate the efficacy of the fetal cardiac circumference derived by spatiotemporal image correlation (STIC) for predicting fetal hemoglobin (Hb) Bart disease.

Methods—Pregnancies at risk of fetal Hb Bart disease at 17 to 22 weeks' gestation were enrolled. All underwent STIC volume acquisition for analysis of fetal cardiac dimensions and cordocentesis for Hb level and Hb typing analysis. Spatiotemporal image correlation volume data sets were subsequently analyzed offline for cardiac circumference measurements, which were used to evaluate the efficacy in predicting fetal anemia and fetal Hb Bart disease.

Results—A total of 88 pregnancies at risk of fetal Hb Bart disease at 17 to 22 weeks were recruited into the study. The cardiac circumference was significantly higher in fetuses with Hb Bart disease than in unaffected fetuses (1.33 and 1.02 multiples of the median [MoM], respectively; $P < .001$). The cardiac circumference effectively predicted fetal Hb Bart disease (area under the receiver operating characteristic curve, 0.85; 95% confidence interval [CI], 0.73–0.97), with sensitivity of 86.4% (95% CI, 72.0%–100%) and specificity of 78.1% (95% CI, 68.0%–88.3%) using a cutoff point of greater than 1.17 MoM, whereas the cardiothoracic ratio had better efficacy, with sensitivity of 90.9% (95% CI, 78.9%–100%) and specificity of 85.9% (95% CI, 77.4%–94.5%) using a cutoff point of greater than 0.50.

Conclusions—At midpregnancy, a cardiac circumference of greater than 1.17 MoM can be used as an alternative sonographic marker for predicting fetal Hb Bart disease, although not perfectly. However, the cardiothoracic ratio seems to be more accurate than the cardiac circumference for such a purpose.

Key Words—cardiac circumference; fetal anemia; hemoglobin Bart disease; homozygous α -thalassemia 1 disease; spatiotemporal image correlation

Fetal homozygous α -thalassemia 1, or hemoglobin (Hb) Bart disease, is the most common inherited hematologic disease in Southeast Asia. As a consequence of fetal anemia, affected fetuses are finally hydropic, which is manifested by cardiomegaly, hepatomegaly, skin edema, ascites, and placentomegaly. Traditionally, prenatal diagnosis usually relied on invasive techniques such as chorionic villous sampling, amniocentesis, and cordocentesis, which can be associated with fetal loss. However, early sonographic signs (cardiothoracic ratio, placental thickness, and peak

doi:10.7863/ultra.32.8.1483

systolic velocity of the middle cerebral artery) have been shown to be helpful for noninvasive prenatal diagnosis among pregnancies at risk.^{1–3}

In our practice, the cardiothoracic ratio (fetal cardiac diameter divided by thoracic transverse diameter) derived from 2-dimensional sonography is a sensitive marker for differentiating unaffected fetuses from those with Hb Bart disease.² Likewise, the cardiac circumference, which represents all dimensions of the fetal heart, may theoretically be useful for such a purpose. However, to our knowledge, no study on the cardiac circumference as an early predictor of fetal anemia or Hb Bart disease has been published. Therefore, the objective of this study was to evaluate the efficacy of the fetal cardiac circumference for predicting fetal anemia secondary to Hb Bart disease. For the best measurement, the fetal cardiac circumference was derived from spatiotemporal image correlation (STIC) volume analysis, which can precisely obtain the correct 4-chamber view in 3 orthogonal planes.^{4,5}

Materials and Methods

A descriptive analysis of the diagnostic test was conducted at Maharaj Nakorn Chiang Mai Hospital, Department of Obstetrics and Gynecology, Faculty of Medicine, Chiang Mai University, with approval by the institute's Ethics Committee. Pregnant women at risk of fetal Hb Bart disease scheduled for cordocentesis at 17 to 22 weeks were recruited into the study after providing written informed consent. Pregnancies at risk of fetal Hb Bart disease were identified by our established screening program as described elsewhere.⁶ Pregnancies at risk were diagnosed when both of the couples were α -thalassemia 1 carriers based on polymerase chain reaction (**S13 avian erythroblastosis** type). Gestational age relied on a certain last menstrual period and early fetal biometric measurement either by the crown-rump length in the first trimester or the biparietal diameter in the second trimester. Exclusion criteria included multiple pregnancies, fetal structural or chromosomal abnormalities, fetal anemia from any causes other than Hb Bart disease, and failure of fetal blood sampling or contamination with maternal blood.

Before cordocentesis, a transabdominal sonographic examination was performed for fetal biometric measurements and a fetal anatomic survey. Spatiotemporal image correlation volumes were acquired with a real-time ultrasound machine and 2–5-MHz curvilinear transabdominal transducers (Voluson E8; GE Healthcare, Milwaukee, WI). The acquisition involved a transverse sweep through the fetal chest, which included the proper apical and trans-

verse 4-chamber views. Acquisition times ranged from 7.5 to 15 seconds depending on fetal movements, and acquisition angles ranged from 20° to 25° depending on cardiac size. All of the STIC volumes were stored in the ultrasound machine's hard drive for subsequent offline analysis.

For measurement of the **cardiothoracic ratio**, the cross-sectional view of the fetal thorax at the level of the 4-chamber view was obtained. The cardiac diameter (outer to outer) was measured at the level of the atrioventricular valves during end diastole, and the transverse thoracic diameter was measured in the same image. The cardiothoracic ratio was then calculated by dividing the cardiac diameter by the thoracic diameter.²

To collect fetal blood samples, cordocentesis was performed under transabdominal real-time sonographic guidance using techniques as previously described.⁷ Fetal blood samples were tested for maternal blood contamination by a simple acid elution test. Hemoglobin typing using high-performance liquid chromatography and DNA analysis (if needed) was performed for diagnosis of the fetal thalassemia status. The fetal complete blood count was analyzed with an automated complete blood count machine (Coulter STKS analyzer; Beckman Coulter, Hialeah, FL). The Hb level was then transformed into multiples of the median (MoM) by dividing the measured Hb value by the median Hb value at each gestational age.⁸ The fetal anemia group was categorized as mild (0.84–0.65 MoM), moderate (0.64–0.55 MoM) or severe (<0.55 MoM) anemia.⁸

Fetal Cardiac Circumference Derived by STIC Analysis

The computerized stored STIC volume data sets were subsequently analyzed offline by one of the authors, who was blinded to the patients' information: either the fetal thalassemia status, fetal complete blood count, or cardiothoracic ratio derived from 2-dimensional sonography. First, the STIC volumes were systematically adjusted to identify the exact 4-chamber view^{9,10} using 4D View version 9.0 processing software (GE Healthcare, Zipf, Austria). To assess the fetal cardiac circumference, the analysis was performed as follows:

1. The volume data sets underwent image processing such as speed or brightness adjustment to optimize tissue contrast resolution as appropriate for analysis.
2. A multiplanar slicing analysis was subsequently performed offline. Dynamic images of the fetal heart were simultaneously visualized in the 3 orthogonal planes (axial, coronal, and sagittal) to get the interventricular septum in the exact horizontal plane in all 3 panels. Images of the 4-chamber view were adjusted and dis-

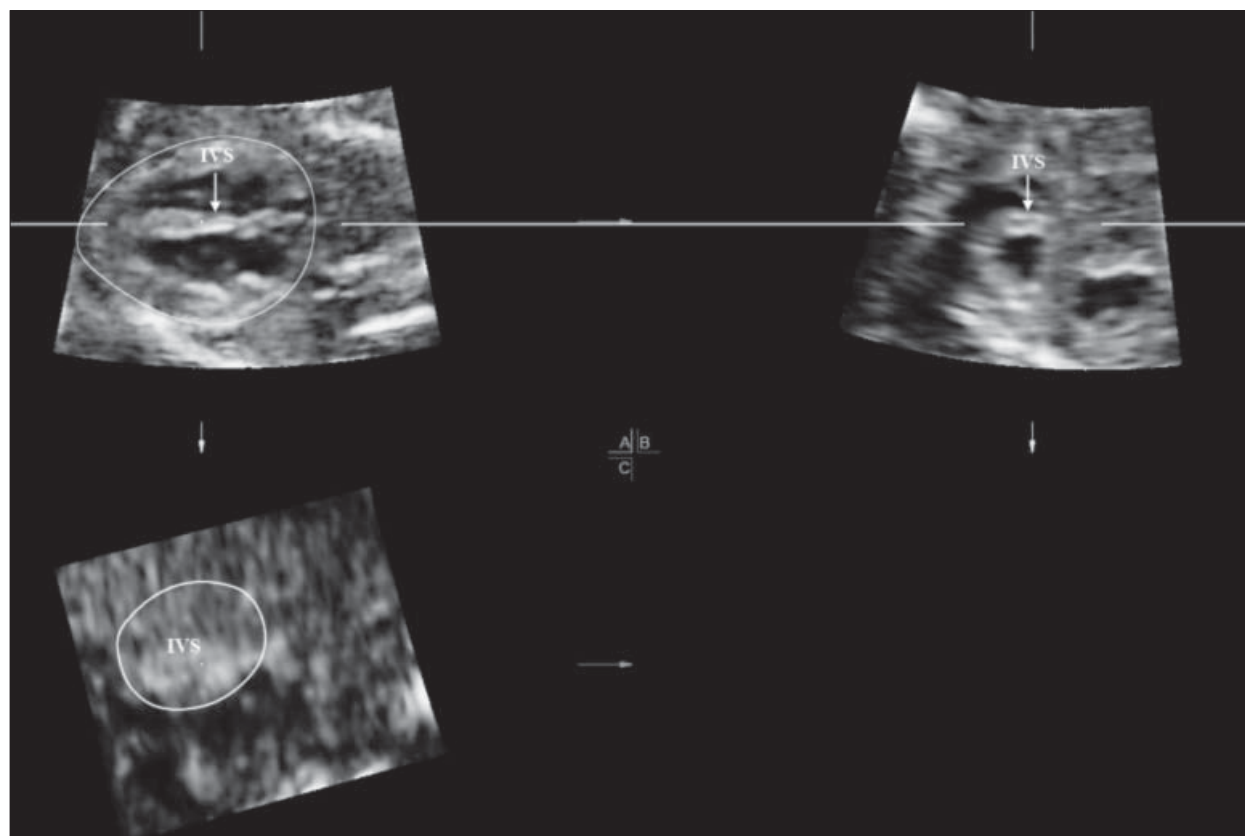
played by moving the reference dot and rotating the fetal heart around the 3 orthogonal axes (x, y, and z). The proper image could be checked by showing that the interventricular septum in panel A was on the same line as in panel B, and a total en face view of the interventricular septum was visualized in panel C.

3. In panel A, the descending aorta was moved to the midline, and then the reference dot was placed at the aorta. The aorta in panel B must be in the horizontal and vertical positions in panel C.
4. The typical 4-chamber view in panel A was frozen at end diastole and then was displayed and magnified as a single image on the screen. The cardiac circumference was measured at end diastole using the area point function in 4D View (Figure 1).
5. The cardiac circumference was then transformed into MoM by dividing the measured value by the median value at each gestational age.⁵

Statistical Analyses

All statistical analyses were performed with SPSS version 17.0 software (SPSS Inc, Chicago, IL). The demographic characteristics were described and compared between the affected group (pregnancies with fetal Hb Bart disease) and the unaffected group (pregnancies with normal fetal Hb typing or α -thalassemia 1 carriers) by an independent samples *t* test. The cardiac circumference derived by STIC (expressed as MoM for each gestational week) was compared between both groups by the Mann-Whitney *U* test. The ability of the cardiac circumference derived by STIC to discriminate the affected from the unaffected pregnancies was evaluated by receiver operating characteristic curve analysis. The performance of the cardiac circumference derived by STIC for identifying affected fetuses was determined by calculating the sensitivity and specificity using a best cutoff value. The relationship between the cardiac circumference and Hb level was evaluated by regression analysis. $P < .05$ was considered statistically significant.

Figure 1. Measurement of the fetal cardiac circumference in the 4-chamber view at end diastole on a multiplanar view after a STIC volume adjustment. IVS indicates interventricular septum.



Results

During the study period, 88 singleton pregnancies at risk of fetal Hb Bart disease were recruited, and fetal STIC volumes were acquired. Two fetuses were excluded because of fetal Hb H disease diagnosed from fetal blood analysis (1 case) and an unsatisfactory volume data set for cardiac circumference measurement (1 case). Of the remaining 86 fetuses, 22 had a diagnosis of fetal Hb Bart disease, and the remainder were unaffected, were α -thalassemia 1 carriers, or had normal Hb typing. The prevalence of fetal Hb Bart disease in this study was 25.6% (22 of 86). The baseline characteristics of the study population were not significantly different between the two groups, as presented in Table 1.

Median and MoM values for the fetal cardiac circumference derived by STIC in the affected group were significantly higher than those in the unaffected group. The median cardiac circumference values were 72.2 mm (95% confidence interval [CI], 65.7–78.8 mm) and 67.1 mm (95% CI, 64.4–69.6 mm) in the affected and unaffected groups, respectively ($P < .05$). Likewise, the MoM values were 1.33 (95% CI, 1.24–1.37) and 1.02 (95% CI, 0.91–1.15) in the affected and unaffected groups ($P < .001$).

The receiver operating characteristic curve of the cardiac circumference (MoM) for predicting fetal Hb Bart disease gave an area under the curve of 0.85 (95% CI, 0.73–0.97), as shown in Figure 2. With a best cutoff point of greater than 1.17 MoM, the cardiac circumference (MoM) had sensitivity of 86.4%, specificity of 78.1%, a positive predictive value (PPV) of 57.6%, and a negative predictive value (NPV) of 94.3% for predicting fetal Hb Bart disease, as presented in Table 2. Intraobserver variability in the cardiac circumference measurements were as follows; mean difference, -1.832 (SD, 5.377; 95% CI, -4.279 – 0.615); intraclass correlation coefficient (internal consistency), 0.831 (95% CI, 0.630–0.927); and Cronbach α , .931.

Table 1. Baseline Characteristics of the Pregnancies Affected and Unaffected by Fetal Hb Bart Disease

Characteristic	Affected (n = 22)	Unaffected (n = 64)	P
Gestational age, wk	18.22 $\pm$ 1.11	18.94 $\pm$ 1.00	.384
Biparietal diameter, mm	41.55 $\pm$ 3.65	44.13 $\pm$ 3.22	.612
Maternal age, y	28.71 $\pm$ 6.25	28.35 $\pm$ 6.04	.931

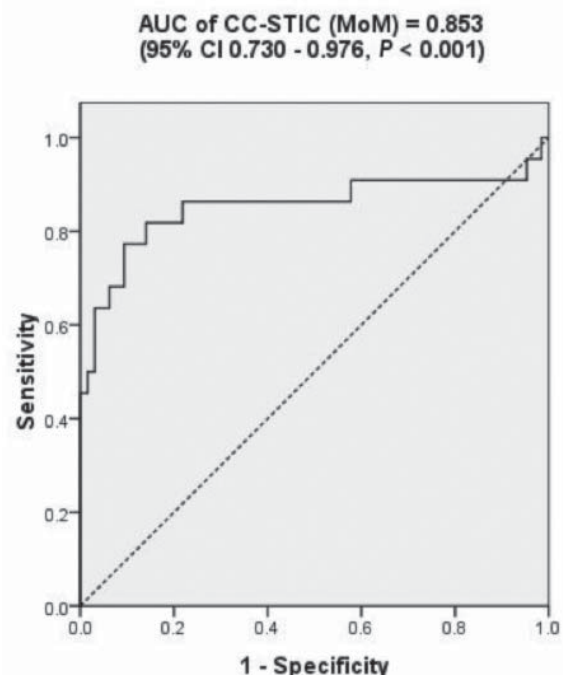
Data are presented as mean $\pm$ SD.

Among the 86 cordocentesis procedures, fetal blood samples were not enough for complete blood count analysis in 11 cases. The median Hb levels in the affected and unaffected groups were 6.50 g/dL (95% CI, 6.1–7.1 g/dL) and 10.6 g/dL (95% CI, 10.2–10.7 g/dL), respectively ($P < .001$). Fetal Hb levels by the mean of the MoM in both groups are categorized and presented in Table 3. With a cutoff point of greater than 1.17 MoM, the cardiac circumference (MoM) had sensitivity of 77.3%, specificity of 75.5%, a PPV of 56.7%, and an NPV of 88.9% for predicting fetal anemia (Hb < 0.84 MoM). The relationship between the cardiac circumference (MoM) and Hb level evaluated by regression analysis is shown in Figure 3 ($r = 0.534$; $P < .001$).

Discussion

In this study, the cardiac circumference derived by STIC was effective in predicting fetuses with Hb Bart disease at 18 to 22 weeks' gestation with sensitivity of 86.4% and specificity of 78.1% based on a cutoff point of greater than 1.17 MoM. However, the cardiothoracic ratio derived by 2-dimensional sonography had better efficacy for predict-

Figure 2. Receiver operating characteristic curve of the fetal cardiac circumference derived by STIC (CC-STIC) by the mean of the MoM for predicting fetal Hb Bart disease. AUC indicates area under the curve.



ing Hb Bart disease, with sensitivity of 91% and a specificity of 86% based on a cutoff point of greater than 0.50. Nevertheless, the cardiac circumference is still reliable and may be used as an adjunct method in fetuses with Hb Bart disease with acceptable accuracy and reproducibility.

The rationale of this study was based on the fact that evaluation of fetal heart size has been proven to be helpful in early diagnosis of fetal Hb Bart disease.^{3,11} As already known, with 2-dimensional sonography, the cardiac diameter and cardiothoracic ratio have been reported as effective sonographic signs for predicting fetal Hb Bart disease.² However, only 1 fetal heart dimension is measured for cardiothoracic ratio calculation, not the whole heart size. Therefore, theoretically, the cardiac circumference may better represent the fetal heart size than the cardiac transverse diameter and can presumably be a more precise marker for early diagnosis of fetal cardiomegaly. Consequently, the primary objective in this study focused on the effectiveness of the cardiac circumference for predicting Hb Bart disease, which to our knowledge has never been reported before.

Table 2. Efficacy of Fetal Cardiac Circumference (cutoff of 1.17 MoM) and Cardiothoracic Ratio in Predicting Fetal Hb Bart Disease

Cardiac Size	Affected (n = 22)	Unaffected (n = 64)	Total
Abnormal cardiac circumference	19	14	33
Normal cardiac circumference	3	50	53
Abnormal cardiothoracic ratio	20	9	29
Normal cardiothoracic ratio	2	55	57
Total	22	64	86

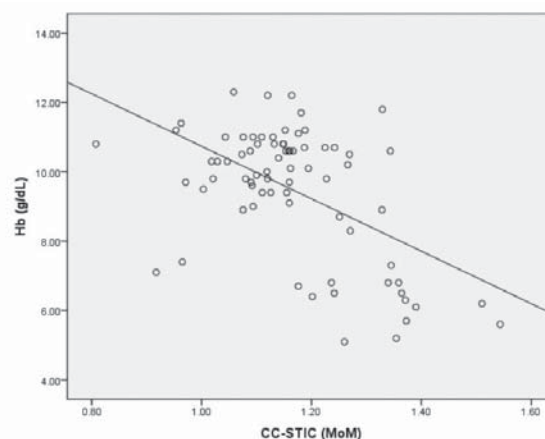
Diagnostic indices for cardiac circumference: sensitivity, 86.4% (19 of 22; 95% CI, 72.0%–100%); specificity, 78.1% (50 of 64; 95% CI, 68.0%–88.3%); PPV, 57.6% (19 of 33; 95% CI, 40.7%–74.4%); NPV, 94.3% (50 of 53; 95% CI, 86.5%–100%). Diagnostic indices for cardiothoracic ratio: sensitivity, 90.9% (20 of 22; 95% CI, 78.9%–100%); specificity, 85.9% (55 of 64; 95% CI, 77.4%–94.5%); PPV, 69.0% (20 of 29; 95% CI, 52.1%–85.8%); NPV, 96.5% (55 of 57; 95% CI, 86.5%–100%).

Table 3. Fetal Hb Level by Mean of MoM in Pregnancies Affected and Unaffected by Fetal Hb Bart Disease

Fetal Anemia	Affected (n = 19)	Unaffected (n = 56)
No anemia (Hb >0.84 MoM), n (%)	0 (0)	53 (94.6)
Mild anemia (Hb 0.84–0.65 MoM), n (%)	4 (21.1)	3 (5.4)
Moderate anemia (Hb 0.64–0.55 MoM), n (%)	11 (57.9)	0 (0)
Severe anemia (Hb <0.55 MoM), n (%)	4 (21.1)	0 (0)

Surprisingly, the findings in this study were contrary to what we had initially expected. Despite a theoretical advantage in terms of better representing heart size, the cardiac circumference seemed to be associated with greater measurement variation and to be less accurate than the cardiothoracic ratio, although the correct plane of the 4-chamber view could be simply and properly shown by STIC. Our findings suggest that, in real practice, the cardiac circumference may not better represent cardiac size than the cardiac diameter. This finding may partly be explained by the fact that the atrial area is more varied in shape and more deformable compared with the transverse cardiac diameter at its greatest dimension. In addition, a poor borderline image was another reason for the error because the heart structures in the second trimester could not be clearly distinguished from the surrounding structures. Finally the poor quality of the STIC volume data sets in some fetuses could also have been responsible for the greater variability. We hypothesize that the cardiac diameter may be more reproducible than the cardiac circumference, since it is more complicated in measurement because of the poor border outline, as mentioned above. The shape of the fetal heart is not always simply geometric, especially the atrium, which may be markedly varied in shape, whereas the cardiothoracic ratio is simpler and more reproducible. In contrast, the greatest transverse diameter of the heart should always be proportionately changed with cardiac size. Although the cardiac circumference seemed to be effective, our findings did not support the use of such a measurement as a primary screening test for fetal Hb Bart disease, since it did not seem to be superior to the cardiac diameter, which was much easier and less time-consuming.

Figure 3. Relationship between the cardiac circumference derived by STIC (CC-STIC) and Hb level by regression analysis ($r = 0.534$; $P < .001$).



Nevertheless, although not a perfect predictor, the cardiac circumference may be used as an adjunct to other sonographic parameters of fetal Hb Bart disease, such as the cardiothoracic ratio and peak systolic velocity of the middle cerebral artery.

Limitations of this study that should be mentioned include the fact that the cardiac size based on the cardiac circumference did not represent the size in relation to the thoracic cage. The cardiac circumference and thoracic circumference ratio may possibly be better than the absolute size of the heart. However, we did not incorporate the thoracic circumference into the analysis, since several volume data sets did not cover the whole thoracic cage of the fetuses. In addition, interobserver variability was not evaluated in this study.

In detection of fetal anemia, the cardiac circumference derived by STIC (MoM) had modest efficacy for determining fetal Hb levels of less than 0.84 MoM. Although it is not necessary to determine the Hb level in a fetus with Hb Bart disease, and the cardiac circumference is not valuable for differentiating anemic levels in this lethal disease, the cardiac circumference could possibly be helpful in the other causes of fetal anemia, such as parvovirus B19 infection and Rh isoimmunization, but this idea needs further study. Although the results of this study were derived from pregnancies at risk of fetal Hb Bart disease, the cardiac circumference might possibly be used to evaluate fetal anemia secondary to other causes, since fetal Hb seems to be inversely proportionate to fetal cardiac size.

The strengths of this study included the following (1) the large and homogeneous sample size of fetuses with anemia due to Hb Bart disease at the same gestational ages; (2) the single examiner, who was blinded to any fetal information, including the thalassemia status and other sonographic markers of fetal anemia such as placentomegaly, a high middle cerebral artery peak systolic velocity, and hydropic changes; and (3) the use of normative reference ranges for the cardiac circumference, which were based on our own data from a previous study.

In conclusion, at midpregnancy, a cardiac circumference of greater than 1.17 MoM can be used as an alternative sonographic marker for predicting fetuses with Hb Bart disease, although not perfectly. However, the cardiothoracic ratio seems to be better than the cardiac circumference for predicting Hb Bart disease among pregnancies at risk.

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ORIGINAL ARTICLE

Effectiveness of the model for prenatal control of severe thalassemia

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ABSTRACT

Objective The aim of the research was to determine effectiveness of the model for prenatal control in reducing new cases of severe thalassemia.

Methods Pregnant women at six tertiary centers were recruited to follow the model, consisting of (1) carrier screening using mean corpuscular volume (for α -thal-1 and β -thal) and CMU-E screen (for HbE trait), (2) carrier diagnosis, (3) the couples at risk were counseled and offered prenatal diagnosis, and (4) termination of affected pregnancy. All neonates were evaluated for thalassemia.

Results Of the 12 874 recruited pregnancies, 7008 were valid for analysis. Of them, 281 couples were identified to be at risk. Of the 281, 58 affected fetuses were identified and 55 pregnancies were terminated, whereas three did not accept pregnancy termination. All 6727 neonates at no risk were proven to be unaffected. The model had sensitivity and positive predictive value of 100% and 20%, respectively. The model could detect all of affected fetuses.

Conclusion The model could prenatally identify affected fetuses with a detection rate and negative predictive value of 100%. The model was highly effective to prenatally detect affected fetuses with an acceptable false positive rate. © 2013 John Wiley & Sons, Ltd.

Funding sources: The Research was supported by the Thailand Research Fund (TRF) and the Commission on Higher Education (CHE) of Thailand, (grant no. RTA55380008- RTA5080011).

Conflicts of interest: None declared

INTRODUCTION

Thalassemia is a very common inherited disease worldwide, especially in South East Asia, Southern Asia, and Mediterranean. In South East Asia, the prevalence of α -thal-1, β -thal, and HbE gene is as high as 3–30%, 2–9%, and 10–50%, respectively,¹ resulting in many births of new cases with severe thalassemia, including homozygous β -thalassemia (β -thal major), β -thalassemia/HbE, and Hb Bart's disease (homozygous α -thal-1). Nationwide programs have launched to prevent and control these three entities by prenatal approach. To achieve this goal, mass screening to identify the carriers and couples at risk of having fetuses with the diseases and prenatal diagnosis is essential. Recent solid evidence suggests that mean corpuscular volume (MCV) be probably most cost-effective for mass screening α -thalassemia-1 and β -thalassemia carriers,²

whereas CMU-E screen is the best screening method for HbE carriers due to its high accuracy without false negative result.³ Regarding diagnostic test for carriers, we prefer HbA2/HbE microcolumn technique for β -thal trait and HbE trait and immuno-chromatographic (IC)-strip/polymerase chain reaction (PCR) set for α -thal-1 trait due to low cost, simplicity, and high effectiveness.^{4,5} In our practice, the cost of this prospective screening model, not including the expense of prenatal diagnosis, is very low.

Although several strategies of prenatal control have been proposed and published,^{6–10} to the best of our knowledge, all such studies focused on detection rates among the couples at risk indicated by the screening tests but none evaluated the occurrence of the disease among pregnancies at no risk. They knew how many cases they could prenatally detect but they

did not know how many cases they missed. Therefore, none of the previous reports could perfectly assess the effectiveness of the model in control of the diseases. This is the first study aimed to assess the effectiveness of the model for prenatal control of severe thalassemia in which the babies of both couples at risk and no risk were tested for the disease. The model in this study consisted of MCV plus CMU-E screen as carrier screening tests, HbA₂/HbE microcolumn plus IC-strip/PCR set as carrier diagnostic tests, and final diagnosis of the severe diseases with high performance liquid chromatography (HPLC) or isoelectric focusing (IEF) as a gold standard.

MATERIALS AND METHODS

This prospective study was conducted at six tertiary centers in Thailand with ethical approval by the Institutional Review Board. The model is shown in Box 1 and Figure 1. Study population was pregnant women attending antenatal care clinic at each center between August 2009 and December 2011. Inclusion criteria are as follows: (1) attending antenatal care clinic in the first half of pregnancies, (2) not anemic, defined by hemoglobin > 10 gm/dl. Exclusion criteria are the following: (1) known cases of thalassemia carrier, or any hematologic diseases, (2) loss to follow-up or not following the protocol or the data of final fetal /neonatal diagnosis could not be obtained.

Some definitions used in this study are as follows: severe thalassemia diseases: homozygous α -thalassemia-1 (Hb Bart's disease), homozygous β -thalassemia (β -thalassemia major) and β -thalassemia/HbE diseases; MCV: measured by automated hematology analyzer and expressed in femtolitre unit, considered abnormal for the measurement of less than 78 fl¹¹; CMU-E-screen test: a technique used to screen HbE carrier, using microcolumn DEAE Sephadex A50 chromatography^{12,13}; PCR for α -thal-1 (SEA type): a technique for amplification and analysis of DNA for α -thalassemia-1 gene, modified from Chang's method^{14,15} by changing the primer specific for α -thal-1 trait; alpha-thal IC strip test: a strip test using monoclonal antibody sandwich technique to identify minute amount of Hb Bart's in maternal blood,⁴ used as an adjunct test with PCR to diagnose α -thal-1 carrier; HbA₂/HbE microcolumn test: a simple quantitative test (using standard A₂ column kit; microcolumn chromatography) was used to identify β -thalassemia trait and HbE trait. The levels of normal, β -thal trait, HbE trait, and homozygous HbE were < 4%, 4–9%, >10%, and >80%, respectively, (HbA₂ and HbE are shown in the same location of the column). Used as a diagnostic test for β -trait or HbE trait in cases of positive MCV or positive HbE screen; IEF: an electrophoretic technique with excellent resolution used to identify and quantify hemoglobin in neonates.

Steps in the model

The steps in the model are the following: (1) women meeting the inclusion criteria were invited to the study and were

Box 1 : The Model for Prenatal Control of Severe Thalassemia

- 1) Genetic counseling
- 2) Screening pregnant women for a carrier using the following screening tests
 - MCV screening for thalassemia trait (α -thal-1, or β -thal)
 - CMU-E screen for HbE trait
- 3) Screening the husbands whose wife had a positive screen (MCV and CMU-E)
- 4) Diagnostic test for a carrier if both had positive screens;
 - Positive MCV (risk for α -thal-1 or β -thal trait):
 - HbA₂/HbE microcolumn for β -thalassemia trait
 - IC strip/PCR test for diagnosis for α -thal-1
 - Positive CMU-E only: (risk for HbE trait but not α -thal-1 or β -thal trait)
 - HbA₂/HbE microcolumn for diagnosis of HbE trait

*if the test was positive in one of the couple then the other was also tested
if the test was negative in one of the couple no further test was needed*
- 5) Identify the matched couples at risk for a certain disease
 - Homozygous β thalassemia:
 - β thal trait and β thal trait
 - β thal trait and β thal / HbE
 - β thalassemia / HbE :
 - β thal trait and HbE trait
 - β thal trait and homozygous HbE
 - β thal trait and β thal/HbE disease
 - Hb Bart's disease
 - α thal 1 trait and α thal 1 trait
- 6) Prenatal diagnosis for pregnancies at risk
 - Risk for β thalassemia major, β -thalassemia / HbE disease
 - CVS or Amniocentesis or Cordocentesis for fetal DNA or blood analysis
 - Risk for Hb Bart's disease
 - Invasive diagnosis as above OR
 - Serial ultrasound
 - No ultrasound marker: repeat ultrasound every 2 weeks
 - +ve for ultrasound marker: invasive fetal diagnosis
- 7) Counseling and options for termination of affected pregnancy

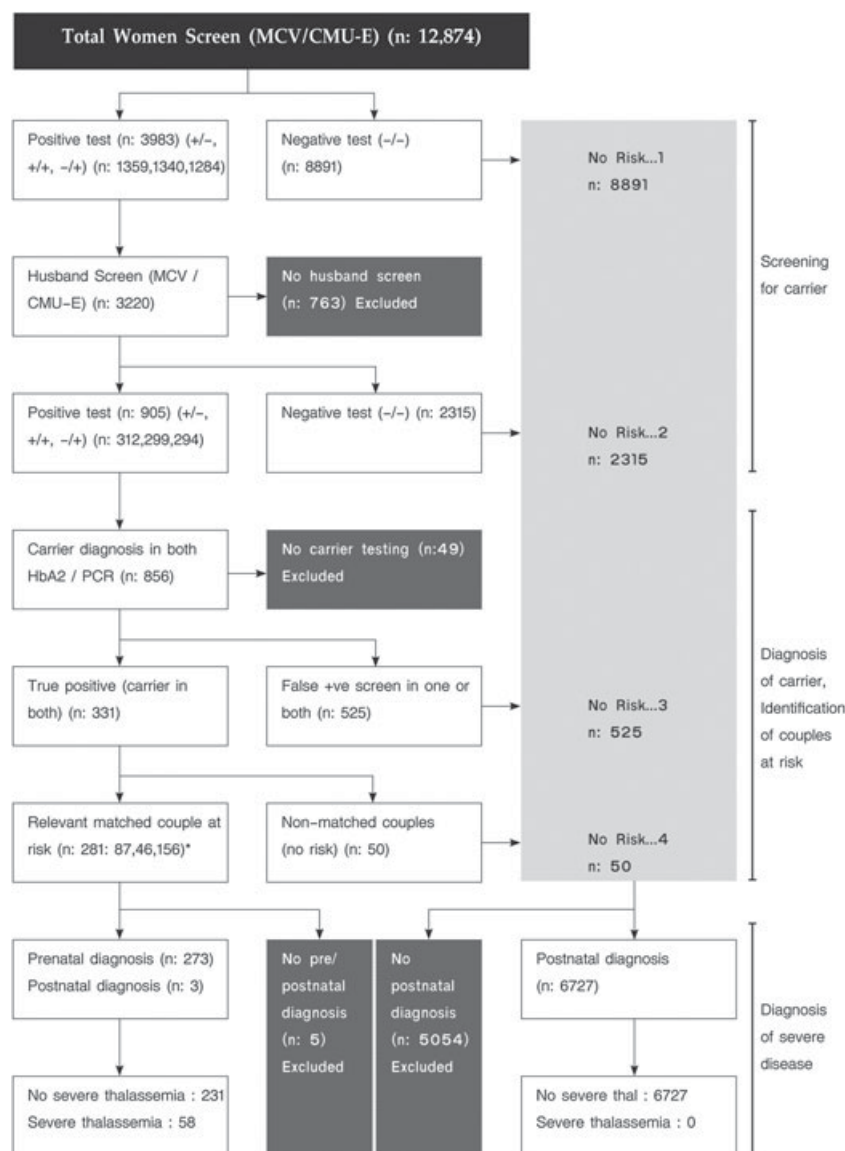


Figure 1 Flow chart of the model (*Some pregnancies had risk for two severe diseases)

enrolled with informed written consent, (2) all recruited women as well as the husbands, if they came together, were counseled for screening at first visit and then 2 mL of blood was obtained and tested for screening test and diagnostic test as needed. The model is shown in Box 1. Most screened women had negative MCV and CMU-E-screen test and needed no husband testing. If the woman was positive, the husband would be tested. If one of them had a negative test, they were not a couple at risk. Only when both had positive relevant tests, diagnostic tests for carrier status were done. Techniques for MCV measurement¹¹ and CMU-E-screen test¹⁶ were described elsewhere. (3) The pregnant women with positive for either test would be asked for taking blood sample of her husband for the same screening (if the husband did not come at the first visit). If the results indicated that the couples were at risk for being a carrier (positive MCV or CMU-E screen), the diagnostic tests for carrier status were performed as follows: HbA2/HbE measurement for β -thal trait or HbE trait in cases of either positive MCV or CMU-E) in both of the couple AND IC-Strip/PCR set for

α -thal-1 trait in cases of positive MCV. One who had only CMU-E screen was not necessary to test for α -thal-1. If IC-strip was negative, α -thal-1 trait could be excluded and if IC strip was positive, α -thal-1 trait would be confirmed by PCR technique (α -thal-1; SEA type), (4) the pregnancies at risk had options for prenatal diagnosis including chorionic villous sampling; chorionic villus sampling (CVS) (10–14 weeks) or amniocentesis (16–18 weeks) for DNA analysis or cordocentesis (18–22 weeks) for fetal blood analysis with HPLC; depending on gestational age, availability of prenatal techniques, and couples' preference. In cases of fetuses at risk for Hb Bart's disease, the option of serial ultrasound every 4 weeks until term was additionally offered. In the presence of sonographic markers of fetal anemia, invasive diagnosis, for example, cordocentesis, for definite fetal diagnosis would be performed. (5) The pregnancies with unaffected fetuses were managed as routine antenatal care. The pregnancies with affected fetuses were offered the choices of continuing pregnancy in high-risk clinic or termination of pregnancy. (6)

All newborns were taken blood (cord blood after birth) for hemoglobin typing analysis with IEF.

Statistical analysis

Effectiveness of the model in preventing new cases of severe thalassemia would be presented as detection rate (sensitivity) and specificity in detecting affected fetuses and newborns. The study needed a sample size of at least 43 affected fetuses for the model sensitivity of 90% at confidence interval of 95% and acceptable error of 10% for sensitivity.

RESULTS

A total of 12 874 pregnant women were prospectively screened at six tertiary centers as shown in Table 1. Of them, 3574 (30.9%) women had a positive screening test (either for thalassemia or HbE) and needed husband testing. Finally, 281 couples were identified to be at risk of severe thalassemia. Of these fetuses at risk, 273 underwent prenatal diagnoses, including chorionic villous sampling (55), amniocentesis (63), cordocentesis (121 cases), ultrasound (for fetuses at risk of Hb Bart's disease; 31 cases), and neonatal diagnosis three cases. The model could identify all of 58 affected fetuses. The prevalence of severe thalassemia is 58/7008 or 0.83% of pregnancies. Among the 6727 neonates of the couples at no risk, no any single case of severe thalassemia was identified by neonatal testing (IEF technique). No any new cases escape from screening program. However, three neonates had severe thalassemia consisting of one baby with β -thalassemia disease, which was born to the parents at risk (both were carriers) but they did not choose to have prenatal diagnosis, another two babies with β -thalassemia/HbE disease. Both couples were identified to be at risk but one did not choose to have prenatal diagnosis and the other had prenatal diagnosis but they did not have termination of pregnancy.

Of the 87 fetuses at risk of Hb Bart's disease, prenatal diagnostic techniques included CVS, amniocentesis, cordocentesis, and serial ultrasound/cordocentesis in 15, 6, 35, and 31 cases, respectively. Of the 31 cases with serial ultrasound, all of the eight affected cases showed some sonomarkers before 22 weeks of gestation and were confirmed by cordocentesis, whereas the remaining were unaffected and needed no invasive diagnosis.

A total of 7008 neonates/fetuses evaluated for thalassemia status, either trait or diseases, is presented in Tables 2 and 3.

Table 2 Prenatal diagnosis of severe thalassemia syndromes among pregnancies at risk

Diseases being at risk (no.)	PND	Unaffected	Affected
β -thalassemia/HbE disease	151	124	27
HbBart's disease	87	67	20
β -thalassemia major	43	35	8
HbBart's disease + β major	3	2	1
HbBart's disease + β -thalassemia/HbE disease	5	3	2
Total ^a	281	231	58

PND, prenatal diagnosis.

^aSome pregnancies had risk for two severe diseases.

Of the recruited pregnant women, 8891 (69.1%) were negative for both screening tests and were considered at no risk at the first step of the model. Therefore, most of husbands needed no screen. Note that 12 874 pregnancies were recruited, only 6727 neonates were successfully taken blood for hematological analysis with IEF. This was due to several factors including transportation problems, failed communication between the researchers' team, and physicians taking care of the parturients and several women did not give birth at the hospitals registered for the project. Therefore, only 7008 pregnancies (281 couples at risk and 6727 couples at no risk) were evaluated for the effectiveness of the program. The remainders who had no final outcomes were excluded from the analysis. The model had a sensitivity and negative predictive value of 100%. No any affected fetus was missed from the detection as presented in Table 3. All of the 58 affected fetuses were prenatally identified, although three of them chose not to have invasive prenatal diagnosis or therapeutic abortion. In real practice, 222 (58/12 874) screens are needed to detect one affected fetus, whereas only 121 (58/7008) screens are needed to detect one affected case in ideal condition of auditing system.

DISCUSSION

Severe thalassemia (Hb Bart's disease, β -thalassemia major, and β -thalassemia/HbE disease) is one of the most common health problems worldwide. Prenatal control is one of the strategies proven effective in preventing new cases of the diseases.^{10,17–20} This study is an example of successful strategy

Table 1 Number of couples and neonates participating through the programs

Institute	No. of woman	No. of positive screened women	No. fetal diagnosis
Bhumibol Hospital	999	286	232
Chiang Mai University	3280	986	1766
Khonkaen University	810	339	778
Pramongkutklao Hospital	3554	955	1455
Prince of Songkla University	722	266	239
Rajavithi Hospital	3509	1151	2538
Total	12 874	3983	7008

Table 3 Effectiveness of the model in prenatal detection of severe thalassemia syndromes

Model prediction	Severe thalassemia	No severe thalassemia	Total
Couples at risk	58	223	281
Couples at no risk	0	6727	6727
Total	58	7008	7008
Prevalence of severe thalassemia	0.83% (58/7008)		
Sensitivity (58/58)	100.0 % (95% CI: 100;100)		
Specificity (6727/6950)	96.8% (95% CI: 96.4;97.2)		
Positive predictive value (58/281)	20.6 % (95% CI: 15.9;25.4)		
Negative predictive value (6727/6727)	100.0 % (95% CI: 100;100)		

in large-scale application. Note that, of the 6727 cases screened, women with negative results, no any single cases of severe thalassemia in newborns were identified, whereas the estimated prevalence of the diseases should probably be 0.5–0.8%. Nearly all, if not all, affected children could prenatally be identified. This result suggested that the model is very highly effective in preventing new cases. Of the screened couples, 35 affected fetuses, not including Hb Bart's, opted to have pregnancy termination, signifying that the direct cost for management (approximately 1 137 500–6 480 810 US\$¹) could be saved and innumerable psychological burden could be obviated. Additionally, several serious morbidities associated with Hb Bart's disease in 20 fetuses were avoided because all were detected and terminated before hydropic changes. If this model had run as routine practice, no neonatal testing, the expense of screening and identifying couples at risk was only about 103 000 US\$ (12874 × 8 US\$), plus approximately 60 000 US\$ for prenatal diagnosis in 281 cases and termination of pregnancies in 55 cases. However, cost-effectiveness of the model is directly associated with prevalence of the diseases. Thus, to assess the cost-effectiveness, prevalence of the disease that varies among geographical areas must be taken into account.

This model emphasizes on the effectiveness of screening and identifying carriers as well as couples at risk leading to prenatal diagnosis with several options. It could prenatally identify affected fetuses with a detection rate and negative predictive value of 100%. Although the true positive prediction was only 20.6 %, this was consistent with theoretical risk of having affected fetuses 25% for autosomal recessive disease, implying very high accuracy of the screening system. The true prevalence of severe thalassemia is likely to be less than 0.8% which is based on 7008 pregnancies. The risk of severe diseases among the excluded cases is very low because the excluded cases were mostly confined to a group of negative screening test. However, effectiveness of the model in terms of detection rate (sensitivity) and specificity could not be affected from such exclusion.

Although the model presented here seems to have no false negative test, no any missing case, this might be due to too small sample size of affected fetuses to express such a low false negative rate that might exist. Additionally, in the real practice where laboratory quality control may probably not perfect, new cases of severe thalassemia may occur. In our experience,

new cases of severe thalassemia may sometimes be encountered in spite of prenatal control strategy. This suggests that the problem in control of the disease is not associated with the model itself but less quality control in implementation. Finally, the model could not completely prevent the new affected cases because some couples did not accept the option of pregnancy termination or prenatal invasive diagnosis.

The most commonly used primary screening tests for α -thal-1 and β -thal carrier are OF or MCV. One test can screen two types of thalassemia carriers. The detection rate may be comparable but MCV provides fewer false positive rates,¹¹ requiring less diagnostic tests. Negative test of OF or MCV can reliably ruled out α -thal-1 and β -thal trait. We prefer to use MCV because of its convenience of automated hematology analyzer and it could be performed in the same time of routine complete blood cell counts. Both MCV and OF may give a false positive test in some medical diseases such as iron deficiency, resulting in unnecessary further work-up. Individuals with HbE trait may have normal MCV or OF in approximately 30% of cases. Thus, they could not be used as a screening test for HbE trait and other more reliable test is needed. Hb E carrier could be screened by several tests such as dichlorophenolindophenol (DCIP), KKL-DCIP, or CMU-E screen. On the basis of our extensive experience and scientific evidence, we preferred CMU-E-screen because it has the highest sensitivity and specificity for Hb E carriers.^{3,13} With negative CMU-E screen, HbE trait can be reliably excluded.

All screening tests are very highly sensitive but less specific. Thus, several non-carriers are also recruited because of false positive tests. Therefore, it is essential that diagnostic tests for carrier be performed. β -thalassemia trait and HbE trait can be effectively confirmed with HbA2/HbE microcolumn as a quantitative assay of HbA2/HbE levels. One test can confirm two types of carrier status. PCR-based diagnosis for α -thal-1 carrier is widely available and not expensive. Our experience show that PCR-based approach will be even cheap when combined with IC-strip test as a single set test.⁵ IC-strip test is much cheaper but confidently excluded α -thal-1 when it is negative. With this approach, only a small portion of positive MCV requires PCR confirmation.

Note that the main fetal diagnostic technique for fetuses at risk of β -thalassemia major and β -thalassemia/HbE disease was cordocentesis with fetal blood analysis with HPLC. This is one of

our limitations because cordocentesis carries a higher rate of fetal loss than amniocentesis and is performed rather late in gestation. A rather small number of cases underwent CVS or amniocentesis with DNA analysis because DNA analysis was not available in most centers. A total of 31 fetuses at risk of Hb Bart's disease were selected for serial ultrasound option. Cordocentesis was performed in only eight pregnancies and all were positive and diagnosed before 22 weeks of gestation. All of the remainders with negative ultrasound markers were finally proven to be normal. Ultrasound can effectively reduce the need of invasive diagnostic techniques which can be related to fetal loss.

Some pitfalls should be mentioned in this study. First of all, not all fetuses were followed-up until the final thalassemia status was tested, resulting in excluding a large number of pregnancies and compromising the power of test. Second, many pregnancies at risk did not undergo prenatal diagnosis due to several reasons, leading to exclusion from analysis. For example, the husbands of the wives with abnormal screening test did not come to be tested, some couples at risk did not receive prenatal diagnosis, and many women first presented in rather late gestation. Therefore, when taking the excluded cases into account, several affected newborns were missed from the detection and we did not know the exact rate of the missing, although this is not directly because of the weakness of the model itself but rather due to the effectiveness of the administration system. Therefore, this study suggests that auditing system is critical to achieve the highest effectiveness.

The risk of prenatal invasive diagnosis is another subject to be seriously considered. Although no procedure-related loss occurred in this study, this may be due to a small number of procedures performed in this study. Additionally, all invasive procedures in the study presented here were performed by experienced perinatologists. However, the procedure-related fetal loss must be taken into account for the model risk. For example, cordocentesis related fetal loss may be 1–2%.^{21–23} From our perspective, in widely implementation, effective screening should be effectively carried out to identify couples at risk in primary health care and invasive prenatal diagnosis should be reserved for tertiary care or referral center.

Fetal diagnosis could be performed by DNA analysis from chorionic villi or amniocytes to identify some specific

mutations. Although this technique has been shown successful for prenatal diagnosis,^{10,20,24} it is sophisticated, expensive, and time-consuming that could not put into practice for routine work, especially in our population which have various mutations of β -genes.²⁵ Because of the fact that fetal blood analysis using HPLC, which could differentiate types of hemoglobin even in small amount with high accuracy,^{26,27} is more available than DNA analysis in our countries, many centers in our country preferred cordocentesis as a primary invasive prenatal technique for such a purpose.

In conclusion, this study strongly suggests that the model consisting of MCV plus CMU-E as screening tests and HbA2/HbE microcolumn plus IC-strip/PCR as diagnostic tests for carrier status be highly effective in reducing the incidence of severe thalassemia, resulting in improving quality of life. Hopefully, when widely implemented, this model could have a great good impact on public health in geographical areas of high prevalence. This study provides scientific evidence supporting potential cost-effectiveness of the model and serves as a basis for assessment of cost-effectiveness in implementation elsewhere. Finally, we expect that this model can be used as reference for national policy in control of severe thalassemia in the future. However, cost-effectiveness of the model is varied with the prevalence; therefore, it must be further evaluated before implementation.

ACKNOWLEDGEMENTS

The authors wish to thank the Pediatric Hematologic Laboratory, Chiang Mai University for contributing to most lab work-up in this study.

WHAT'S ALREADY KNOWN ABOUT THIS TOPIC?

- Several screening systems have been proposed in the literature.

WHAT DOES THIS STUDY ADD?

- Effectiveness of the screening system, in term of sensitivity, specificity, positive and predictive value, is first determined.

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Chorionic Villous Sampling: Experience of 636 Cases

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Objective: To describe the technical aspects and complications of chorionic villous sampling (CVS).

Material and Method: The database of CVS procedures performed between January 2004 and August 2011 at Maharaj Nakorn Chiang Mai Hospital was assessed prospectively. Consecutive procedures during 10 to 14 gestational weeks were included into the present study. Indications, results, and complications of the CVS were extracted and analyzed.

Results: All 636 CVS procedures were successful. Indications for CVS were fetal karyotyping, DNA analysis for severe thalassemia disease, and for both in 36.5%, 50.6%, and 12.9%, respectively. However, 3.4% had inconclusive CVS result, and the second trimester prenatal diagnosis procedures needed to be done. There were five cases (0.8%) of fetal loss in the present study. Only two cases (0.3%) that fetal loss happened within two weeks of CVS procedures. Other minor complications such as vaginal bleeding and amniotic fluid leakage were found in 0.3 to 1.3% and had no long-term effect. No case with anomaly and procedure-related infection following the procedure was seen.

Conclusion: CVS is a safe and reliable prenatal diagnosis procedure in the first trimester. In experienced operators, fetal loss rate was less than 1%. However, a few cases will have inconclusive CVS results and may need a confirmation during the second trimester diagnostic procedures.

Keywords: Chorionic villous sampling, Prenatal diagnosis, Fetal loss

J Med Assoc Thai 2013; 96 (4): 383-8

Full text. e-Journal: <http://jmat.mat.or.th>

Over the past decades, chorionic villous sampling (CVS) has emerged as an alternative to amniocentesis for women who wish to have an early prenatal diagnosis. In addition, due to an increase in a number of pregnancies with advanced of maternal age and availability of first trimester aneuploidy screening program, demand of prenatal diagnosis procedure during the first trimester is increased accordingly. CVS is a procedure that retrieves cells from the developing placenta for genetic or chromosome analysis during first trimester. There are two approaches of CVS depending on placental accessibility, transabdominal and transcervical, of which safety and diagnostic accuracy are comparable⁽¹⁾.

The main advantage of CVS over amniocentesis or cordocentesis is that the definitive diagnosis could be made earlier in gestation. However, an invasive procedure carries a risk of pregnancy loss varying from 0.6 to 3% and needs more expertise⁽²⁻⁶⁾. Although there has been concern that CVS may lead

to limb reduction defects, current data suggest that CVS performed between 10 to 14 weeks of gestation is not significantly associated with a higher incidence of such a condition^(7,8). However, if CVS was done before 10 weeks of gestation, the risk of limb reduction defect may probably increase to 1 to 2%^(9,10). The aim of the present study was to describe the experience of transabdominal and transcervical CVS at Maharaj Nakorn Chiang Mai Hospital, in the aspects of indications, success rate, and complications of the procedure.

Material and Method

The present retrospective descriptive study recruited consecutive cases of singleton pregnant women with indication for CVS at 10 to 14 weeks gestation at Maharaj Nakorn Chiang Mai Hospital, between January 2004 and August 2011. All procedures, either by transabdominal or transcervical approach, were performed with informed consent. The six operators who performed CVS in the present study were familiar with ultrasound guided prenatal diagnostic procedures and had performed over 100 cases of cordocentesis before performing CVS. For transabdominal CVS, the procedure was performed

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under ultrasound guidance, supine position under local anesthesia, using 20 gauge spinal needle with back and forth movement technique. For transcervical approach, the patient was placed in lithotomy position and the procedure was performed using Rodeck CVS biopsy forceps under continuous ultrasound guidance. Once obtained, the sample was immediately examined under a microscope to determine if the amount of villi were adequate and remove the decidua (maternal cells) from the villi. Then the tissues were sent to laboratories for processing. For karyotyping, the chorionic villi were cultured and analyzed. For DNA analysis, extraction of DNA was performed and PCR-base technique was used for mutation detection. Success rate of procedures, pregnancy outcomes, and procedure related complications were prospectively assessed and recorded. The data were analyzed using the statistical

package for the social science (SPSS, Chicago), version 15.0. Descriptive data were presented as percentage, mean, and standard deviation (SD) and 95% confidence interval (95% CI). The present study was approved by the Institutional Review Boards (IRB) of ethics committee and financially supported by the National Research University Project under Thailand's office of the Higher Education Commission.

Results

During the study period, 636 pregnancies were recruited into the study. The mean maternal age was 32.3 ± 5.5 years, and the mean gestational age was 12.1 ± 0.9 weeks. Ninety two point five percent (588 cases) and 7.5% (48 cases) had transabdominal and transcervical CVS, respectively. The procedure was successful in all cases, and 89.6% (570 cases) were success with one attempt of puncture. Only four cases (0.6%) needed three or more punctures to retrieve adequate samples. The indications for CVS were fetal karyotyping, DNA analysis for severe thalassemia diseases and for both karyotype and DNA analysis in 36.5% (232 cases), 50.6% (322 cases), and 12.9% (82 cases), respectively. However, the final results could not be obtained in 3.1% (20 cases), consisting of two cases (0.3%) with maternal cell contamination and 18 cases (2.8%) with problems in laboratory techniques including inadequate metaphase for karyotyping (12 cases, 1.9%), unable to identify globin gene mutation (5 cases, 0.8%), and inconclusive result for DNA analysis of severe thalassemia disease in one case (0.1%).

Table 1. Results of CVS

Results	Cases (%)
Karyotype	314 (100)
Normal	275 (87.6)
Autosomal trisomy (trisomy 21, 18, 13, 7)	15 (4.8)
45,XO	5 (1.6)
Mosaicism	3 (1.0)
Others	4 (1.2)
No results due to laboratory failure	12 (3.8)
Severe thalassemia	404 (100)
Normal	86 (21.3)
Trait	203 (50.2)
Affected	109 (27.0)
No results due to laboratory failure	6 (1.5)

Table 2. Details of cases with abnormal karyotypes from CVS (n = 27)

CVS results	Number (cases)	US findings	Second trimester PND	Pregnancy outcome
Trisomy 21	6	Thick NT all	-	Therapeutic abortion
Trisomy 18	6	Thick NT all	-	Therapeutic abortion
Trisomy 13	2	Thick NT all	-	Therapeutic abortion
Trisomy 7	1	Normal US	46,XX	Normal female newborn
45,XO	4	Cystic hygroma	-	Therapeutic abortion
45,XO	1	Normal US	46,XX	Normal female newborn
46,XX/47,XXY	1	Normal US	46,XY	Normal male newborn
46,XY/47,XX +13	1	Normal US	46,XY	Normal male newborn
46,XY/47,XX +7	1	Cystic hygroma	-	Therapeutic abortion
47,XXY	1	Normal US	-	Therapeutic abortion
47,YYY	1	Normal US	-	Normal male newborn
46,XX, del (22) (q11.2)	1	Normal US	46,XX, del (22) (q11)	Normal female newborn
46,XY, inv (6)	1	Normal US	46,XY	Normal male newborn

The results of CVS are shown in Table 1. Abnormal karyotypes from CVS results were found in 8.6% (27 cases) and details of individual case are presented in Table 2.

Twenty-two cases (3.4%) needed further prenatal diagnosis procedures in the second trimester to clarify the CVS results. The reasons for confirmatory tests were failure in laboratory techniques (14 cases; 2.2%), maternal cell contamination (2 cases; 0.3%), and abnormal karyotypes without any sonographic soft signs or structural abnormalities (6 cases; 0.9%). Among these six cases, one case with microdeletion of chromosome 22, which was confirmed by cordocentesis, was proven to be inherited from the mother and the pregnancy outcome was uneventful. In contrast, the other five cases had normal karyotype from cordocentesis, though CVS showed abnormal results. Additionally, 27.0% (109 cases) of fetuses at risk of severe thalassemia diseases were identified as affected fetuses by CVS. There were 11.6% (47 cases), 11.4% (46 cases), and 4.0% (16 cases) of fetuses with homozygous alpha thalassemia-1 (Hb Bart's), beta-thalassemia/Hemoglobin E disease, and homozygous beta-thalassemia diseases, respectively. However, CVS could not diagnose for thalassemia status in 1.5% (6 cases) due to failure in laboratory techniques as shown in Table 1.

The complications of CVS are shown in Table 3. There were missing data in 22 cases (3.4%). Therefore, 614 cases were left for analysis. Five cases (0.8%) had fetal loss. Of them, two fetal losses occurred within two weeks after the transabdominal CVS procedures, which were most likely to be procedure-related fetal loss. The first case had two attempts of CVS puncture but there was failure in laboratory technique due to inadequate metaphase of villi and the other case was fetal trisomy 18. However, there were three cases that fetal loss occurred more than two weeks after CVS, which were two cases of transabdominal and one case of transcervical approach and puncture in one attempt for all cases. Fetal loss occurred at 17, 24, and 16 gestational weeks, respectively. Both abortuses had no gross anomaly and CVS results showed normal in one case and thalassemia trait in the other two cases. Additionally, there were some minor complications following CVS procedure such as minimal vaginal bleeding that found in 1.3% (8 cases), which most of them were transcervical CVS. Amniotic fluid leakage also found in 0.3% (2 cases), the leakage occurred within a few days after procedure and ceased spontaneously, both of them had normal term

Table 3. Complications of CVS

Complications	Transabdominal CVS 568 cases (missing data 20 cases) n (%) (95% CI)	Transcervical CVS 46 cases (missing data 2 cases) n (%) (95% CI)	Total 614 cases (missing data 22 cases) n (%) (95% CI)
Fetal loss within 2 weeks after CVS	2 (0.4) (-0.12 to 0.92)	-	2 (0.3) (-0.13 to 0.73)
Fetal loss more than 2 weeks after CVS	2 (0.4) (-0.12 to 0.92)	1 (2.2) (-2.04-6.44)	3 (0.5) (-0.06 to 1.06)
Vaginal bleeding	3 (0.5) (-0.08 to 1.08)	5 (10.9) (1.89-19.91)	8 (1.3) (0.4 to 2.2)
Amniotic fluid leakage	2 (0.4) (-0.12 to 0.92)	-	2 (0.3) (-0.13 to 0.73)
Small for gestational age	12 in 447 cases (2.7) (1.2-4.2)	-	12 in 480 cases (2.5) (1.1 to 3.9)
Deliver before 34 weeks	6 in 447 cases (1.3) (0.25-2.35)	1 in 33 cases (3) (-2.82-8.82)	7 in 480 cases (1.5) (0.41 to 2.59)
Deliver between 34-36 weeks	29 in 447 cases (6.4) (4.13-8.67)	2 in 33 cases (6) (-2.1-14.1)	31 in 480 cases (6.5) (4.29 to 8.71)

newborns. There was no procedure-related infection in the present study. Therapeutic abortion due to abnormal chromosome, severe thalassemia disease, and severe fetal anomaly was carried out in 134 (21%) cases. Therefore, 480 cases (447 cases of transabdominal and 33 cases of transcervical CVS) were left for analysis of long-term complications. In assessment of newborns, no case of structural defect was found to be related to the CVS procedures. The prevalence of newborns with small for gestational age was only 2.5% (12 cases) and prevalence of preterm delivery was 8.0% (38 cases), which only 1.5% (7 cases) that delivered before 34 weeks, both of these events were unlikely to be affected by CVS procedure. Based on the outcomes, using clinical estimation of the newborns, no cases of severe thalassemia or abnormal major chromosomal abnormalities were missed.

Discussion

CVS is one of the prenatal diagnosis procedures that have been widely accepted for more than decades. Many publications have been reported about its safety and accuracy of the result^(8,11,12). CVS-related fetal loss was reported in the range of 0.6-3%^(5,6,8,11,13,14) and incidence of confined placental mosaicism was 1 to 2%⁽¹⁵⁾.

From the 636 CVS procedures in the authors' study, most of the procedures were transabdominal approach. The procedure was successful in all cases, which almost 90% were successful in one attempt of transabdominal puncture or using Rodeck CVS biopsy forceps in transcervical cases. Most cases that required more than one puncture were usually due to the difficulty to access placental location or the period of operators' learning curve in performing CVS. DNA analysis for severe thalassemia disease was the most common indications for CVS in the present study with the benefit of early diagnosis and less procedure-related fetal loss compared to cordocentesis during mid-pregnancy^(15,16). However, due to limitations and failure in laboratory techniques, data from the present study found that CVS results could not be obtained in around 3% of the cases, which is consistent with other studies^(17,18). This is one important information that should be given to patients about limitation of CVS procedure.

Few cases also need confirmatory prenatal diagnosis procedures due to inconsistent between CVS results and ultrasound findings. Most of these cases had abnormal karyotype from CVS such as autosomal trisomy, Turner's syndrome, mosaicism, deletion of

chromosome and chromosomal inversion. However, all of these cases had normal ultrasound findings, which were unlikely if the CVS results were correct. Therefore, it was reasonable to repeat the prenatal diagnosis procedure to confirm the abnormalities of fetal karyotype in such a circumstance. Finally, either amniocentesis or cordocentesis in these cases showed normal karyotype in most of them, except for one case with microdeletion of chromosome 22 that was inherited from the mother. Thus, interpretation of CVS results should be done together with ultrasound findings. Not only mosaicism from CVS results that should be repeated to exclude confined placental mosaicism, but also in case of abnormal karyotype by CVS with no fetal structural defects. Therefore, after excluding five cases of normal chromosome after repeat testing, the rate of true abnormal chromosome in the present study was 7.0%. Moreover, in the aspect of DNA analysis in fetuses at risk of severe thalassemia diseases, CVS cannot identify gene mutation in 1.5% of the cases and cordocentesis for fetal hemoglobin typing need to be done during mid-pregnancy. Therefore, this data is also an important issue for counseling the patients about the limitation of CVS in case of fetal at risk for severe thalassemia diseases. Of note, such a problem was not associated with CVS itself but rather laboratory technique, signifying that improvement in laboratory quality control may be more important than CVS techniques.

From the result, total fetal loss rate from CVS is 0.8% (5 cases), which is lower than previous studies^(3,4) and comparable to fetal loss rate from second trimester amniocentesis⁽¹¹⁾. Moreover, only two in five cases of fetal loss occurred within two weeks after CVS, which defined as procedure-related fetal loss. In addition, one of these two cases was trisomy 18 fetus, which might have been the cause of fetal loss rather than procedure-related. Therefore, the true rates of CVS-related fetal loss in the present study might possibly be only 0.2 to 0.3%. The reason that fetal loss rate in the present study is very low may be related to experience of the operators and most of the cases in the present study are transabdominal CVS, which is relatively safer than transcervical approach. All operators who performed CVS in the present study were familiar with ultrasound guided prenatal diagnostic procedures such as amniocentesis and cordocentesis, and all of them had performed over 100 cases of cordocentesis before performing CVS. In the aspect of other complications, incidence of minor complications such as leakage of amniotic fluid

or vaginal bleeding were around 0.3 to 1.3% and had no long-term effect on the fetuses. Vaginal bleeding is the complication that was common in transcervical approach. CVS-related fetal anomaly as limb reduction defect, and infection after the procedures were not found in the present study. The incidences of small for gestational age babies and preterm birth were only 2.5% and 8.0%, respectively, which are very low and unlikely to be the result from CVS procedure.

With the limitation of descriptive study, the authors did not have the control group to compare the complications especially fetal loss rate and long-term effect of the CVS procedure. However, the present study had a large sample size of more than 600 cases of CVS and included almost 50 cases of transcervical CVS. Moreover, the study population is homogenous and confined within the period of 10 to 14 gestational weeks, which are the most safe and common periods of performing CVS. Therefore, the information from the present study is valid and may be useful in clinical practice.

Conclusion

The present study suggests that both transabdominal and transcervical CVS are relatively safe and reliable methods of prenatal diagnosis in late first trimester. In experienced hands, fetal loss rate is less than 1% and no long-term complications for fetuses. However, a minority of cases with inconclusive CVS results may need a confirmation by second trimester diagnostic procedures.

Acknowledgement

The authors wish to thank the National Research University Project under Thailand's Office of the Higher Education Commission for financial support.

Potential conflicts of interest

None.

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การตัดชิ้นเนื้อรก: ประสบการณ์ 636 ราย

อุไรวรรณ ลักษณะวิสัย, วีรวิทย์ ปิยะมงคล, สุพัตรา ศิริโชติยะกุล, เพ็ญธิดา ทองประเสริฐ, สุขยา ลือวรรณ, เกษมศรี ศรีสุพรรณดิฐ

วัตถุประสงค์: บรรยายเทคนิคและภาวะแทรกซ้อนจากการตัดชิ้นเนื้อรก

วัสดุและวิธีการ: วิเคราะห์ฐานข้อมูลการตัดชิ้นเนื้อรกในช่วงอายุครรภ์ 10-14 สัปดาห์ ของโรงพยาบาลมหาราชนครเชียงใหม่ ตั้งแต่ มกราคม พ.ศ. 2547 ถึงสิงหาคม พ.ศ. 2554 โดยประเมินเกี่ยวกับข้อบ่งชี้ผลลัพธ์และภาวะแทรกซ้อน

ผลการศึกษา: สตรีตั้งครรภ์ 636 ราย ข้อบ่งชี้ในการตัดชิ้นเนื้อรกคือตรวจโครโมโซมทารก, ตรวจโรคธาลัสซีเมีย และตรวจทั้งสองชนิด คิดเป็นร้อยละ 36.5, 50.6 และ 12.9 ตามลำดับ อย่างไรก็ตามมีร้อยละ 3.4 ที่สรุปผลไม่ได้จากการตัดชิ้นเนื้อรก ทำให้ต้องวินิจฉัยก่อนคลอดอีกครั้งในช่วงไตรมาสที่สอง พบว่ามีทารกที่แท้งหลังการเจาะร้อยละ 0.8 โดยมีเพียงร้อยละ 0.3 ที่การแท้งเกิดขึ้นภายใน 2 สัปดาห์ เลือดออกหรือน้ำเดินพบได้ร้อยละ 0.3-1.3

สรุป: การตัดชิ้นเนื้อรกมีความปลอดภัย ถ้าทำโดยแพทย์ผู้ชำนาญจะมีการแท้งน้อยกว่าร้อยละ 1 อย่างไรก็ตามอาจมีผู้ป่วยจำนวนหนึ่งที่ต้องรับการวินิจฉัยก่อนคลอดด้วยวิธีอื่นซ้ำ เนื่องจากผลของการตัดชิ้นเนื้อรกไม่ชัดเจน

Fetal Red Blood Cell Hematology at Mid-Pregnancy Among Fetuses at Risk of Homozygous β -Thalassemia Disease

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Objective: To compare red blood cell hematology among fetuses at risk of homozygous β -thalassemia disease at mid-pregnancy.

Materials and Methods: Eighty-six fetuses, 18 to 22 gestational weeks, at risk of homozygous β -thalassemia disease undergoing cordocentesis between December 2010 and June 2012 were recruited in the study. Red blood cell parameters were measured and final diagnosis of thalassemia status was based on fetal hemoglobin typing by high performance liquid chromatography technique and DNA analysis. The fetuses were categorized into 3 groups as normal β -globin genotype, β -thalassemia trait, and homozygous β -thalassemia disease.

Results: Mean maternal age and mean gestational age were 26.56 ± 6.36 and 19.12 ± 1.06 weeks, respectively. The prevalence of fetuses with homozygous β -thalassemia disease, β -thalassemia trait, and normal β -globin genotype fetuses were 29.07% (25 cases), 20.93% (18 cases), and 50% (43 cases), respectively. All of red blood cell parameters were not significantly different among the 3 groups of fetuses. No affected fetus had anemia during mid-pregnancy.

Conclusion: No significant difference in red blood cell parameters among unaffected and affected fetuses with homozygous β -thalassemia disease was found.

Key Words: β -thalassemia disease, fetus, red blood cell hematology (*J Pediatr Hematol Oncol* 2013;00:000–000)

Theoretically, β -globin function begins to play an important role after birth and fetuses with homozygous β -thalassemia are not anemic in utero. However, the studies of fetal hematology have been published in very limited numbers,^{1–8} and none of them has studied fetuses with β -thalassemia disease. Better understanding of the nature of the disease in utero is essential for development of fetal therapy, particularly stem cell transplantation in fetuses with homozygous β -thalassemia disease.⁹ Therefore, the objective of this study was to establish normative data of red blood cell parameters of fetuses with homozygous β -thalassemia disease, compared with unaffected fetuses at mid-pregnancy.

MATERIALS AND METHODS

The prospective study was conducted on pregnant women at risk of fetal homozygous β -thalassemia disease undergoing prenatal diagnosis with cordocentesis at Maharaj Nakorn Chiang Mai hospital, between December 2010 and June 2012. The participants were invited to the study with written informed consent. Inclusion criteria were (1) singleton pregnancy; (2) gestational age of 18 to 22 weeks based on fetal sonographic biometry in the first half of pregnancy; (3) no hematologic disorders; and (4) the couple at risk of fetal homozygous β -thalassemia diagnosed by a screening program in our center as described elsewhere.^{10–14} Fetuses with anomaly or any hematologic disorder other than β -thalassemia, failure of blood sampling with cordocentesis, or incomplete data for analysis were excluded from the study. Cordocenteses were performed by 7 experienced perinatologists under real-time ultrasound guidance using Aloka SSD Prosound α -10 or Aloka Prosound α -6 model (Tokyo, Japan) with transabdominal 3.5 MHz curvilinear transducers. Fetal blood samples in amount of 0.5 to 1.0 mL were collected for analysis of red blood cell hematology including hemoglobin (Hb) levels, hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), red blood cell distribution width (RDW), and severity of fetal anemia, defined by fetal Hb of less than the fifth percentile for each gestational week, using reference ranges reported by Mari et al.¹⁵ All hematological parameters were measured within 24 hours after cordocentesis using an automated complete blood count machine (Coulter STKS analyzer; Beckman, CA). All fetuses were categorized into 3 groups according to fetal Hb typing analysis by high performance liquid chromatography technique,¹⁶ together with DNA analysis to determine the gene mutation, including (1) normal β -globin genotype; (2) β -thalassemia trait; and (3) homozygous β -thalassemia disease (affected fetuses). This study was approved by the institute ethics committee and financially supported by the Thailand Research Fund and National Research University Project under Thailand's Office of the Higher Education Commission.

Statistical Analysis

The data were analyzed using the statistical package for the social science (SPSS, Chicago), version 17.0. Descriptive data were presented as percentage, means, and SD for parametric data, whereas median and range were used for nonparametric data. Analysis of variance test and Kruskal-Wallis test were used to compare means or medians among the 3 groups as appropriate.

Received for publication November 16, 2012; accepted June 25, 2013. From the Department of Obstetrics and Gynecology, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand.

The authors declare no conflict of interest.

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TABLE 1. Hematology of Red Blood Cell Indices in Mid-Pregnancy Fetuses (Analysis of Variance and Kruskal-Wallis Test)

Parameters (Mean ± SD)	Fetuses (n = 86)			P
	Normal β -Globin Genotype (n = 43)	β -Trait (n = 18)	Homozygous β -Thalassemia Disease (n = 25)	
GA (median [range]) (wk)	19 (17-22)	19 (18-21)	19 (18-22)	0.6
Hb (median [range]) (g/dL)	11.1 (6.8-12.6)	10.85 (9.2-13.2)	11.4 (9.6-13.2)	0.81
Hct (median [range]) (%)	33.4 (20-38.0)	32.9 (27.5-38.7)	34.2 (29-39.9)	0.4
MCV (fL)	124.86 ± 1.3	126.67 ± 5.7	123.48 ± 8.5	0.42
MCH (pg)	41.44 ± 3.4	41.77 ± 1.9	40.37 ± 3.2	0.27
MCHC (g/L)	33.17 ± 0.9	33.0 ± 0.6	32.67 ± 0.8	0.06
RDW (%)	7.4 ± 1.3	7.36 ± 0.9	7.64 ± 1.2	0.68

GA indicates gestational age; Hct, hematocrit; MCH, mean corpuscular hemoglobin; MCHC, mean cell hemoglobin concentration; MCV, mean corpuscular volume; RDW, red blood cell distribution width.

RESULTS

Eighty-six fetuses at risk of homozygous β -thalassemia disease were recruited into the study and no cases were excluded. Mean maternal age was 26.56 ± 6.36 years and mean gestational age was 19.12 ± 1.06 weeks. The prevalence of fetuses with homozygous β -thalassemia disease, β -thalassemia trait, and normal β -globin genotype fetuses was 29.07% (25 cases), 20.93% (18 cases), and 50% (43 cases), respectively. Red blood cell parameters including; Hb levels, Hct, MCV, MCH, MCHC, and RDW of the fetuses in each group are shown in Table 1.

All of the red blood cell parameters among the 3 groups of fetuses were not significantly different ($P > 0.05$). Although MCHC values tends to be different among fetuses but it is not statistically significant ($P = 0.06$). Incidentally, the results showed that 4 fetuses with normal β -globin genotype were anemic, mild degree of anemia in 3, and moderate degree in 1 fetus. All of the 4 pregnant women with anemic fetuses were healthy without any signs of anemia, infection, or hematologic diseases. The 3 cases of mild anemic fetuses had uneventful pregnancy outcome with term normal newborns, whereas the other fetus with moderate anemia was prematurely delivered at 34 gestational weeks, birth weight of 1900 grams. Except for prematurity and suspected of neonatal sepsis at birth, the baby had no hematological disorders or any sign of anemia after 6 months of age.

DISCUSSION

This study is the first report establishing ranges of fetal hematologic indices in homozygous β -thalassemia fetuses and unaffected fetuses which derived from adequate sample size and homogeneous study population. Our results showed no statistical difference in Hb level, Hct, MCV, MCH, MCHC, and RDW between affected and unaffected fetuses. Therefore, it may be presumed that fetuses with homozygous β -thalassemia disease or β -thalassemia trait have normal hematologic parameters and have no signs of anemia at mid-pregnancy. Interestingly, MCHC values of affected fetuses tended to be significantly different between affected and unaffected fetuses ($P = 0.06$). This finding is consistent with the result in a study in China reported by Huang et al,¹⁷ but further studies with a larger sample size are needed to elucidate such a finding because there is substantial overlap in MCHC values between affected and unaffected fetuses.

The weakness of this study may include incomplete work-up and incomplete follow-up of the 4 fetuses with

normal globin genotype but anemia at mid-pregnancy. However, all these 4 pregnant women had uneventful pregnancy outcomes, except 1 fetus was delivered preterm. Although the cause of fetal anemia was unexplained, it was only subtle and was unlikely to have significant impact on perinatal outcome.

The data from this study is useful in clinical practice to confirm that fetal hematology during mid-pregnancy of fetuses with β -thalassemia trait or disease shows no significant differences from fetuses with β -thalassemia trait or a normal β -globin genotype.

ACKNOWLEDGMENT

The authors thank the Thailand Research Fund and the National Research University Project under Thailand's Office of the Higher Education Commission for financial support.

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Reference Ranges for Fetal Septum Primum Excursion From 14 to 40 Weeks' Gestation

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Objectives—The purpose of this study was to establish reference ranges for septum primum excursion and the septum primum excursion index.

Methods—Normal singleton pregnancies with certain dates from 14 to 40 weeks' gestation were enrolled to acquire spatiotemporal image correlation volume data sets. The stored data sets were subsequently displayed offline to measure septum primum excursion and the left atrium diameter. The measured values were regressed to identify the best-fitted model as a function of gestational age and biparietal diameter.

Results—A total of 624 volumes were successfully measured, and normal reference ranges for predicting means and standard deviations of septum primum excursion and the septum primum excursion index were established based on best-fitted equations. The septum primum excursion index was relatively constant throughout pregnancy (mean $\pm$ SD, 0.474 ± 0.082), whereas septum primum excursion increased with gestational age and biparietal diameter as follows: (1) septum primum excursion = $-6.30 + 0.667 \times \text{gestational age} - 0.009 \times \text{gestational age}^2$; SD of septum primum excursion = $0.219 + 0.02 \times \text{gestational age}$; and (2) septum primum excursion = $-3.342 + 1.933 \times \text{biparietal diameter} - 0.102 \times \text{biparietal diameter}^2$; SD of septum primum excursion = $0.330 + 0.065 \times \text{biparietal diameter}$. Percentile charts for predicting septum primum excursion and equations for Z score calculation were also provided.

Conclusions—Normal reference ranges for fetal septum primum excursion and the septum primum excursion index have been provided. These normative data may be useful tools for assessment of hemodynamics through the foramen ovale or left ventricular diastolic function.

Key Words—fetal septum primum excursion; 4-dimensional sonography; prenatal; reference range; spatiotemporal image correlation; Z scores

Received December 20, 2012, from the Department of Obstetrics and Gynecology, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand. Revision requested January 23, 2013. Revised manuscript accepted for publication March 18, 2013.

We thank the Thai Research Fund and the National Research University Project under Thailand's Office of the Higher Education Commission for financial support.

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Abbreviations

STIC, spatiotemporal image correlation

Septum primum excursion is a distance of maximal linear displacement of the flap of the foramen ovale projecting into the left atrium, and the septum primum excursion index is a ratio of septum **primum excursion** and the left atrium diameter. Septum primum excursion and the septum primum excursion index can reflect the flow shunting through the foramen ovale and may be used as simple tools for assessment of cardiac function.¹ It has been postulated that the septum primum excursion index should be increased in physiologic states marked by increased cardiac output such as fetal anemia. Similarly, fetal hypoxemia can lead to redistribution and an increased amount of blood shunting through the ductus venosus and the foramen ovale.² For example, Firpo and Zielinsky³ reported that septum primum excursion was significantly

reduced in the fetuses of diabetic mothers with septal hypertrophy. Moreover, Kiserud et al⁴ found that septum primum excursion was also reduced in a growth-restricted fetus with pronounced hemodynamic impairment of the placenta.

The use of septum primum excursion or the septum primum excursion index is a very simple method compared to Doppler techniques, such as the Tei index. Thus, theoretically, this method may possibly be a simple tool for general practitioners in assessment of cardiac function. However, to evaluate the effectiveness of septum primum excursion and the septum primum excursion index, normal reference ranges of septum primum excursion and the septum primum excursion index must first be developed. In a literature review, we found only a few studies on normal reference ranges for septum primum excursion and the septum primum excursion index,^{5,6} and they were based on a very limited number of cases or gestational ages. Therefore, we conducted this study to establish reference ranges for septum primum excursion and the septum primum excursion index in the hope that these might serve as simple tools for evaluation of diastolic cardiac function.

Materials and Methods

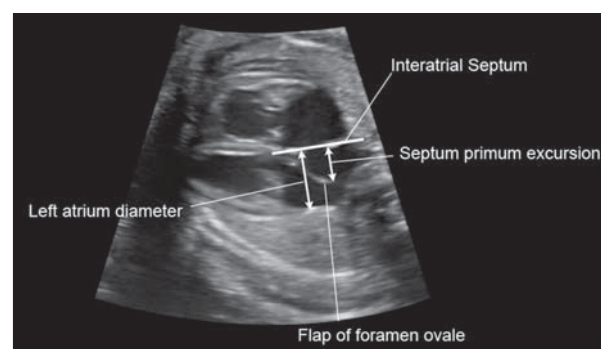
We conducted a prospective descriptive analysis with approval of the Institutional Review Board for Ethics at the Department of Obstetrics and Gynecology, Chiang Mai University, between September 1, 2007, and August 31, 2012. Healthy pregnant women attending an antenatal care clinic were enrolled in the study and provided written informed consent. The inclusion criteria were as follows: (1) singleton pregnancy; (2) certain gestational age based on sonographic dating in the first half of pregnancy and a reliable last menstrual period; (3) gestational age between 14 and 40 weeks; and (4) low-risk pregnancy without known obstetric or medical complications, eg, anemia, cardiac disease, diabetes mellitus, and chronic hypertension. The exclusion criteria were as follows: (1) fetal structural or chromosomal abnormalities; (2) fetal growth restriction (<10th percentile) or macrosomia (>90th percentile); and (3) cardiac spatiotemporal image correlation (STIC) volume data sets of poor quality.

The participants underwent a standard sonographic examination for fetal anomaly screening and biometric measurements. During the examination, STIC volumes were acquired on a real-time machine with 2–5-MHz transabdominal 4-dimensional curvilinear transducers (Voluson E8; GE Healthcare, Zipf, Austria). After the typical 4-chamber views, both apical and transverse planes were visualized

during fetal quiescence, and STIC was activated to acquire the volume data sets by sweeping through the fetal chest. The angle of acquisition ranged from 20° to 40°, mainly depending on the fetal heart size, and the acquisition time accordingly ranged from 7.5 to 15 seconds. All of the acquired volume data sets were stored in the hard drive of the scanning machine and then were transferred to the hard drive of a personal computer for later offline analysis.

The STIC volume analysis was systematically performed on multiplanar views with 4D View version 9.0 software (GE Healthcare). The analysis was based on only the acquired volumes of the transverse 4-chamber view, in which the flap of the foramen ovale or the septum primum was usually perpendicular to the ultrasound beam. Each volume data set was manipulated to visualize the perfect 4-chamber view, following guidelines described elsewhere.^{7,8} With the cine loop function, the volume was searched for the frame with maximal excursion of the septum primum projecting to the left atrium. On such a frame, the measurements of septum primum excursion and the septum primum excursion index were performed on panel A of the screen. Septum primum excursion is the distance from the interatrial septum to the point of maximal extension of the septum primum, as shown in Figure 1. The left atrium diameter was also measured on the same frame. The diameters of the septum primum and left atrium were perpendicular to the interatrial septum. The septum primum excursion index was calculated by dividing the septum primum **excursion** by the left atrium diameter. The first 50 volume data sets were used to determine intraobserver and interobserver variability and were not included in the analysis for construction of the reference ranges.

Regression analysis of fetal septum primum excursion and the septum primum excursion index was done to identify the optimal model using SPSS version 17.0 software



(SPSS Inc, Chicago, IL). The statistical procedure for constructing normal ranges for septum primum excursion and the septum primum excursion index followed the guidelines suggested by Royston and Wright.⁹ Briefly, regression models for both the mean and standard deviation of the fetal parameters were fitted separately to generate the best-fitted equations for Z score calculation. Normality and goodness-of-fit regression models were validated by checking the scatter patterns of points relative to fitted means and standard deviations, expressed as Z scores (actual measured value – estimated mean/estimated standard deviation). Z scores were also tested for normality by the Shapiro-Wilk *W* test and Q-Q plots. Intraobserver variability and interobserver variability were identified by intraclass correlation coefficients.

Results

A total of 656 STIC volume data sets were successfully acquired with satisfactory quality and available for offline analysis. However, 32 data sets were later excluded, since the flap of the foramen ovale was not clearly visualized. Therefore, the remaining 624 data sets with successful measurements were used for statistical analysis. Most of the participants (341 [54.65%]) were nulliparous. The mean maternal age $\pm$ SD was 26.38 ± 6.77 years (range, 15–43 years). All were healthy Thai women residing in the northern part of Thailand. The mean birth weight was 2910 ± 488 g. The male to female ratio was 320:304 (51.28%:48.72%). The distribution of participants by gestational age is presented in Table 1. Intraobserver variability and interobserver variability in septum primum excursion measurements were found to have good agreement, with intraclass correlation coefficients of 0.968 (95% confidence interval, 0.944–0.982; $P < .001$) and 0.949 (95% confidence interval, 0.910–0.971; $P < .001$), respectively. Although the septum primum excursion index was relatively constant or minimally decreased with gestational age, as shown in Figure 2 (mean, 0.474 ± 0.082 ; range, 0.20–0.71; $R^2 = .004$), **septum primum excursion increased significantly with gestational age.**

A quadratic regression model was the best equation for the predicted mean of septum primum excursion as a function of gestational age (Figure 3) and biparietal diameter (Figure 4). The standard deviation of septum primum excursion also varied with gestational age and biparietal diameter, as presented in Table 2 and Figures 2 and 3. These equations were validated by constructing a Z score for each variable. The normalcy of the Z scores was demonstrated in a Q-Q plot. The Shapiro-Wilk *W* test showed nor-

mality in the distribution ($P = .20$). In addition, the Z scores were equally distributed above and below 0 across the entire range of gestational ages and biparietal diameters, as well as following a standard normal distribution. The number of Z scores distributed outside the range of ± 2 SD was as expected: approximately 10% of the values as seen in Figure 5. Reference ranges for septum primum excursion by percentile as a function of gestational age and biparietal diameter are presented in Tables 1 and 3, respectively.

Z scores for each measured value of septum primum excursion can readily be calculated as follows: $Z \text{ score} = (\text{measured septum primum excursion value} - \text{predicted value}) / \text{predicted standard deviation}$ (the predicted septum primum excursion value and standard deviation are available from the equations in Table 2). Example of Z score calculation for a given measurement: actual measured septum primum excursion = 6.4 mm at 20 weeks' gestation. Using equations in Table 2: predicted septum primum excursion at 20 weeks' gestation = $-6.30 + 0.667 \times \text{gestational age} - 0.009 \times \text{gestational age}^2 = 3.44$ mm; predicted SD of septum primum excursion at 20 weeks'

Table 1. Septum Primum Excursion as a Function of Gestational Age

GA, wk	n	Septum Primum Excursion, mm				
		5th	10th	50th	90th	95th
14	13	0.4	0.6	1.2	1.9	2.0
15	14	0.8	1.0	1.6	2.3	2.5
16	13	1.1	1.3	2.0	2.7	2.9
17	19	1.5	1.7	2.4	3.1	3.3
18	19	1.8	2.0	2.7	3.5	3.7
19	18	2.1	2.3	3.0	3.8	4.0
20	21	2.4	2.6	3.4	4.1	4.4
21	23	2.6	2.8	3.6	4.5	4.7
22	24	2.8	3.1	3.9	4.8	5.0
23	26	3.1	3.3	4.2	5.0	5.3
24	28	3.3	3.5	4.4	5.3	5.5
25	27	3.5	3.7	4.6	5.5	5.8
26	26	3.6	3.9	4.8	5.7	6.0
27	33	3.8	4.0	5.0	6.0	6.2
28	31	3.9	4.2	5.2	6.1	6.4
29	26	4.0	4.3	5.3	6.3	6.6
30	30	4.1	4.4	5.4	6.5	6.7
31	31	4.2	4.5	5.5	6.6	6.9
32	28	4.2	4.5	5.6	6.7	7.0
33	21	4.3	4.6	5.7	6.8	7.1
34	24	4.3	4.6	5.7	6.9	7.2
35	29	4.3	4.6	5.8	6.9	7.2
36	25	4.2	4.6	5.8	7.0	7.3
37	25	4.2	4.6	5.8	7.0	7.3
38	25	4.2	4.5	5.7	7.0	7.3
39	16	4.1	4.4	5.7	7.0	7.3
40	9	4.0	4.4	5.6	6.9	7.3

GA indicates gestational age.

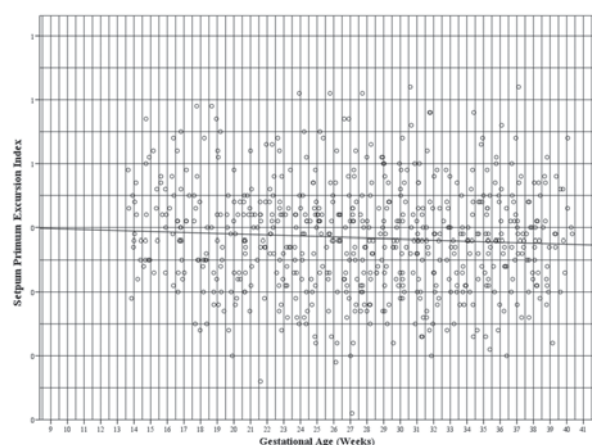


Figure 2. Fetal septum primum excursion index as a function of gestational age.

gestation = $0.216 + (0.020 \times 20) = 0.619$ mm; Z score = $(6.4 - 3.44) / 0.619 = 4.78$. Therefore, this fetus had wide septum primum excursion, at 4.78 SD above the predicted mean excursion for a fetus at 20 weeks' gestation, which was out of the normal range.

Discussion

In this study, septum primum excursion increased significantly with gestational age, consistent with previous reports,^{1,5} and the reference ranges were alike, although the best-fitted model was different. In contrast, the septum primum excursion index was relatively constant throughout

gestational age. In fact, it had a tendency to decreased minimally with gestational age, but the extent of the decrease was too small to have any clinical significance. Therefore, this study suggests that only one reference value (mean $\pm$ SD, 0.474 ± 0.082) may be used as a date-independent parameter. However, our value is somewhat lower than that reported by Zielinsky et al,¹⁰ which may be associated with differences in sample sizes, sonographic techniques, and possibly racial factors.

It is possible that excursion of the septum primum flap may partly depend on the left atrial diastolic pressure.³ This possibility is supported by some clinical observations that the more hypertrophic the interventricular septum, the worse the restriction to ventricular filling, and the smaller the excursion of the septum primum flap valve within the left atrium.³ Thus, Zielinsky et al¹ suggested that assessment of septum primum excursion and the septum primum excursion index may be used as a simple tool for evaluation of left ventricular diastolic function. In addition to evaluation of left ventricular diastolic function, especially in cases of fetal interventricular hypertrophy due to maternal diabetes or in fetuses with growth restriction, the additional rationale of this study was based on our observation that mobility of the septum primum seems to be exaggerated in hypervolemic or hyperhemodynamic states, as in fetuses with hemoglobin Bart disease, and septum primum excursion may have potential for early detection of fetal anemia. If this rationale is true, septum primum excursion may possibly be a promising sonographic marker for fetal anemia, especially in pregnancies at risk, since the measurement is simple and reproducible. Before we explore the

Figure 3. Quadratic function relationship between gestational age and septum primum excursion. The lines represent the 5th, 10th, 50th, 90th, and 95th percentiles.

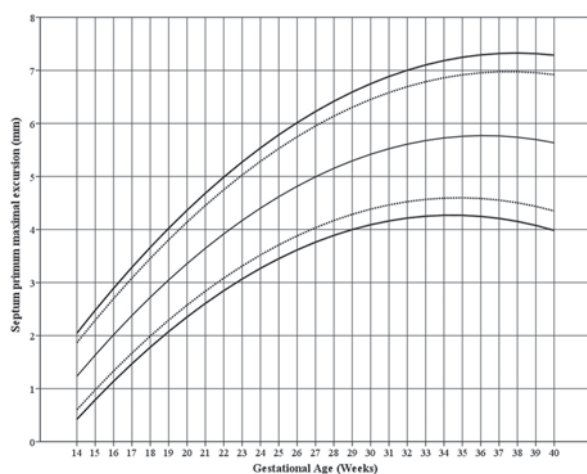


Figure 4. Linear relationship between biparietal diameter and septum primum excursion. The lines represent the 5th, 10th, 50th, 90th, and 95th percentiles.

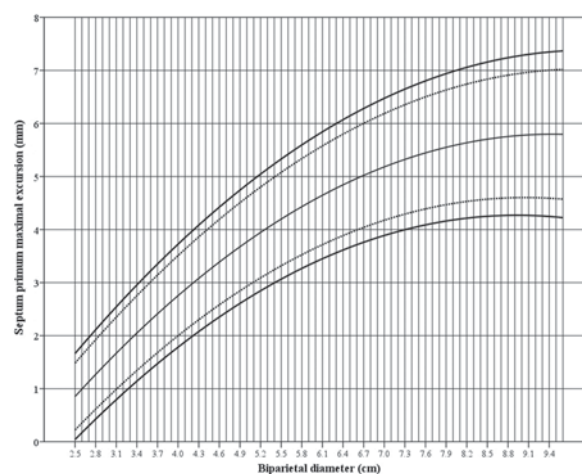


Table 2. Regression Models for Prediction of the Mean and SD of Septum Primum Excursion Based on Gestational Age and Biparietal Diameter

Parameter	Model Derived From Regression Analysis	R ²
Gestational age (GA), wk		
Septum primum excursion, mm	$-6.30 + 0.667 \times \text{GA} - 0.009 \times \text{GA}^2$	0.711
SD of septum primum excursion, mm	$0.219 + 0.02 \times \text{GA}$	0.217
Biparietal diameter (BPD), cm		
Septum primum excursion, mm	$-3.342 + 1.933 \times \text{BPD} - 0.102 \times \text{BPD}^2$	0.711

potential use of this marker for such a purpose, we need to construct normal reference ranges.

To date, only a little evidence is available on the use of septum primum excursion and the septum primum excursion index as a simple alternative for assessment of fetal left ventricular diastolic function. The data presented here can probably serve as baseline data for prenatal evaluation of hemodynamics through the foramen ovale, especially for assessment of left ventricular diastolic function in fetuses of diabetic mothers and those with severe growth restriction. In addition, septum primum excursion may possibly be used as a marker of a high-output state, although this possibility is yet to be elucidated in future studies.

Unlike reference ranges in previous studies, in which the measurements were only compared with gestational ages, the ranges in this study were also constructed as a function of the biparietal diameter. This process may theoretically be more reliable in cases of uncertain dates, since gestational age is a derived value from regression analysis of measurements of the fetal head that introduces an additional error. In the postnatal assessment, cardiac measurements are usually compared with the size of the individual, not the age. Thus, it is preferable to compare the measurements with the fetal biparietal diameter, independent of age.

One limitation of this study that should be mentioned was that the resolution of STIC volume data sets may have been somewhat compromised when compared to 2-dimensional sonography, resulting in exclusion of many cases from the analysis. In addition, this study only demonstrated a normal pattern of septum primum excursion and the septum primum excursion index without testing their accuracy in identifying abnormal cases or comparing them to other tests such as the Tei index. Therefore, further studies to elucidate their clinical application are needed.

The strengths of this study were as follows: (1) the sample size was large; (2) intraobserver variability and interobserver variability were validated and found to have good agreement; (3) the measurements were based on offline analysis of volume data sets without time limits, permitting us to manipulate the data sets in all axes to get the

exact 4-chamber views; (4) our reference ranges included fetuses at early gestational ages; and (5) equations for Z score calculation were provided. We tried our best to establish reliable reference ranges. Although these values were based on STIC, they could also be clinically applied to 2-dimensional sonography. Nevertheless, 2-dimensional sonography may be associated with greater technical difficulties in getting the proper plane, but once the proper plane is achieved, the interpretation should be reliable. The reliability of the measurement is likely to depend on the method used itself rather than the reference ranges.

In conclusion, we have constructed normative data for fetal septum primum excursion and the septum primum excursion index as a function of gestational age and biparietal diameter on the basis of STIC volume data sets. These reference ranges may serve as simple tools for assessment of ventricular diastolic function, especially in fetuses of diabetic mothers and those with growth restriction. Finally, these ranges may be useful tools for further studies on hemodynamics and blood flow shunting through the foramen ovale, especially in fetal anemia.

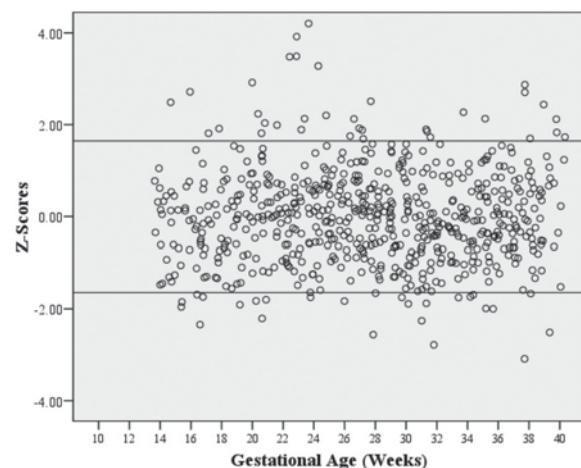
Figure 5. Distribution of calculated Z scores for septum primum excursion against gestational age, indicating an adequate level of fit.

Table 3. Septum Primum Excursion as a Function of Biparietal Diameter

BPD, cm	Septum Primum Excursion, mm					BPD, cm	Septum Primum Excursion, mm				
	5th	10th	50th	90th	95th		5th	10th	50th	90th	95th
2.5	0.1	0.2	0.9	1.5	1.7	6.1	3.5	3.7	4.6	5.6	5.8
2.6	0.2	0.4	1.0	1.6	1.8	6.2	3.5	3.8	4.7	5.7	5.9
2.7	0.3	0.5	1.1	1.8	2.0	6.3	3.6	3.8	4.8	5.7	6.0
2.8	0.4	0.6	1.3	1.9	2.1	6.4	3.6	3.9	4.8	5.8	6.1
2.9	0.6	0.7	1.4	2.1	2.3	6.5	3.7	3.9	4.9	5.9	6.1
3.0	0.7	0.9	1.5	2.2	2.4	6.6	3.7	4.0	5.0	5.9	6.2
3.1	0.8	1.0	1.7	2.3	2.5	6.7	3.8	4.0	5.0	6.0	6.3
3.2	0.9	1.1	1.8	2.5	2.7	6.8	3.8	4.1	5.1	6.1	6.3
3.3	1.0	1.2	1.9	2.6	2.8	6.9	3.8	4.1	5.1	6.1	6.4
3.4	1.1	1.3	2.0	2.8	3.0	7.0	3.9	4.2	5.2	6.2	6.5
3.5	1.3	1.5	2.2	2.9	3.1	7.1	3.9	4.2	5.2	6.2	6.5
3.6	1.4	1.6	2.3	3.0	3.2	7.2	4.0	4.3	5.3	6.3	6.6
3.7	1.5	1.7	2.4	3.1	3.4	7.3	4.0	4.3	5.3	6.4	6.6
3.8	1.6	1.8	2.5	3.3	3.5	7.4	4.0	4.3	5.4	6.4	6.7
3.9	1.7	1.9	2.6	3.4	3.6	7.5	4.1	4.4	5.4	6.5	6.8
4.0	1.8	2.0	2.8	3.5	3.7	7.6	4.1	4.4	5.4	6.5	6.8
4.1	1.9	2.1	2.9	3.6	3.8	7.7	4.1	4.4	5.5	6.5	6.9
4.2	2.0	2.2	3.0	3.7	4.0	7.8	4.1	4.4	5.5	6.6	6.9
4.3	2.1	2.3	3.1	3.9	4.1	7.9	4.2	4.5	5.6	6.6	6.9
4.4	2.2	2.4	3.2	4.0	4.2	8.0	4.2	4.5	5.6	6.7	7.0
4.5	2.3	2.5	3.3	4.1	4.3	8.1	4.2	4.5	5.6	6.7	7.0
4.6	2.4	2.6	3.4	4.2	4.4	8.2	4.2	4.5	5.6	6.7	7.1
4.7	2.4	2.7	3.5	4.3	4.5	8.3	4.2	4.5	5.7	6.8	7.1
4.8	2.5	2.8	3.6	4.4	4.6	8.4	4.2	4.6	5.7	6.8	7.1
4.9	2.6	2.8	3.7	4.5	4.7	8.5	4.3	4.6	5.7	6.8	7.2
5.0	2.7	2.9	3.8	4.6	4.8	8.6	4.3	4.6	5.7	6.9	7.2
5.1	2.8	3.0	3.9	4.7	4.9	8.7	4.3	4.6	5.7	6.9	7.2
5.2	2.8	3.1	3.9	4.8	5.0	8.8	4.3	4.6	5.8	6.9	7.2
5.3	2.9	3.2	4.0	4.9	5.1	8.9	4.3	4.6	5.8	6.9	7.3
5.4	3.0	3.2	4.1	5.0	5.2	9.0	4.3	4.6	5.8	6.9	7.3
5.5	3.1	3.3	4.2	5.1	5.3	9.1	4.3	4.6	5.8	7.0	7.3
5.6	3.1	3.4	4.3	5.2	5.4	9.2	4.3	4.6	5.8	7.0	7.3
5.7	3.2	3.5	4.4	5.3	5.5	9.3	4.3	4.6	5.8	7.0	7.3
5.8	3.3	3.5	4.4	5.3	5.6	9.4	4.2	4.6	5.8	7.0	7.3
5.9	3.3	3.6	4.5	5.4	5.7	9.5	4.2	4.6	5.8	7.0	7.4
6.0	3.4	3.7	4.6	5.5	5.8	9.6	4.2	4.6	5.8	7.0	7.4

BPD indicates biparietal diameter.

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A comparison of Cardio-STIC (Cardio-Spatio-Temporal Image Correlation) segmental analysis between cardiac-based and body-based scrolling-up

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Short title: Cardio-STIC segmental analysis

Key Words: Body-based scrolling-up, Cardiac-based scrolling-up, Spatio-temporal image correlation (STIC), 4D-ultrasound

A comparison of Cardio-STIC (Cardio-Spatio-Temporal Image Correlation) segmental analysis between cardiac-based and body-based scrolling-up

Abstract

Objective: To compare the successful rates in demonstration of the five typical transverse planes in the fetal upper thorax using only scrolling-up between the cardiac-based and body-based scrolling-up technique

Materials and Methods: The cardio-STIC volume datasets acquired at the four-chamber view (4CV) level were recruited from the low-risk singleton pregnancies with normal fetuses. Each volume dataset was analyzed by the authors using both cardiac-based and body-based techniques. With cardiac-based technique, the exact four-chamber view was first identified as an initial plane before scrolling whereas, with body-based technique, a volume dataset was first manipulated to get fetal orientation in the exact dorsal supine position as an initial plane. In both techniques the volumes were then scrolled through the upper thorax to identify the standard planes, including 4CV, five-chamber view (5CV), three-vessel view (3VV), three-vessel-trachea view (3VT), and the aortic arch view (AoA).

Results: The analysis of 50 volume datasets to evaluate intra- and inter-observer variability in the successful rates of 4CV, 5CV, 3VV, 3VT and AoA views showed good agreement. In analysis of the separate 296 volume datasets, the cardiac-based scrolling was associated with significantly higher successful rates than the body-based scrolling in all standard planes.

Conclusion: Cardiac-based scrolling resulted in more successful rates in obtaining the standard planes of cardio-STIC than body-based scrolling. Therefore, the examiners in early learning curve should be encouraged to firstly identify the perfect four-chamber view at the start of analysis.

Introduction

In real practice, analysis of volume datasets in the multiplanar view is simple for cardio-STIC experts who can control the volumes to display the required cardiac planes regardless of fetal positions. Several proposed techniques are certainly not necessary for such experts who are familiar with 3D/4D US, since they can demonstrate any plane they want in no time. However, systematic approach can help the beginners, who are not familiar with cardio-STIC consistently, obtain the standard planes from a cardiac volume dataset (1;2). The simple approach to encourage or facilitate the amateur to be happy with practice of 3D/4D US is needed. The technique to demonstrate the four standard transverse cardiac planes (the four-chamber view, the five-chamber view, the three-vessel view and the three-vessel and trachea view), suggested by DeVore and et al(3), is mainly based on simply scrolling or navigating from the initial acquired transverse plane in Panel A together with rotation of the volumes around the X-, or Y-axis to more clearly display the vessel at any point of interest. Nevertheless, the initial plane prior to navigation is essential for effective display of such standard planes. Unfortunately, the initial plane in Panel A of multiplanar view is not always perfect for navigation, often required some adjustment before scrolling-up. This is due to variations of fetal positions, and the scanning is not exactly perpendicular to the fetal thorax. An examiner who is not familiar with 3D/4D ultrasound will have a hard time adjusting the volume to get the optimal initial images in Panel A before navigation.

Initial adjustment of the volume datasets as a standardization before navigation through the volume has been suggested to simplify the cardio-STIC orientation and enable a reproducible planes for analysis (4;5), though these techniques are not necessary for skillful or experienced sonographers. For cardio-STIC beginners, initial adjustment of the volume datasets is recommended. Such an adjustment may be categorized into two approaches, cardiac-based and body-based techniques. The first approach involves manipulating the volume dataset to depict the typical 4CV and then, scrolling through inside the volume in the multiplanar display mode. The different cardiac planes could be visualized in the transverse plane just only by scrolling in Panel A. The second approach or the body-based scrolling-up (2;5;6) involves firstly maneuvering the volume dataset to place the fetus in a standardized position as if it is lying horizontally on an examination bed in the exact dorsal supine position, by demonstrating the descending aorta as a cross-sectional view in Panel A and longitudinal views in Panel B and C.

The objective of this study was to compare the successful rates in demonstration of the five typical transverse planes in the fetal upper thorax using only scrolling-up between the cardiac-based and body-based technique.

Materials and Methods

The volume datasets were prospectively collected between August 1st, 2008 and July 31st, 2011 at Maharaj Nakorn Chiang Mai Hospital, Chiang Mai University, Thailand with approval of the institute review board ethical committee. The women attending our antenatal care clinic were enrolled with written informed consent. The inclusion criteria consisted of the followings: 1) low risk pregnancy without known medical and obstetric complications, 2) accurate gestational age based on sonographic fetal biometry in the first half of pregnancies, and 3) gestational age between 16 and 37 weeks. The exclusion criteria were 1) multifetal pregnancies; 2) fetal abnormalities either structural anomalies or abnormal growth, and 4) cardio-STIC volume datasets with poor quality. The invited participants underwent standard ultrasound examination, including fetal biometry, anomaly screen, and acquisition for cardio-STIC volumes, using real-time machine with transabdominal 2-5 MHz curvilinear transducers, Voluson E8 (GE Medical Systems, Zipf, Austria). The volume datasets were acquired with transverse sweeps through the fetal thorax after the four-chamber view (4CV) was clearly visualized on 2D ultrasound. Acquisition time ranged from 7.5 to 15 seconds and the angle of sweeping ranged from 20° and 40°, depending on cardiac size and gestational age. All of the volume datasets were stored in the ultrasound machine hard disk and subsequent transfer to a PC computer for off-line analysis.

On phase I of analysis aimed to validate intra- and inter-observer variations, the first 50 volume datasets were blinded to both authors to analyze separately, using a 4D view version 9.0 (GE Medical Systems, Zipf, Austria). Each set was analyzed by both authors using both cardiac-based and body-based techniques. On phase II of analysis aimed to compare the successful rates between the two techniques, each set of the data volume was analyzed by only one of the authors. With cardiac-based technique, analysis was performed to identify the exact four-chamber view. The volume was firstly manipulated the Panel A in the multiplanar views to demonstrate the typical 4CV, regardless of fetal spine position and then navigating through the volume, following the instruction described elsewhere(3;7). With body-based technique, a volume dataset was first manipulated to get fetal orientation in the exact dorsal supine position as described elsewhere(6), as shown in Figure 1. The exact supine position was confirmed by demonstration of cross-sectional view of the descending

Figure 1 *Upper:* Initial plane prior to scrolling for cardiac-based technique; Panel A shows four-chamber view, *Lower:* Initial plane prior to scrolling for body-based technique; descending aorta is adjust to be in exact supine regardless of cardiac position.

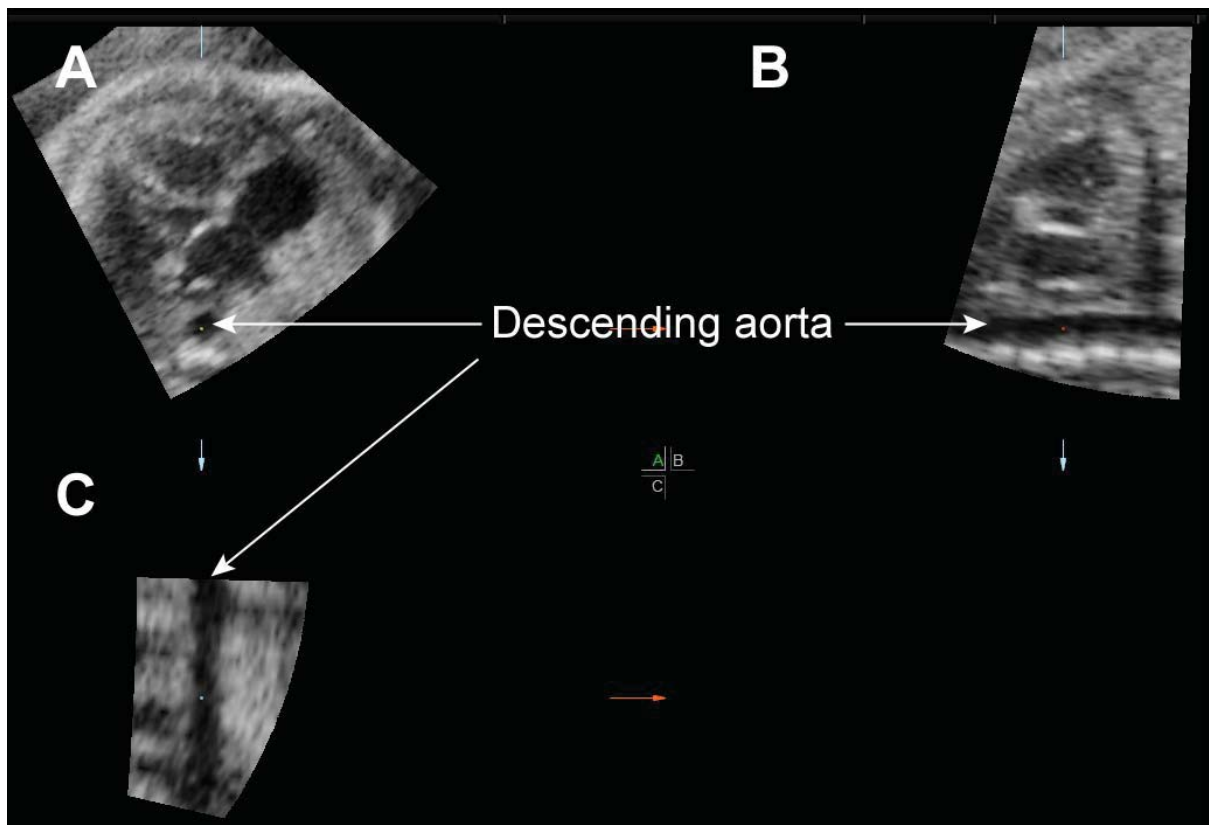
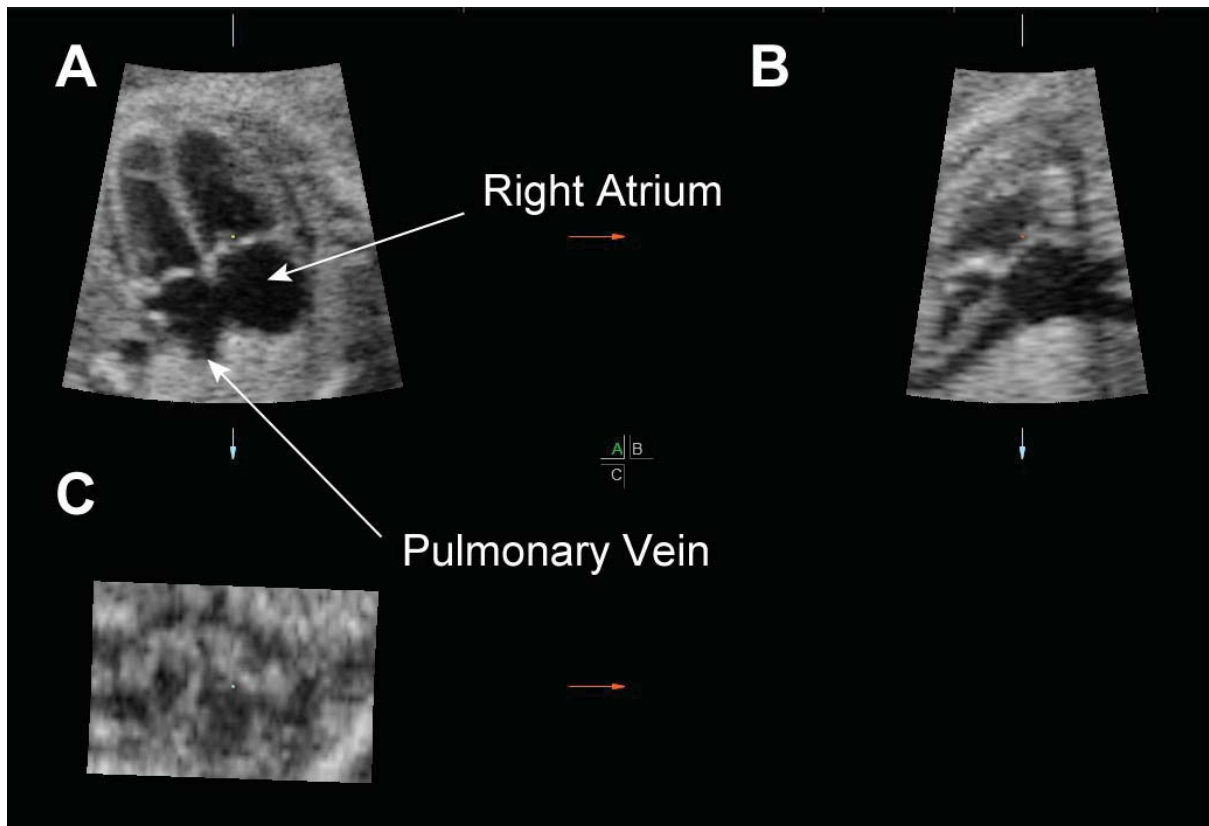
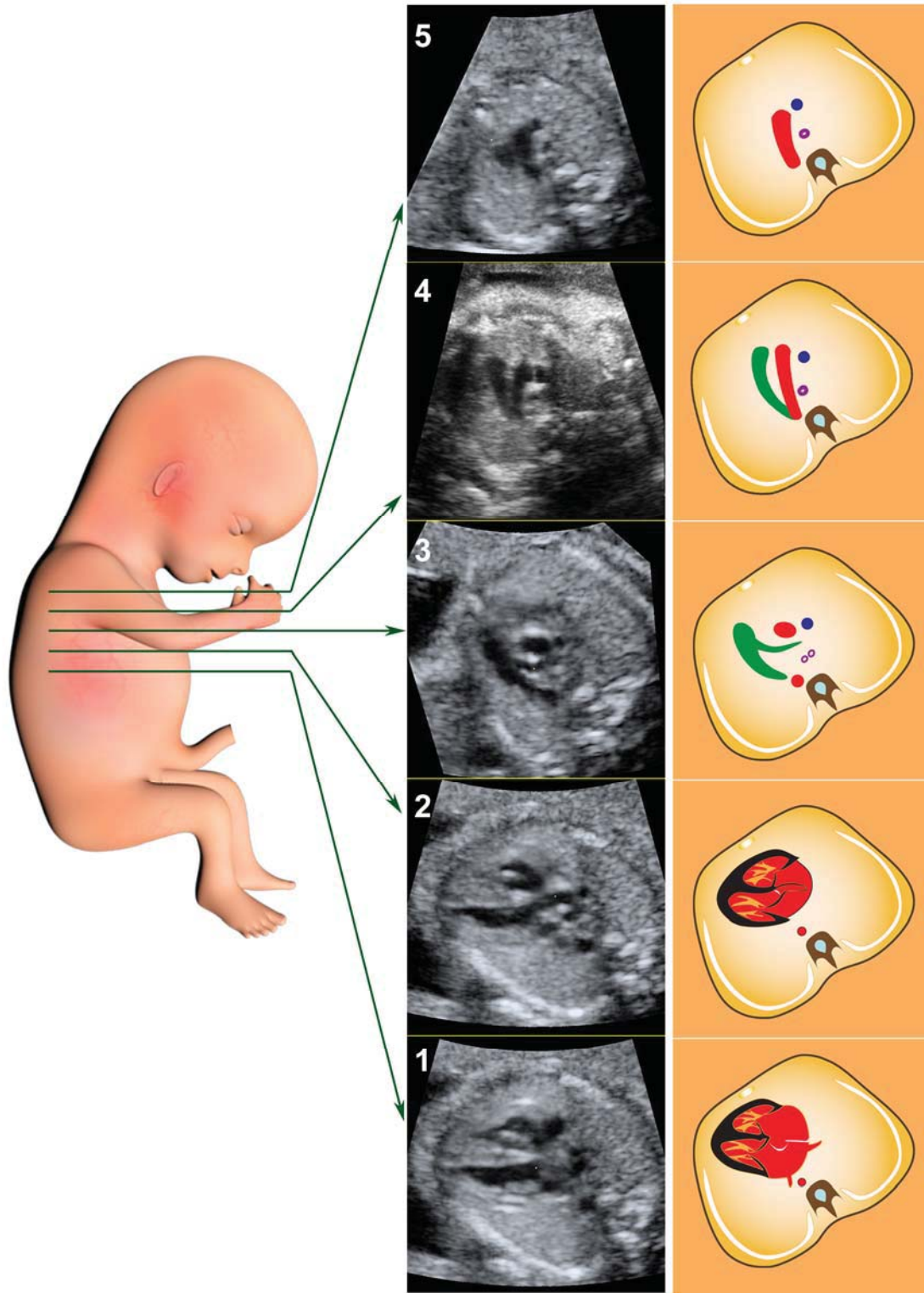


Figure 2 Standard images: 1) four-chamber view; 2) five-chamber view; 3) three-vessel view; 4) three-vessels trachea view; 5) aortic arch view



aorta in Panel A, longitudinal view of the aorta in Panel B and C. The dynamic images of the fetal heart were simultaneously visualized in the three orthogonal planes on the panel A, B, and C of the multiplanar views. With either technique, the five standard axial planes were evaluated during navigation through the fetal thorax, including four-chamber view (4CV), five-chamber view (5CV), three-vessel view (3VV), three-vessel-trachea view (3VT), and the aortic arch view (AoA), as shown in Figure 2.

Statistical analysis: Intra- and inter-observer variability was determined using kappa analysis. Chi-square McNemar test was used to compare the successful rates in visualization of each plane between the two techniques. All statistical analyses were performed using the statistical package for the social sciences (SPSS) software version 17.0 (Chicago, IL).

Result

The total of 50 volume datasets (a total of 100 navigations for both cardiac-based and body-based scrolling) were used to validate for intra-and inter-observer variability. Intra- and inter-observer variability in the successful rates of 4CV, 5CV, 3VV, 3VT and AoA views were good agreement or high reproducibility, as suggested by Kappa indices shown in Table 1 and Table 2.

The separate 296 volume datasets were used for a comparison of the successful rates between both techniques. The number of volume datasets acquired at gestational age of 16-20, 21-25, 26-30, and 31-36 gestational weeks were 74, 72, 91, and 59 respectively. The cardiac-based scrolling was associated with significantly higher successful rates than the body-based scrolling in all standard planes, as shown in Table 3. Note that at initial planes of the body-based approach or planes of exactly supine position, the 4CVs were identified only nearly 70% of cases whereas nearly all cases were visualized with cardiac-based approach.

Table 1: Intra-observer variability in demonstration of the various standard planes by scrolling between cardiac-based and body-based technique (50 volumes; 100 scrolling)

Standard planes	Number of positive agreement	Number of negative agreement	Total	Kappa value	p-value
4CV	88	9	100	0.840	< 0.001
5CV	91	7	100	0.864	< 0.001
3VV	93	5	100	0.823	< 0.001
3VT	89	8	100	0.826	< 0.001
AoA	76	19	100	0.852	< 0.001

Table 2: Inter-observer variability in demonstration of the various standard planes by scrolling between cardiac-based and body-based technique (50 volumes; 100 scrolling)

Standard planes	Number of positive agreement	Number of negative agreement	Total	Kappa value	p-value
4CV	86	9	100	0.755	< 0.001
5CV	90	6	100	0.728	< 0.001
3VV	92	5	100	0.753	< 0.001
3VT	88	8	100	0.778	< 0.001
AoA	75	18	100	0.793	< 0.001

Table 3: Comparison of successful rates in demonstration of the various standard planes by scrolling between cardiac-based and body-based technique

	Cardiac- based Scrolling (Number / %)		Body-based scrolling (Number / %)		Total (Number)	P value *
4CV	293	99.0%	207	69.9%	296	< 0.001
5CV	293	99.0%	266	89.9%	296	< 0.001
3VV	293	99.0%	276	93.2%	296	< 0.001
3VT	266	89.9%	249	84.1%	296	0.008
AoA	209	70.6%	187	63.2%	296	0.003

* Chi-square McNemar test

Discussion

Currently, 3D-US (three-dimensional ultrasound) including cardio-spatio-temporal image correlation (Cardio-STIC) is commonly used, but it seems to be complex and difficult to understand and practice for un-experienced sonographers. Though, practice on cardio-STIC is actually simple for an expert but it always needs a learning curve for beginners.

The most basic approach for analysis of volume datasets acquired with cardio-STIC is to simply scroll or sweep through the volume along the original plane of acquisition. With scrolling, several interesting planes will be completely or partially visualized and then the further manipulate such planes by spinning or rotating along X- or Y-axis for complete demonstration. Theoretically, the standard planes of 4CV, 5CV, 3VV, 3VT could be obtained by scrolling-up the volume datasets from 4CV through the fetal upper chest as described by DeVore and et al(3). However, each such a plane usually needs some adjustment to visualize a typical image. For the beginners, it would be better if scrolling-up firstly provides the number of typical planes as much as possible. This study focused on simple scrolling-up without spinning at all, aimed to compare the effectiveness between the cardiac-based and body-based scrolling.

Based on this study, cardiac-based scrolling was obviously better than body-based scrolling since it had much higher successful rates in demonstration of all standard planes needed for fetal cardiac study. Certainly, it may be easier to get the exact dorsal supine position used in body-based scrolling-up by manipulating the descending aorta, compared to identifying the 4CV plane. Nevertheless, the exact dorsal supine position does not seem to offer the 4CV first due to variations in heart position. As seen in the results of this study, body-based approach could miss several initial 4CV planes. Likewise, visualization of the other standard planes could be less predictable.

Another advantage of the cardiac based scrolling-up is no need to set new initial plane before scrolling-up in case that perfect 4CV can be acquired. In other words, the first steps for scrolling-up has already been set once you activate the acquisition on the typical 4CV on the 2D US screen, whereas it needs to manipulate the volume datasets to set the exact dorsal supine position, used as the initial plane in body-based scrolling up. However, an attempt to adjust the acquired initial image to be a perfect 4CV first will facilitate the beginner to shorten the learning curve for cardio-STIC experience. On our opinion, this study supports examiners to acquire the perfect plane of 4CV with 2D US prior to acquisition and display the volume datasets using cardiac-based approach irrespective of exact fetal dorsal position.

Our findings signify that cardiac-based scrolling facilitates the beginners to achieve the standard planes with less maneuvering the volume datasets, contributing to shorten a learning curve. In other words, this technique will allow examiners to quickly learn the other additional techniques, especially spin techniques described by DeVore(3). Examiners could get nearly all standard planes first just only by scrolling, requiring only minor adjustment to display the entire vessel by placing a reference dot over the vessel of interest and spinning along the X- or Y-axis to get the full length of the vessel completely visualized.

With advanced cardio-STIC, volume datasets can now be displayed as automated slicing or TUI (tomographic ultrasound imaging). Once the proper initial transverse plane or 4CV is selected as a reference plane, several parallel planes to the 4CV could simultaneously be displayed with only one click of activation for TUI mode. These series of tomographic parallel images are displayed on a single screen, as commonly seen in computed tomography and magnetic resonance imaging. However, we use manual scrolling in this study since it can better display and provide more details. Unlike TUI display, manual scrolling allows the examiners to evaluate the volume datasets continuously. Some typical planes of the great arteries may be skipped by TUI because of its limitation in the number of slices.

One limitation of this study should be mentioned was that the application in practice could probably be applied only for the examiners in early learning curve since the cardio-STIC experts can manipulate the volume in any which way they want. Moreover, this study did not include other useful techniques other than scrolling, as well as not including analysis of sagittal planes of the volume datasets. The strength of this study may include 1) adequate sample size for evaluation, as well as distribution to nearly all gestational age in the second and third trimester, and 2) intra- and inter-observer variability was validated and proven to be good agreement.

In conclusion, cardiac-based scrolling resulted in more successful rates in obtaining the standard planes of cardio-STIC than body-based scrolling. Therefore, the examiners in early learning curve should be encouraged to firstly identify the perfect four-chamber view at the start of analysis, as well as at the time of acquisition. We hope that the cardiac-based technique can simply be applied to the beginners in cardio-STIC practice to shorten their learning curve and for educational purpose.

Acknowledgement

The authors wish to thank the Thailand Research Fund for financial support.

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CASE REPORT

Cervical pregnancy with massive bleeding after treatment with methotrexate

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ttongson@mail.med.cmu.ac.th**SUMMARY**

This report describes a catastrophic bleeding following methotrexate treatment of cervical pregnancy and dramatic response to Bakri surgical obstetric silicone (SOS) balloon tamponade in controlling massive bleeding. A 42-year-old woman was diagnosed for cervical pregnancy with a viable fetus at 12 weeks by transvaginal ultrasound. Conservative treatment with intrafetal potassium chloride injection and systemic methotrexate were instituted. On the sixth day of therapy, catastrophic bleeding lead to hypovolemic shock. After resuscitation and blood transfusion, we attempt to control the bleeding with evacuation and curettage but was unsuccessful. Bakri SOS balloon tamponade was applied with immediate and effective response. Nevertheless, total abdominal hysterectomy was performed. In conclusion, conservative treatment of cervical pregnancy with systemic methotrexate could be catastrophic in some patients. Control of active bleeding with Bakri SOS balloon tamponade may possibly be helpful in case of uncontrolled bleeding in selected cases.

BACKGROUND

Cervical pregnancy is a rare type of ectopic pregnancy, but potentially life-threatening. It is defined as an implantation of a fertilised ovum in the cervical canal, occurring between 1 in 1000 and 1 in 95 000 pregnancies, accounting for less than 1% of all ectopic pregnancies.^{1 2} Traditionally cervical pregnancy has been managed surgically, typically by total abdominal hysterectomy, owing to higher risk of massive bleeding. Nevertheless, with the advent of transvaginal ultrasound and the rapid sensitive assay of serum β human chorionic gonadotropin (β hCG) in the past two decades, most ectopic pregnancies, including cervical pregnancies, have often been diagnosed in earlier gestation. Consequently, management of cervical pregnancy with radical modalities has shifted to more conservative approaches.^{3–9} Most reports have demonstrated high efficacy and safety of conservative treatment of cervical pregnancy either by medical treatment with methotrexate administration, uterine artery embolisation, or hysteroscopic resection or dilation and curettage with Foley's balloon tamponade. None in such reports found serious complications with conservative management, though failure might be seen in few cases. The objectives of this report were to describe catastrophic bleeding secondary to methotrexate treatment of cervical pregnancy and to illustrate the dramatic response to Bakri surgical obstetric silicone (SOS) balloon

tamponade in controlling massive bleeding from cervical pregnancy, rarely described elsewhere.

CASE PRESENTATION

A 42-year-old woman, G2 P0010, first presented for antenatal care at 12 weeks of gestation. She was healthy and asymptomatic at first visit without history of in vitro fertilisation treatment and with no vaginal bleeding. Her first pregnancy ended with spontaneous abortion at 9 weeks of gestation, without any interventions. On physical examination, the patient was healthy with stable vital signs. Pelvic examination revealed enlarged barrel-shaped cervix, 1 cm dilation, minimal effacement and no sign of vaginal or cervical bleeding. Transabdominal ultrasonography was performed for nuchal translucency, using 3.5 MHz transducer (Voluson E8; GE Medical Systems, Zipf, Austria). An ultrasound examination revealed a gestational sac with fetal cardiac activity situated 1 cm above the external os. A cervical ectopic pregnancy was diagnosed and was confirmed by transvaginal ultrasound (5 MHz transducer) based on the findings of an empty uterus, an enlarged barrel-shaped cervix which was larger than the uterine corpus. The gestational sac was located below the level of the uterine arteries, absence of the sliding sign (the gestational sac did not slide against the endocervical canal when pressure from the transducer was applied to the cervix) and high vascularisation around the gestational sac on colour flow mapping (figure 1 and video 1). The fetus had cardiac activity with crown-rump length of 5.5 cm. The placenta was localised in the markedly enlarged cervical canal. The uterine corpus and fundus were

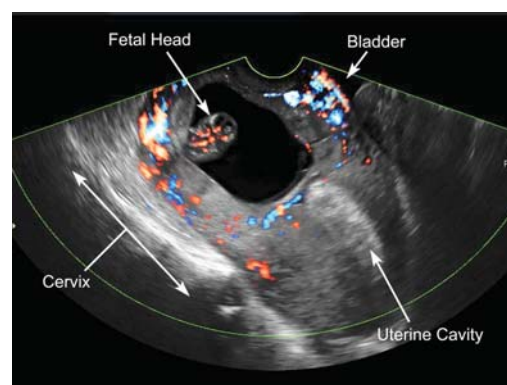
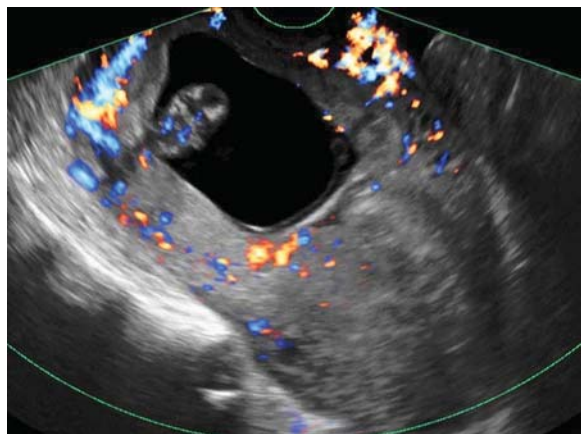


Figure 1 Transvaginal colour Doppler ultrasound, sagittal scan of the uterus, showing conceptive product occupies the whole cervix; note: vascularisation of the placental site and empty uterine corpus.

To cite: Saeng-anan U, Sreshthaputra O, Sukpan K, et al. *BMJ Case Rep* Published online: [please include Day Month Year] doi:10.1136/bcr-2013-200440



Video 1 Transvaginal color Doppler ultrasound, sagittal scan of the uterus, shows conceptive product occupies the whole cervix, Note: vascularization of the placental site and empty uterine corpus.

empty. The outline of uterine cavity and conceptive products were clearly delineated.

After proper counselling, the couples strongly requested for conservative management and the plan of management was outlined as follows. The patient was admitted to the hospital for inpatient management. Serum β -hCG was scheduled for follow-up as a standard protocol. Intrafetal potassium chloride (KCL) injection was administered under ultrasound guidance and the fetal activity disappeared immediately, followed by administration of systemic multidose regimen of methotrexate. She was haemodynamically stable, pain free, had no haemoperitoneum on ultrasound examination and had normal kidney and liver function. She received four doses of methotrexate (50 mg/m² of body surface area) given intramuscularly, alternating with four doses of oral folinic acid 5 mg daily.

Apart from mild nausea and gastritis, she tolerated the treatment well. Serum β -hCG levels slightly declined during the first, fourth and sixth day of treatment, 60 826, 53 630, and 47 498 IU/L, respectively. On the sixth day of treatment, the patient had sudden massive bleeding per vagina leading to hypovolemic shock within a short time. Fluid replacement and blood transfusion was instituted and the patient was transferred to the operative room for surgical control of bleeding, soon after

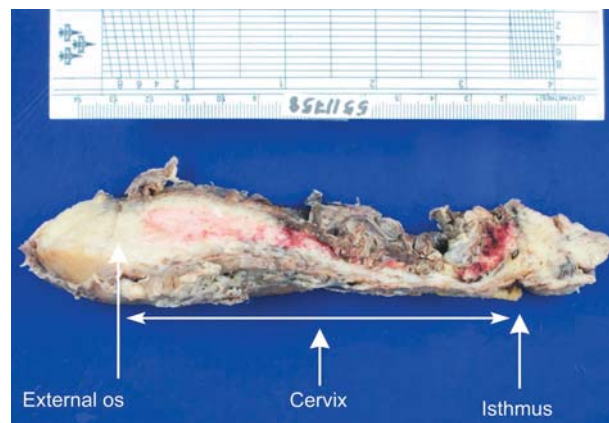


Figure 3 Longitudinal section of the cervix depicts area of the placental site with adherens in the elongated cervix (12 cm in length).

resuscitation, to maintain haemodynamics. Under general anaesthesia, conceptive product was evacuated through cervical os followed by curettage to remove the remaining placental tissue. However, active bleeding still continued throughout the procedure and finally the vital signs became unstable again, despite blood transfusion. To control the active local bleeding, Bakri SOS balloon tamponade was applied, with inflation by normal saline (200 mL), at the implantation site in the cervix. The bleeding dramatically stopped. However, due to unstable vital signs and unsure of further bleeding after the balloon removal, decision to perform total abdominal hysterectomy was made. The balloon was removed after both of the uterine arteries were secured. No significant bleeding from the cervix was observed during hysterectomy. Overall estimated blood loss was approximately 5000 mL. Postoperatively, the patient suffered from acute tubular necrosis and disseminated intravascular coagulation, a consequence of the hypovolemic shock due to massive blood loss. However, the patient gradually improved to complete recovery without any serious sequelae and was discharged on the twelfth day after operation.

Pathological examination revealed markedly enlarged cervix containing cervical pregnancy with placenta increta along circumferential cervical canal with underlying typical cervical



Figure 2 Bivalved gross specimen showing conceptive product occupies the whole cervix; note: empty uterine corpus.

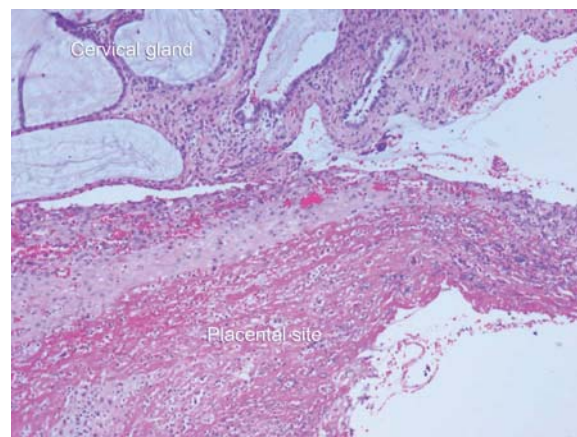


Figure 4 Microscopic findings of the cervix showing placental site with underlying connective tissue and glandular structures of the cervix.

tissue characterised by cervical connective tissue and mucin-secreting endocervical glands (figures 2–4). The cervical canal was stretched to 12 cm in length.

OUTCOME AND FOLLOW-UP

The patient was healthy without any serious sequelae on the follow-up visit at 6 weeks after operation.

DISCUSSION

Of note, the most common presenting symptom is vaginal bleeding, with previous curettage and caesarean delivery being the most common risk factors, which supports what has been reported previously in the literature.^{10–11} However, cervical pregnancy in the case presented here was asymptomatic at first visit and had no known risk factor, spontaneously occurring in the first pregnancy.

The diagnosis of cervical pregnancy in the case presented here was based on diagnostic criteria using transvaginal ultrasonography,⁵ including (1) empty uterine cavity above the internal os; (2) barrel-shaped cervix; (3) typical gestational sac was located below the level of the uterine arteries; (4) the absence of the 'sliding sign' (when pressure from the transducer is applied to the cervix, the gestational sac slides against the endocervical canal in case of imminent abortion but not in an implanted cervical pregnancy; and (5) the vascularisation around the gestation sac demonstrated by Doppler colour flow mapping.

Most cases of cervical pregnancy occur in women of reproductive life, many of whom desire to preserve fertility. In the past two decades with the advent of transvaginal sonography, which can facilitate an earlier diagnosis, conservative management is therefore more commonly practiced. A variety of approaches have been proposed,¹⁰ including dilation and evacuation combined with Foley balloon tamponade, methotrexate with/without KCL injection, uterine artery embolisation^{4–12} and ligation of cervical branches of uterine arteries. The most commonly reported medical treatments are methotrexate, either local injection (intra-amniotic or intrafetal)^{1–13–14} or systemic administration,^{2–3–7–9–15–17} commonly used in conjunction with fetal KCL injection.^{15–18} The outcomes of conservative management with methotrexate have been reported to be relatively safe and highly effective in more than 90% of cases earlier than 12 weeks of gestation,¹⁹ though the time to resolution varies largely.¹⁸ Furthermore, no evidence of adverse effects on subsequent pregnancies was shown.

Unlike reports between 1960 and 1980 in which most cases of cervical pregnancy underwent total abdominal hysterectomy,¹⁰ most recent studies have shown successful conservative treatment of cervical pregnancy without complications.^{1–2–5–9–11–13–14–20–22} In the past decade, failure cases of conservative treatment resulting catastrophic haemorrhage have been very rarely reported in literature. It is possible that the cases of failure and massive bleeding, leading to hysterectomy, might recently tend to be unreported or there is less opportunity to be published compared with those with successful treatment. Therefore, the number of fatal cases with conservative treatment may be underestimated and the effectiveness of medical treatment could be overestimated. Unlike several reports, our case signifies that cervical pregnancy could be catastrophic even in case of well-prepared, especially the cases with higher gestational age like our case. Medical treatment with methotrexate after fetal destruction with KCL is not always successful.

Owing to its large size, effectiveness in bleeding control was unlikely to be achieved with Foley catheter balloon fixation.²¹ We used Bakri SOS tamponade balloon for temporary bleeding control during total abdominal hysterectomy and such a

procedure yielded a high efficacy. The active and massive bleeding immediately stopped. To the best of our knowledge, this is the first report on controlling bleeding due to cervical pregnancy with Bakri SOS tamponade balloon. This experience suggests that Bakri SOS tamponade may be applied in conservative treatment as well, though we temporarily used it for preventing further blood loss during the switching of the procedure due to active bleeding with unstable vital signs in our case. Of note, we did not use an inflated Foley balloon catheter as a local tamponade in this case, as in previous reports, since it was expected to be too small to be effective in the markedly enlarged cervix.

While conservative management of cervical pregnancy in early gestation may be safe and effective, it seems to us that such a treatment at late first trimester or early second trimester, like our case, must be performed with extreme caution in well-prepared settings with available surgical team, anaesthetists and adequate blood components. It should be kept in mind that during the treatment of cervical pregnancy with methotrexate, catastrophic bleeding can unexpectedly occur, extreme cautions must be exercised, especially in the case of large gestational sac, like in our case. The acute massive bleeding without any warning signs in the case presented here was likely to be associated with some degree of partial separation of the placenta in response to methotrexate.

Insights gained from the case presented may be concluded as follows: (1) conservative treatment of cervical pregnancy with systemic methotrexate could be catastrophic and must be carried out with extreme precaution especially in case of higher gestational age. (2) Control of active bleeding with Bakri SOS balloon tamponade can be helpful and should be considered in case of uncontrolled bleeding especially from enlarged cervical canal, while fertility may possibly be preserved in some selected cases.

Learning points

- ▶ Ultrasound findings of cervical pregnancy in this case have been instructive, informative and inspire sonographers to alert on this problem.
- ▶ Catastrophic bleeding can suddenly occur during treatment with methotrexate.
- ▶ Bakri surgical obstetric silicone balloon tamponade can dramatically stop bleeding from large cervical pregnancy.

Acknowledgements The authors would like to thank the Thailand Research Funds for financial support.

Contributors US and TT were involved in the writing of the manuscript. US and OP were involved in the management of the patients. TT contributed in the sonographic diagnosis and autopsy was performed by KS.

Competing interests None.

Patient consent Obtained.

Provenance and peer review Not commissioned; externally peer reviewed.

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Reminder of important clinical lesson

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Comparison of cardiac troponin T and N-terminal pro-B-type natriuretic peptide (nt-proBNP) between fetuses with Hb Bart's disease and non-anemic fetuses

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Running title:

cTnT and nt-proBNP in Hb Bart's fetuses

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Funding source:

Thailand Research Fund (TRF).

Abstract:

Objective: To compare the levels of fetal serum cTnT and nt-proBNP between fetuses with Hb Bart's diseases and non-anemic fetuses.

Methods: Pregnancies at risk for fetal Hb Bart's disease scheduled for cordocentesis at 18 to 22 weeks were recruited into the study. Fetal blood was collected for cTnT, nt-proBNP, hemoglobin level and hemoglobin typing.

Results: Serum nt-proBNP was significantly higher in fetal Hb Bart's disease (24 cases) than those with unaffected fetuses (63 cases) ($P = < 0.001$), whereas cTnT was significantly lower ($P < 0.001$) in the affected group than the unaffected group. The increased serum nt-proBNP and the decreased cTnT were correlated with the degree of fetal anemia.

Conclusion: At midpregnancy, nt-proBNP in fetal Hb Bart's disease is significantly higher whereas cTnT is significantly lower than that in the non-anemic fetuses. This study suggests that cardiomegaly from fetal anemia in early gestation is not associated with fetal cardiac injury or myocardial dysfunction but represents as the cardiac volume overload from compensatory process to maintain adequate tissue oxygenation.

Keywords: fetal anemia, hemoglobin Bart's disease, homozygous alpha-thalassemia-1 disease, cardiac troponin T, N-terminal pro-B-type natriuretic peptide, cardiac marker

Introduction:

Fetal homozygous α -thalassemia-1 or hemoglobin (Hb) Bart's disease is one of the common causes of hydrops fetalis in South East Asia.¹⁻³ The hydropic changes manifested by cardiomegaly, hepatomegaly, skin edema and ascites are characteristics of this disease as a consequence of fetal anemia.⁴ To maintain adequate tissue oxygenation, the cardiac output is increased, leading to cardiomegaly. As a result, the myocardial wall is stretched due to the dilated cardiac volume overload. In addition, the hypoxic myocardial cells can be ischemic and injured. In neonates and adults case series, abnormal biochemical markers have been reported to be associated with congestive heart failure and myocardial ischemia. N-terminal pro-B-type natriuretic peptide (nt-proBNP) is a diagnostic marker used in individuals with the increased cardiac output or the cardiac volume overload while cardiac troponin T (cTnT) is a sensitive marker used in individuals with myocardial cells damage.⁵ An abnormal increase in these two markers has been found in fetal anemia caused by rhesus isoimmunization, parvovirus-B19 infection, hemorrhagic tumors and chronic feto-maternal hemorrhage^{6, 7}. Hypothetically, an increased cardiac workload and anemic-hypoxic myocardial injury secondary to fetal anemia in fetuses with Hb Bart's disease may probably increase the levels of nt-proBNP and cTnT in fetal circulation. Though, these two markers have been extensively explored in adults, the prenatal study, as markers of early congestive heart failure, has never been reported. The aim of this study was to compare the fetal serum levels of nt-proBNP and cTnT between fetuses with Hb Bart's disease and the unaffected fetuses. Furthermore, the relationship between both cardiac markers and the degree of fetal anemia was also investigated. This is the first study on cardiac biochemical markers early in gestation to identify how they changes before and impending congestive heart failure, unlike in previous studies in which the markers were obtained from cord blood after delivery and when the fetuses became obviously hydropic.

Materials and Methods:

This study was prospectively conducted at Maharaj Nakorn Chiang Mai Hospital, Department of Obstetrics and Gynecology, Faculty of Medicine, Chiang Mai University with approval of the Ethical Institutional Review Board. Pregnant women at risk of fetal Hb Bart's

disease, scheduled for cordocentesis at 18 to 22 weeks of gestation between January 2011 and July 2012 were enrolled to the study. The pregnancy at risk was diagnosed when both of the couples were α -thalassemia-1 carriers, confirmed by PCR (SEA-type) technique. Gestational age was based on early fetal biometry either by crown-rump length in the first trimester or biparietal diameter in the second trimester. Exclusion criteria included: 1) multiple pregnancies; 2) fetal anomalies or chromosomal abnormalities and 3) failure of fetal blood sampling or inadequate amount of blood sample for cTnT and nt-proBNP analysis, and contamination with maternal blood.

After informed and written consent was obtained, the demographic characteristics and medical history were recorded. A transabdominal sonographic examination was performed for fetal biometry and fetal anatomical survey. Cardiothoracic ratio (CTR), as an ultrasonographic marker, was also performed using techniques as described in the previous reports.⁸⁻¹⁰ Cordocentesis was then carried out under transabdominal real-time ultrasound guidance, using techniques as previously described.¹¹

Two millimeters of fetal blood sample was collected and tested for maternal blood contamination using a simple acid elution test. Each blood sample was divided into 3 sampling tubes and then immediately sent to the laboratories for 1) hemoglobin typing using high-performance liquid chromatography and DNA analysis (if needed) for the fetal thalassemia status; 2) hemoglobin levels and red blood cell indices analysis and 3) cTnT and nt-proBNP concentrations. Fetal serum cTnT and nt-proBNP concentrations were measured using the Elecsys® troponin T hs (high sensitive) STAT (Short Turn Around Time) and Elecsys® proBNP II on the automated Roche immunoanalyzers Elecsys® 2010 (Roche Diagnostics, Mannheim, Germany) using an electrochemiluminescence sandwich immunoassays. Fetal complete blood count was analyzed on an automated complete blood count (CBC) machine (Coulter STKS analyzer; Beckman, USA). Measured hemoglobin level was then transformed into MoM by dividing by the nomogram of Hb level in each gestational age.¹² Fetal anemia group was categorized as mild (0.84 - 0.65 MoM), moderate (0.64 - 0.55 MoM) or severe (< 0.55 MoM) anemia.¹²

Statistical analysis: All statistical analyses were performed using SPSS 17.0 (SPSS Inc, Chicago, IL). The demographic characteristics were described and analyzed to compare the difference between the affected group (Hb Bart's fetuses) and the unaffected group (normal Hb

typing or α -thalassemia-1 carrier fetuses) by the independent samples T-test. The maternal serum cTnT and nt-proBNP concentrations, CTR, PT and MCA-PSV were compared for medians between both groups using the Mann-Whitney U test. The fetal serum cTnT and nt-proBNP concentrations were compared for medians between each groups of fetal anemia using the Kruskal Wallis test. $P < 0.05$ was considered statistically significant.

Results:

One-hundred and fourteen pregnant women were recruited to the study and cordocentesis procedures were performed. Of them, 27 were excluded because cord blood samples were not enough for cTnT or nt-proBNP analysis. Of the remaining 87 cases, the fetuses affected by Hb Bart's disease and unaffected fetuses (α -thalassemia-1 carriers and normal hemoglobin typing) were identified in 24 cases (27.6%) and 63 cases (72.4%), respectively. Their baseline characteristics are shown in table 1.

Of 24 fetuses with Hb Bart's disease, 94.4% had cardiomegaly defined as the cardiothoracic ratio of more than 50%. Placentomegaly defined as the placental thickness of more than 3 cm had been found 56.3% of the affected cases and 82.4% had the high MCA-PSV of more than 1.5 multiple of median (MoM). None of the affected fetuses had diagnosed for hydrops fetalis defined as the presence of fluid within two body cavities or one cavity fluid collection in the presence of anasarca. Serum concentrations of nt-proBNP were significantly higher in fetuses affected by Hb Bart's disease than in those with unaffected fetuses ($P < 0.001$), whereas the concentrations of cTnT were significantly lower ($P < 0.001$) as presented in table 2 and figure 1.

Among 87 fetuses of analysis, fetal blood samples were not enough for complete blood count analysis in 2 cases. Of the 63 fetuses that had the diagnosis of α -thalassemia-1 carriers and normal hemoglobin typing, 93.5% had normal hemoglobin levels and 4 fetuses (6.5%) were categorized as mild anemia. In the group of Hb Bart's disease fetuses, none of them had normal hemoglobin levels and they were categorized as mild, moderate and severe anemia in 21.7%, 52.2% and 26.1%, respectively. The fetal serum cTnT and nt-proBNP concentrations were compared between each groups of fetal anemia after four mild anemic fetuses in the unaffected group were excluded as presented in table 2 and figure 2.

Table 1: Baseline characteristics of the pregnancies affected and unaffected by fetal Hb Bart's disease

Characteristics	Unaffected group (n = 63)	Affected group (n = 24)	P-value
Maternal age; yr, mean $\pm$ sd	26.2 $\pm$ 1.2	30.5 $\pm$ 1.1	0.084
GA at sampling; wk, mean $\pm$ sd	18.4 $\pm$ 0.5	18.7 $\pm$ 0.2	0.531
CTR; %, median (range)	47.5 (35.0 – 53.0)	58.0 (48.0 – 65.0)	< 0.001
HB; g/dl, median (range)	10.6 (8.70; 12.30)	6.4 (4.80; 8.90)	< 0.001
HCT; %, median (range)	32.9 (26.2 – 38.0)	24.4 (18.40; 31.00)	< 0.001
MCV; fl/cell, median (range)	112.5 (90.0 – 138.0)	97.0 (87.0 – 116.0)	< 0.001
MCH; pg/cell, median (range)	36.3 (27.2 – 47.0)	25.8 (23.4 – 37.4)	< 0.001
MCHC; g/dl, median (range)	32.2 (30.2 – 34.0)	26.4 (23.3 – 32.4)	< 0.001

Table 2: Maternal serum cardiac troponin T (cTnT) and N-terminal pro-B-type natriuretic peptide (nt-proBNP) concentrations in pregnancies affected and unaffected by fetal Hb Bart's disease and each groups of fetal anemia.

	n	cTnT	P-value	nt-proBNP	P-value
Thalassemia status	87		< 0.001		< 0.001
- Unaffected fetuses	63	354.0 (177.7 – 716.1)		1441.0 (813.8 – 4241.0)	
- Affected fetuses	24	244.5 (167.0 – 432.9)		11642.0 (2588.0 – 21124.0)	
Degree of fetal anemia	81		0.002		< 0.001
- Non-anemic fetuses	58	361.0 (208.8 – 707.0)		1462.0 (846.7 – 3341.0)	
- Mild anemia	5	320.0 (227.9 – 393.0)		6328.0 (2588.0 – 9903.0)	
- Moderate anemia	12	237.0 (172.0 – 395.0)		12606.0 (5769.0 – 21124.0)	
- Severe anemia	6	258.4 (167.0 – 432.9)		13206.5 (7151.0 – 19803.0)	

Figure 1 Boxplots of fetal serum cardiac troponin T (cTnT) and N-terminal pro-B-type natriuretic peptide (nt-proBNP) concentrations in pregnancies affected and unaffected by fetal Hb Bart's disease.

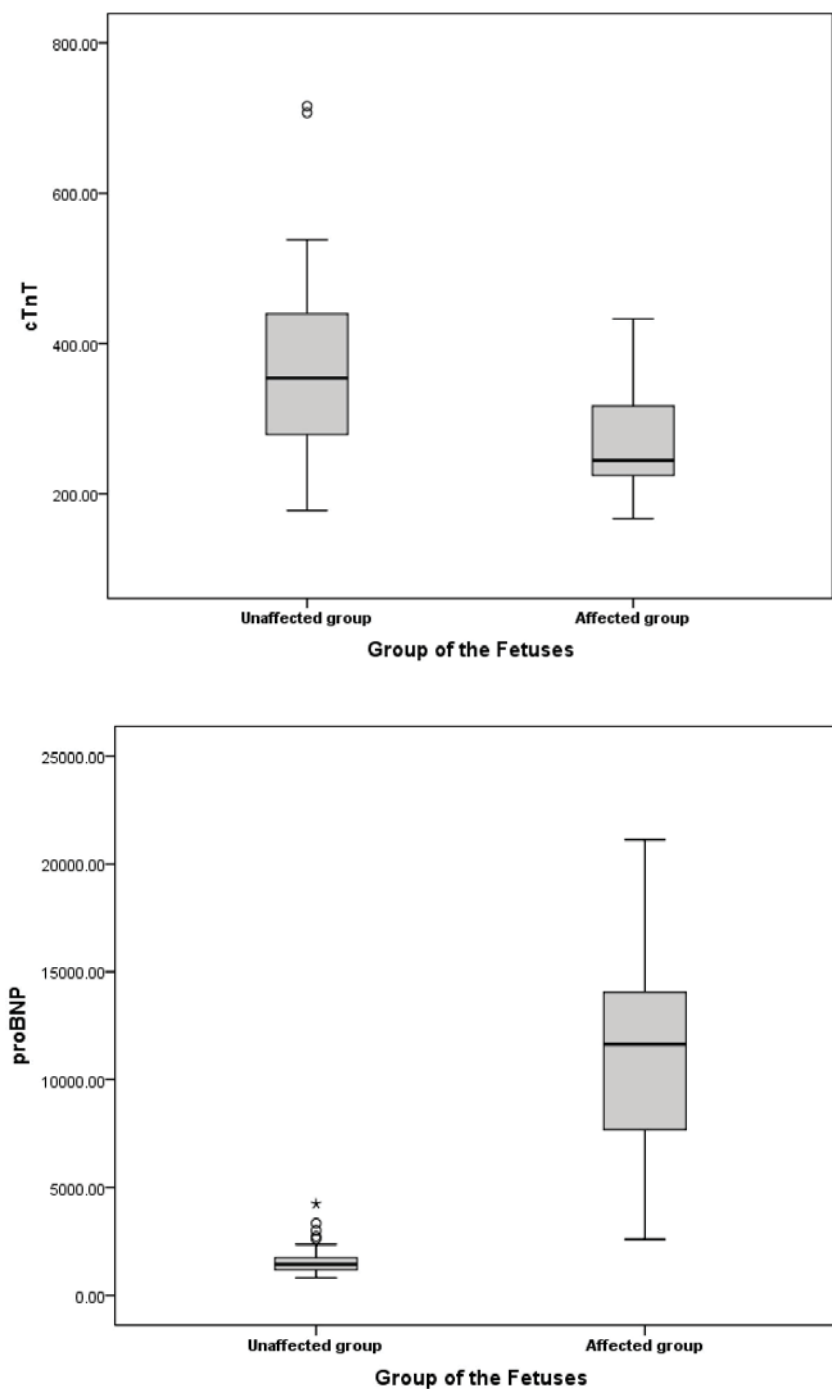
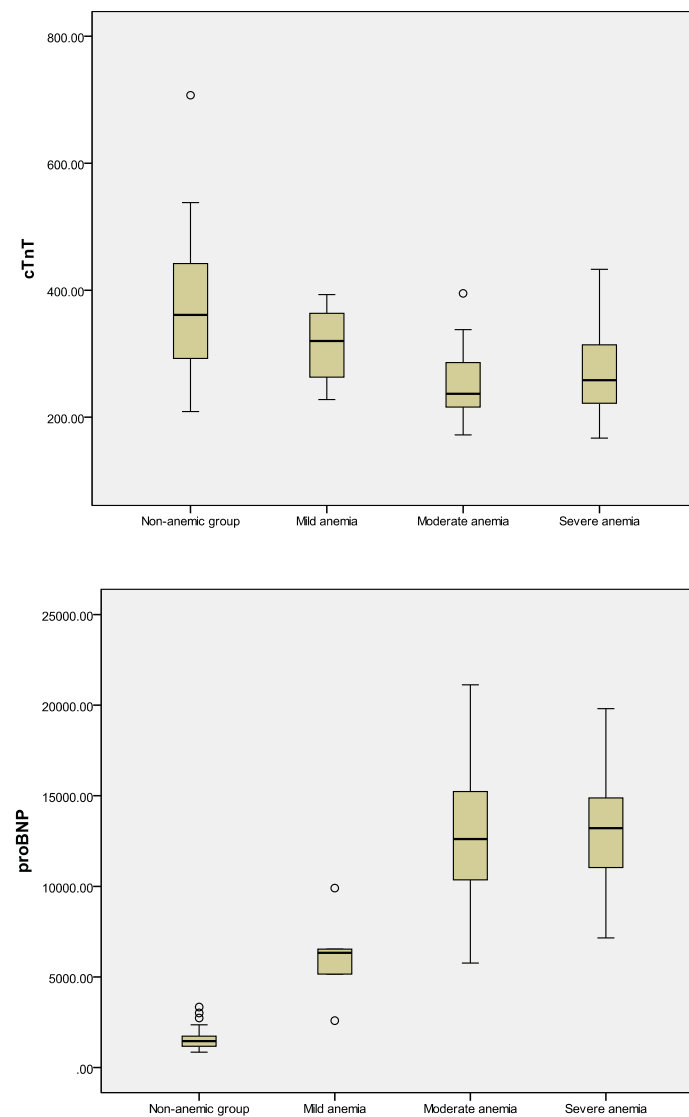


Figure 2 Boxplots of fetal serum cardiac troponin T (cTnT) and N-terminal pro-B-type natriuretic peptide (nt-proBNP) concentrations in each groups of fetal anemia.



Discussion:

Fetal hemoglobin (Hb) Bart's disease is one of the common causes of hydrops fetalis secondary to fetal anemia, particularly in South East Asia.¹⁻³ In the early state or at mid-pregnancy, the affected fetuses usually manifest by mild cardiomegaly or increased cardiothoracic ratio (CTR), thickened placenta and increased middle cerebral artery peak systolic velocity (MCA-PSV).⁸⁻¹⁰ The full manifestation of hydrops fetalis such as generalized skin edema or anasarca, pleural effusion, pericardial effusion or ascites are not commonly found in our study populations between 18 – 22 weeks of gestation. All of the fetuses with Hb Bart's disease in this study did not present any signs of hydropic changes although the low hemoglobin levels were demonstrated in all of them, mostly in the moderate to severe degree of fetal anemia. As the consequence of fetal anemia, cardiac output was compensatory increased to maintain tissue oxygenation resulting in cardiomegaly.

According to the volume overload, the cardiac chambers are dilated and myocardial wall is stretched. The natriuretic peptides, the antagonists of the renin-angiotensin-aldosterone system, are secreted in response to the fluid and electrolyte imbalance.¹³ B-type natriuretic peptide (BNP) which is mainly secreted by the ventricles into the circulation is cleaved into physiologically active BNP and the inactive N-terminal fragment; nt-proBNP.¹⁴ In adult studies, nt-proBNP has been used as the diagnostic and prognostic marker in congestive heart failure patients.^{15, 16} The increased serum concentration of nt-proBNP correlates with the prognosis of the left ventricular dysfunction.¹⁷ In fetal studies, the abnormal increase of nt-proBNP has been found in cases of ventricular wall tension caused by fetal growth restriction, fetal cardiac malformations and fetal anemia.^{5, 6, 18} In fetal anemia secondary to different conditions such as rhesus alloimmunization, parvovirus-B19 infection, hemorrhagic tumors and chronic fetomaternal hemorrhage, nt-proBNP was markedly elevated especially in cases of hydrops, and then returned to normal level after multiple intrauterine transfusions.⁶ In our study of fetal anemia specifically from Hb Bart's disease, we found the similar finding of the significantly higher serum nt-proBNP concentrations in the affected fetuses than in the controls. The rising nt-proBNP level in mild anemia group may indicate that the ventricular wall tension is increased in response to the hyperdynamic state even in early state of fetal anemia during mid-pregnancy. Moreover, the higher nt-proBNP levels in moderate and severe anemia group may imply that the

degree of fetal anemia correlates with the degree of myocardial workload or cardiac volume overload.

In cases of cardiac volume overload secondary to fetal anemia, the myocardial cells may have hypoxia, ischemia and injury from the deoxygenation process. As a result, a cardio-specific marker for myocardial damage, cardiac troponin T (cTnT) is secreted into the circulation.¹⁹ In adults, cTnT is a sensitive marker of myocardial injury in the guidelines for the diagnosis and treatment of acute myocardial infarction.²⁰ In fetuses and newborns, elevated levels of cTnT were found in nonimmune hydrops fetalis, perinatal asphyxiated infants, fetal growth restriction fetuses, neonates who receive intravenous magnesium sulfate infusion and congenital heart defects with intrauterine cardiac compromise.^{5, 21} In a study of fetal anemia from rhesus alloimmunization, fetal blood cTnT concentration was inversely correlated to hemoglobin level before intrauterine transfusion.⁷ The perinatal death was higher in fetuses with abnormally elevated cTnT than those who had normal cTnT.⁷ However, the characteristics of the populations in that report were different from this study. They studied in pregnant women complicated by rhesus alloimmunization who underwent intrauterine transfusion with the mean gestational age of 28.1 weeks, the mean fetal hemoglobin at first intrauterine transfusion of 6.8 g/dL and the 15% rate of fetal hydrops. Although the similar degree of fetal anemia in the affected cases (the median hemoglobin level = 6.45 g/dL) was demonstrated, the mean gestational age in our study was 18.69 weeks and none showed hydropic changes.

Unlike the other previous studies, our result showed discordant pattern of cTnT and nt-ProBNP. Nearly all affected fetuses in this study had cardiomegaly as well as markedly elevated nt-ProBNP, but they had low cTnT concentrations. Thus, our observation signifies that fetuses with Hb Bart's disease at mid-pregnancy show well-compensated cardiomegaly to keep tissue oxygenation without ischemic myocardial damage. Together with well-established knowledge on cTnT as a marker of congestive heart failure, this study gives insights that the two biochemical cardiac markers represent two phases of cardiac adaptation to anemia. In the first phase, elevated nt-ProBNP level represents volume overload or hyperdynamic stage accompanying with stretched myocardium, while the second phase of an elevated cTnT level represents the phase of myocardial damage or true congestive cardiac failure. Our observations may be clinically significant since these two biochemical markers may be potential markers implying the prognosis and providing options in management, either blood transfusion in utero or termination of pregnancy

or expectant management. Though fetuses with Hb Bart's disease are always lethal and the markers are unlikely to be helpful in clinical application, they may be useful in management of fetal anemia or hydrops fetalis due to non-lethal causes such as Rh isoimmunization or parvovirus B19 infection and etc. Nevertheless further investigations are needed to elucidate this issue.

Unfortunately, we did not evaluate fetal venous Doppler as a cardiac function in this study. Nevertheless, our previous studies(XX) showed that in early phase of fetal anemia in fetuses with Hb Bart's disease at mid-pregnancy, like in this study, were not associated with abnormal venous Doppler indicating increased preload in early gestation. Therefore, our new findings are consistent with previous evidence of no cardiac compromise at this stage of fetal anemia. In contrast, in late gestation, Nomura et al(X) have demonstrated that fetal cTnT obtained during cesarean section was significantly correlated with abnormal venous Doppler, representing severe cardiac compromise with increased systemic venous pressure.

The strengths of this study were the adequate number of affected cases and homogeneity of the specific cause of fetal anemia at the exact pregnancy period. Fetal hemoglobin levels were also categorized according to the degree of fetal anemia in the affected group and to exclude fetal anemia cases in the unaffected group. Therefore, the findings of this study can be applied to any causes of fetal anemia other than Hb Bart's disease. However, fetal cardiac function or fetal blood volume were not assessed which was the limitation of this study.

Conclusion:

At midpregnancy, nt-proBNP in fetal Hb Bart's disease is significantly higher whereas cTnT is significantly lower than that in the non-anemic fetuses. This study suggests that cardiomegaly from fetal anemia in early gestation is not associated with fetal cardiac injury or myocardial dysfunction but represents as the cardiac volume overload from compensatory process to maintain adequate tissue oxygenation.

Acknowledgement:

The authors wish to thank the Thailand Research Fund (TRF) for financial support in the conduct of this research.

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Prenatal ultrasound evaluation of Fetal Hb Bart's Disease among Pregnancies at Risk at 11-14 Weeks of Gestation

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Running title: First trimester diagnosis of Hb Bart's disease

Words count = 2045, **Table count** = 2, **Figure count** = 6

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Funding: Thailand Research Fund (TRF)

Conflict of Interest: None

What's already known about this topic?

Sonomarkers at mid-pregnancy can effectively predict Hb Bart's disease among fetuses at risk

What does this study add?

CT ratio plus MCA-PSV can effectively differentiate fetuses with HbBart's disease from unaffected pregnancies at 11-14 weeks of gestation.

Prenatal ultrasound evaluation of Fetal Hb Bart's Disease among Pregnancies at Risk at 11-14 Weeks of Gestation

Abstract

Objective: To evaluate efficacy of the first trimester sonomarkers (11-14 weeks) in predicting Hb Bart's disease among fetuses at risk

Materials and Methods: Prospective analysis was conducted on pregnancies at risk of fetal Hb Bart's disease at 11-14 weeks of gestation. Sonographic markers including cardiothoracic ratio (CT) ratio, middle cerebral artery peak systolic velocity (MCA-PSV), placental thickness and nuchal translucency were prospectively assessed and recorded. The definite diagnosis of fetal Hb Bart's disease was based on DNA analysis (CVS), or subsequent fetal Hb typing (HPLC; cordocentesis).

Results: Among 104 pregnancies at risk with complete sonographic assesement at 11-14 weeks of gestation, 30 fetuses were finally proven to be affected. The cardiothoracic ratio gave the highest sensitivity, 93.3%, with specificity of 93.2%, followed by placental thickness and MCV-PSV respectively. NT had a very low sensitivity of 16.7 %. The combination of CT ratio and MCA-PSV increased the sensitivity to be 96.7% but somewhat compromise specificity.

Conclusions: At 11-14 weeks of gestation, sonographic markers can effectively differentiate affected from unaffected pregnancies. The most sensitive marker was CT ratio plus MCA-PSV. Of couples at risk with no any sonographic markers, the risk of having an affected fetus is nearly eliminated.

Key words: cardiothoracic ratio; middle cerebral artery peak systolic velocity; first trimester; hemoglobin Bart disease; hydrops fetalis; thalassemia; nuchal translucency

Introduction

Reliable early detection of Hb Bart's disease before development of hydrops fetalis is essential, since they are inevitably either stillborn or die soon after birth and early pregnancy termination can prevent serious obstetric complications, including preeclampsia, dystocia, postpartum hemorrhage due to a large placenta, and the psychological burden of carrying a nonviable fetus to term. Therefore, the ability to make early prenatal diagnosis is essential and offer of pregnancy termination to the couples is ethically justified.

Traditionally, clinicians have relied on invasive methods to detect fetal anemia either indirectly by measurement of amniotic fluid bilirubin levels or cordocentesis. Both methods pose a risk to the fetus. Amniocentesis is related to 0.5-1% fetal loss (1;2) and cordocentesis is associated with 1.4-2.7% fetal loss (3;4). As a screening tool, ultrasound examination could prevent these complications by decreasing the number of invasive procedures required in the management of fetal anemia. Therefore, several attempts have been made to find any other tests enabling us to make the diagnosis earlier. Our extensive experience suggests that, not only sonomarkers like cardio-thoracic (CT) ratio(5;6) or middle cerebral artery peak systolic velocity (MCA-PSV)(7) could effectively predict such a fetus by mid-pregnancy, they are also likely to be useful for such purpose even in late first trimester. Hemodynamic changes caused by Hb Bart's disease can obviously appear as early as 12-13 weeks' gestation (8-11). However, to date, there have been only a limited number of studies on effectiveness of these sonographic markers in differentiating the affected and unaffected fetuses in the first trimester.

We postulate that such hemodynamic alterations can probably be sonographically assessed in early gestation. Therefore, we conducted this study to evaluate the efficacy of the first trimester sonomarkers (11-14 weeks) in predicting fetal Hb Bart's disease among fetuses at risk. These sonographic markers include: cardiothoracic ratio, middle cerebral artery peak systolic velocity (MCA-PSV), placental thickness and nuchal translucency.

Materials and Methods

A prospective descriptive analysis (diagnostic test) was conducted between September 2010 and December 2012 at Maharaj Nakorn Chiang Mai hospital, with approval of the ethical institute review boards. The study patients were recruited by our program to control severe thalassemia (12), consisting of a screening program to identify carriers and pregnancies at risk, prenatal diagnosis, and pregnancy termination for the affected fetuses. The pregnancies were recruited to the study with written informed consent and met the following inclusion criteria: 1) fetuses at risk of Hb Bart's disease (both of the couple were a carrier of alpha-thalassemia-1), 2) gestational age of 11-14 weeks, based on crown-rump length measurement, 3) singleton pregnancies, and 4) fetal diagnosis of Hb Bart's disease. Exclusion criteria consisted of 1) fetal structural anomaly as well as fetal anemia secondary to any other causes, 2) poor quality of ultrasonographic images, especially in case of thickened maternal abdominal wall or poor fetal posture, etc, and 3) loss to follow-up or the final diagnosis could not be made.

All pregnancies underwent sonographic examination for fetal biometry and sonographic markers of fetal Hb Bart's disease. Transabdominal sonographic (TAS) examinations with ProSound SSD-5000 scanners (Aloka Co, Ltd, Tokyo, Japan) or Voluson E8 (GE Medical Systems, Zipf, Austria), having a transducer frequency of 3.5 MHz were performed by the authors before definite diagnoses via CVS or cordocentesis was known. If good quality image could not be obtained, transvaginal sonography (TVS) with a 5 MHz transducer was then performed with patient's permission, or repeated TAS in several hours later for the better fetal position. The sonographic data were prospectively recorded for subsequent analysis. Sonographic markers including cardiothoracic ratio (CT) ratio, middle cerebral artery peak systolic velocity (MCA-PSV), placental thickness and nuchal translucency were assessed and recorded. The techniques of measurements were described elsewhere (6;7;13). The definitions used in this study were as follows: 1) **Fetuses at risk:** Fetuses of the couple who both were carriers of alpha-thalassemia-1 gene, diagnosed by PCR (SEA type), 2) **Fetuses with Hb Bart's disease:** Any fetus who have Hb Bart's diseases diagnosed by DNA analysis (via chorionic villous sampling or amniocentesis) or by cord blood analysis (via cordocentesis) using HPLC, 3) **Normal fetus:** Any fetus who have normal or trait genotype of alpha-thalassemia1 diagnosed by DNA analysis (via chorionic villous sampling or

amniocentesis) or cord blood analysis using HPLC, 4) **Abnormal MCA-PSV**: The MCA-PSV of greater than 1.5 MoM of each gestational age (**Figure 1**), 5) **Nuchal translucency (NT)**: The maximum thickness of the subcutaneous translucency between the skin and the soft tissue overlying the cervical spine measured by standard technique described previously, 6) **Cardio-thoracic (CT) ratio (Figure 1)**: The ratio of a diameter of the heart (outer edge to outer edge) measured at the level of the atrioventricular valves during the end diastole, to a transverse thoracic diameter measured on the same image (outer edge to outer edge of the ribs). 7) **Hydrops fetalis**: A fetus at risk who had one or more of the following sonographic signs: subcutaneous edema, pericardial effusion, pleural effusion, or ascites.

Statistical analysis: The stored data were analyzed for the efficacy of each sonographic marker in predicting fetal Hb Bart's diseases, using the statistical package for the social sciences (SPSS) version 17.0 (Chicago, IL). The measurement outcomes were presented as sensitivity, specificity, positive and negative predictive value. Based on previous studies of MCA-PSV and cardio-thoracic ratio in early second trimester(14), sensitivity of ultrasound markers in late first trimester was expected to be approximately 94%. At a maximum all allowable error was 0.1, a 10% of precision, at 95% confidence level, the study needed a sample size of at least 21 affected fetuses.

Results

Among 111 pregnancies at risk of fetal Hb Bart's disease, 104 fetuses had a successful sonographic assessment at 11-14 weeks of gestation and available for analysis. Seven cases, which were in 11-12 weeks of gestation, were excluded because of inability to obtain good quality images. Of the remainders, 30 fetuses were finally proven to be affected. The distribution of gestational age is presented in **Table 1**. Demographic data of all women were as follows; mean ($\pm$ SD) maternal age was 28.23 ± 4.68 years, 63 (60.6%) were nulliparous, and the mean ($\pm$ SD) gestational age at ultrasound examination was 12.79 ± 1.002 weeks.

The diagnostic indices of each sonomarkers were presented in **Table 2**. Of the four single sonomarkers, cardiothoracic (CT) ratio gave the highest accuracy with a sensitivity of 93.3%, with specificity of 93.2%, followed by placental thickness, MCV-PSV, and NT respectively.

Based on ROC curves, the best cut-off point of CT ratio was 48 % (area under curve 0.963; 95% CI: 0.927-0.999), as presented in **Figure 2** and that of placental thickness was 18

mm, gave a sensitivity of 70% and specificity of 63.2% (area under curve 0.721; 95%CI : 0.605-0.839) as shown in **Figure 3**.

When combined CT ratio with placental thickness, the two components did not improve the sensitivity in prediction, either considered abnormal when both are abnormal or one of them is abnormal.

The combination of CT ratio and MCA-PSV, considered abnormal when either MCA-PSV or CT ratio was abnormal, increased the sensitivity to be 96.7% with minimal compromise of the specificity. However, if the combination was considered abnormal when both MCA-PSV and CT ratio were abnormal the sensitivity was decreased to be only 53.3%.

NT (nuchal translucency) had a low sensitivity of 16.7 %. Only 5 out of 30 affected cases showed thickened NT, based on 95th percentile of our nomogram.

The combination of the four sonomarkers, considered positive if either of them was positive and negative if all were negative, had a high negative predictive value (97.4%) but low sensitivity (50.0%).

Of all, three fetuses had hydrops fetalis (ascites or pleural effusion) and all were affected by Hb Bart's disease, as well as increased CT ratio. Notably all fetuses with hydrops fetalis showed normal NT measurements.

Of all, 55 fetuses underwent CVS at 12-14 weeks and 33 had cordocentesis at 17-22 weeks of gestation. The remaining 16 cases opted to have serial ultrasound and finally were proven to be unaffected. All of latter group had no any sonomarkers.

Figure 1 An example of normal and increased CT ratio at 12 weeks (a) and 11 weeks (b), and normal MCA-PSV(c) and increased MCA-PSV (d) at 12 weeks of gestation (*CT: cardio-thoracic; MCA-PSV: peak systolic velocity of the middle cerebral artery, SP: spine*)

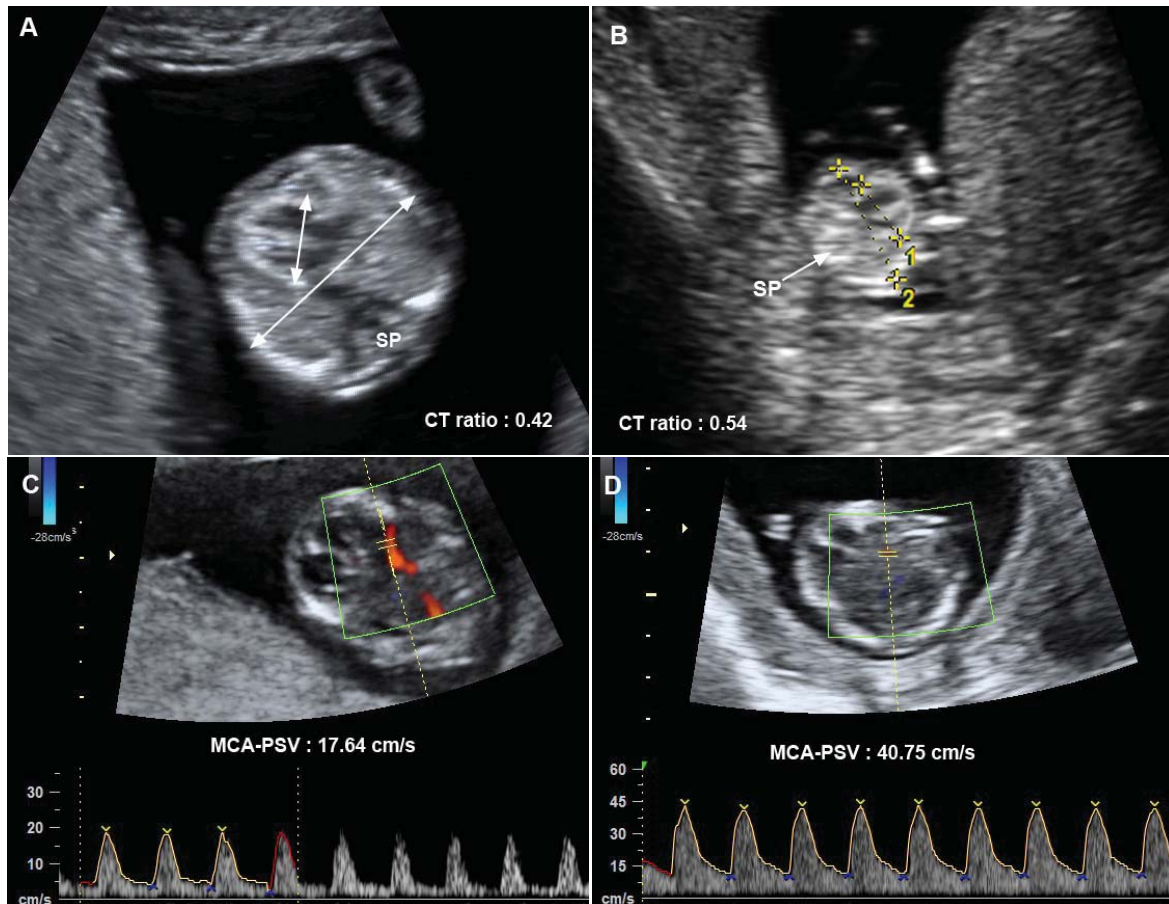


Table 1 Distribution of the number of cases at various gestational weeks

Gestational Weeks	Number (%) of affected fetuses	Number (%) of unaffected fetuses	Total (%)
11	3 (10.0%)	7 (9.5%)	10 (9.6%)
12	13 (43.3%)	22 (29.7%)	35 (33.7%)
13	6 (20.0%)	20 (27.0%)	26 (25.0%)
14	8 (26.7%)	25 (33.8%)	33 (31.7%)
Total	30 (100.0%)	74 (100.0%)	104 (100.0%)

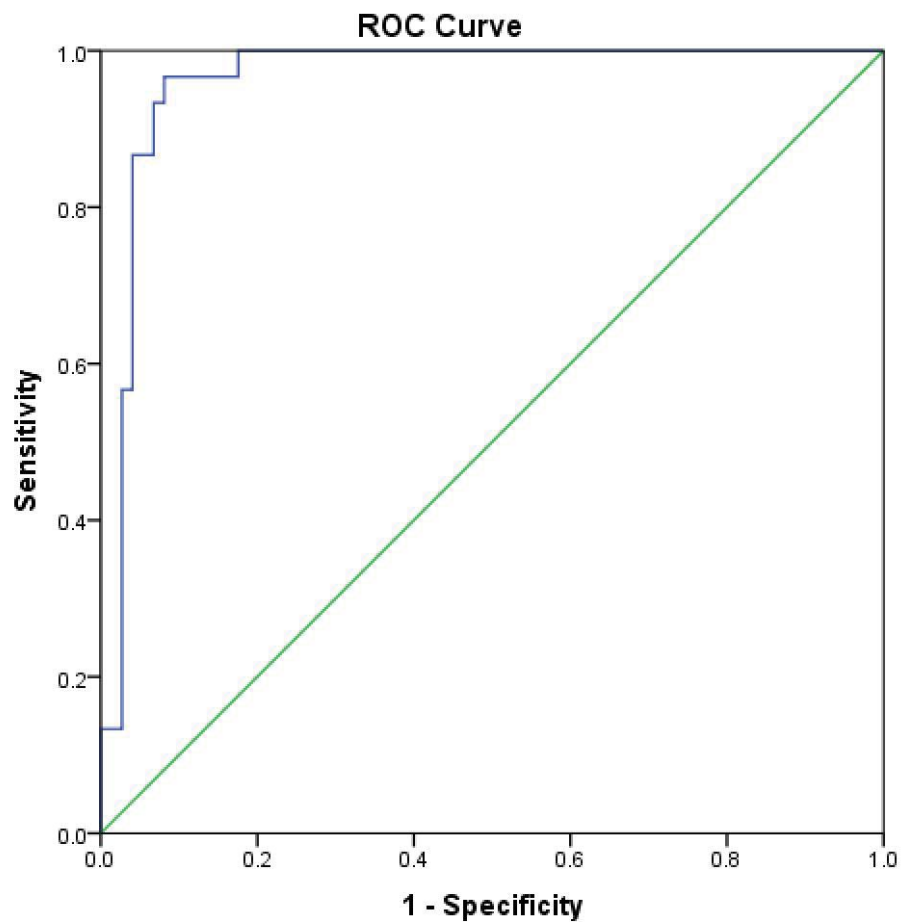
Figure 2 ROC curve of cardio-thoracic ratio in predicting Hb Bart's disease at 11-14 weeks of gestation (the best cut-off point : 0.478, area under curve 0.963; 95%CI : 0.927-0.999)

Figure 3 ROC curve of placental thickness in predicting Hb Bart's disease at 11-14 weeks of gestation (the best cut-off point : 1.8 cms, area under curve 0.721; 95%CI : 0.605-0.839)

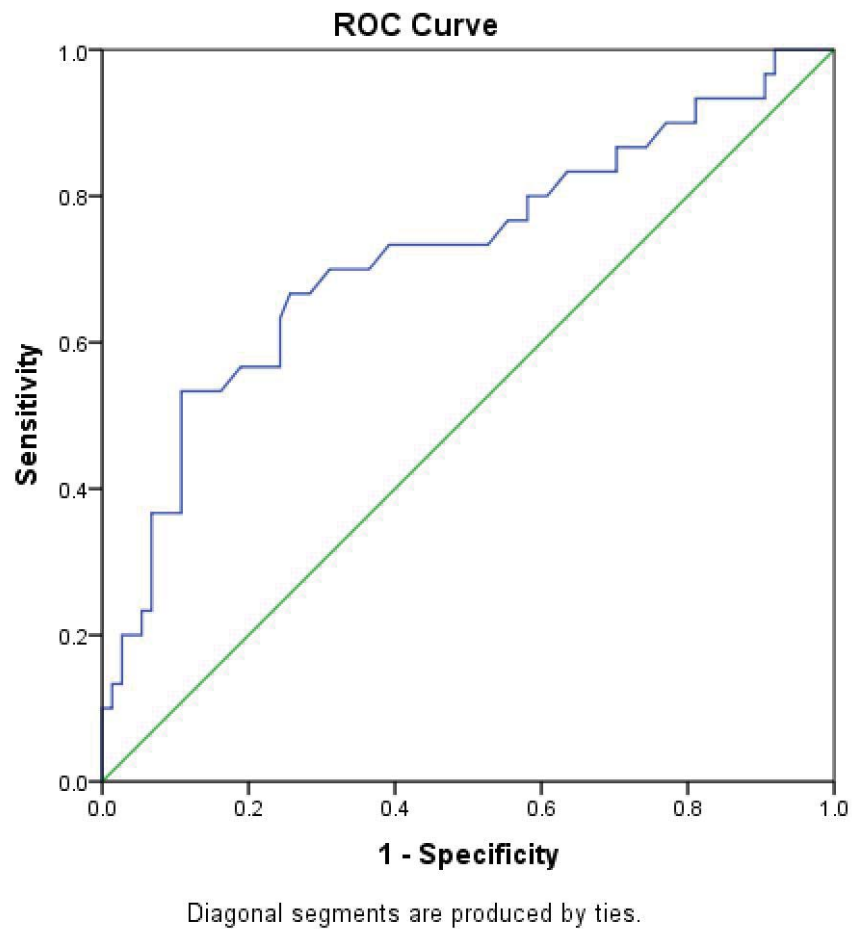


Table 2 Sensitivity and specificity of various sonographic markers in predicting Hb Bart's disease at 11-14 weeks of gestation

Sonographic Markers (cut-off points)	Affected (n = 30)	Unaffected (n = 74)	Sensitivity, % (95% CI)	Specivicity, % (95% CI)	Positive predictive, value % (95% CI)	Negative predictive, value % (95% CI)
Thickened nuchal translucency; NT (95 th percentile)	5	1	16.7 (3.3-30.0)	98.6 (96.0-100.0)	83.3 (53.5-100.0)	74.5 (39.6-100.0)
Increased cardio-thoracic thoracic ratio; CT (48%)	28	69	93.3 (84.4-100.0)	93.2 (87.5-99.0)	84.8 (72.6-97.1)	97.2 (91.5-100.0)
Increased placental thickness (1.8 cm)	21	47	70.0 (53.6-86.4)	63.5 (52.5-74.5)	43.8 (29.7-57.8)	83.9 (73.5-94.3)
Increased MCA-PSV (1.5 MoM)	17	59	56.7 (38.9-74.4)	79.7 (70.6-88.9)	53.1 (35.8-70.4)	81.9 (68.6-95.3)
Increased CT ratio OR Increased placental thickness	28	45	93.3 (84.4-100.0)	60.8 (49.7-71.9)	49.1 (36.1-62.1)	95.7 (90.5-100.0)
Increased CT ratio AND Increased placental thickness	21	71	70.0 (53.6-84.6)	95.9 (91.5-100.0)	87.5 (74.3-100.0)	88.8 (76.1-100.0)
Increased CT ratio OR Increased MCA-PSV	29	55	96.7 (90.2-100.0)	74.3 (64.4-84.3)	60.4 (46.6-74.3)	98.2 (94.5-100.0)
Increased CT ratio AND Increased MCA-PSV	16	73	53.3 (35.5-71.2)	98.6 (96.0-100.0)	94.1 (82.9-100.0)	83.9 (66.4-100.0)
Increased CT ratio OR increased NT OR increased MCA-PSV OR increased Placental thickness	37	29	96.7 (90.2-100.0)	50.0 (38.6-61.4)	43.9 (32.0-55.9)	97.4 (93.5-100.0)

Discussion

This study showed that sonomarkers at 11-14 weeks of gestation could effectively differentiate affected from unaffected pregnancies. The most sensitive single marker was CT ratio. In combination of CT ratio and MCA-PSV, it could increase sensitivity but decreased specificity if considered abnormal when either was abnormal whereas it decreased sensitivity and increased specificity when both were considered abnormal. These findings may be clinically useful. If both are abnormal, early invasive diagnosis should strongly be considered, while only MCA-PSV is abnormal in the first trimester, serial ultrasound may be preferable because of its less specificity. Of the couples at risk without any sonographic markers, the risk of having an affected fetus was nearly eliminated. Our data suggest that in the area of high prevalence of Hb Bart's disease, especially in the couples at risk, when NT measurement is performed in late first trimester, the examiners should also be encouraged to assess the sonographic markers for the disease.

Though we have extensively explored in effectiveness of sonomarkers of fetal Hb Bart's disease during mid-pregnancy (5-7;15), their effectiveness at 11-14 weeks' gestation has never been thoroughly evaluated. Leung KY et al(14) conducted a large study in assessment of sonomarkers in predicting fetal Hb Bart's disease, from 12-20 weeks of gestation. They also included a large number of patients at 12-15 weeks of gestations and their study suggested that adding MCA-PSV to CT ratio in the prediction of Hb Bart's disease can increase the sensitivity at 12-15 weeks. The overall effectiveness of sonomarkers in our study might not be as good as that in Leung's Study. This may be due to the fact that we included fetuses in earlier gestational age and the two studies cannot be perfectly compared because of the different baseline characteristics. The main differences between our study and Leung's one were as follows: this study included cases in earlier gestation, 11-14 weeks vs 12-15 weeks, in our study, MCA-PSV measurements were prospectively performed but retrospective analysis in Leung's study, and we also included NT as a sonomarker of fetal Hb Bart's disease. Additionally, they studied in a long timeframe from 1995-2006 while we collected cases in only three years of study. Like in Leung's study, CT ratio was the best single parameter in predicting Hb Bart's disease but the best cut-off level was different. The cut-off of 48% based on ROC curve was the best in our study but 50% in Leung's study. This

was likely to be the earlier gestational age in our study since CT ratio is typically greater with advanced gestational age(16).

While CT ratio in our study was somewhat less accurate than that in Leung's study, MCA-PSV in our study had a higher sensitivity (56.7% vs 17.6%). Nevertheless, MCA-PSV was relatively not sensitive when compared to that performed at mid-pregnancy(7). This finding signified that fetal anemia in fetal Hb Bart's disease might not be obvious until mid-pregnancy. However, when combined with CT ratio, MCA-PSV could increase the sensitivity of CT ratio from 93.3% to 96.7%. This implied that some affected fetuses with normal CT ratio may demonstrate an abnormal MCA-PSV. Therefore MCA-PSV measurement is helpful in predicting fetal Hb Bart's disease. In contrast, placental thickness did not add sensitivity to CT ratio. This is due to the fact that nearly all cases of affected fetuses with thickened placenta also had an increased CT ratio or cardiomegaly, leading to no additional diagnostic values.

Cardiac dysfunction may be implicated in fetal Hb Bart's disease as early as late first trimester or early second trimester. Theoretically, it is possible that thickened NT as seen in other fetal cardiac abnormalities may probably be observed in these fetuses. Moreover, the heterogeneity of conditions associated with increased nuchal translucency suggests that there may not be a single underlying mechanism for the collection of fluid in the skin of the fetal neck. Early anemia and hypoproteinemia as seen in Hb Bart's disease may also be a possible mechanism. Nevertheless our findings did not support our hypothesis. Based on our findings, though the percentage of affected cases with thickened NT was significantly higher than that in the unaffected cases, its sensitivity was very low (16.7%) and was unlikely to be helpful in clinical practice. Such a finding was consistent with that reported by Lam et al(17). All three fetuses with hydrops fetalis showed cardiomegaly but normal NT measurement. This finding indicated that the hydropic changes or cardiomegaly in fetal anemia might not be mainly associated with pathophysiology of thickened NT, though it has already known that fetal cardiac abnormalities are usually related to thickened NT(18).

The strength of this study may include the following aspects. 1) The sample size was large enough to draw the conclusion. 2) the participants were highly homogeneous, focusing on late first trimester, the best time for screening nuchal translucency in most cases. Nevertheless, a small sample size at 11 weeks of gestation is a limitation of this study.

Because of high efficacy of sonographic signs in differentiating affected and unaffected fetuses in late first trimester, this may help us identify hemoglobin Bart's disease among the fetuses at risk in earlier gestation than before. Furthermore, this study may possibly enable investigators and clinicians to better understand the patho-physiological processes involved in developing hydrops fetalis secondary to fetal anemia and to plan appropriate treatment or interventions.

Acknowledgement

The authors wish to thank the Thailand Research Fund for financial support.

What's already known about this topic?

Sonomarkers at mid-pregnancy can effectively predict Hb Bart's disease among fetuses at risk

What does this study add?

CT ratio plus MCA-PSV can effectively differentiate fetuses with HbBart's disease from unaffected pregnancies at 11-14 weeks of gestation.

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Fetal Inferior Vena Cava Doppler Indices in Low Output and High Output Hydrops Fetalis

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Words count = 2273 (excluding abstract & references)

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Short title: IVC Doppler in Hydrops Fetalis

Key Words: Doppler velocity, Heart failure, Hydrops fetalis, Inferior vena cava, Prenatal Diagnosis, Ultrasound

Abstract

Aim: To compare fetal inferior vena cava (IVC) Doppler indices of fetuses between hydropic fetuses with low cardiac output and those with high cardiac output.

Methods: This prospective cross-sectional analysis included fetuses with hydrops fetalis secondary to congenital heart defects (low output group) and anemia (high output group). The recruited fetuses underwent ultrasound and IVC Doppler indices were measured. The cases of unsatisfactory measurements were excluded. The indices were compared to those of normal reference range and were also compared between both groups.

Results: Sixty-nine hydropic fetuses had satisfactory measurements, 51 in the high output group (fetal anemia secondary to hemoglobin Bart's disease), and 18 in the low output group. Compared to normal reference ranges, the preload index, peak velocity index, and the pulsatility index were significantly lower in the high output group ($p < 0.001$) whereas they were significantly higher in the low output group ($p < 0.001$).

Conclusions: Fetuses with high output hydrops fetalis did not show high preload, peak velocity index and pulsatility index for IVC, suggesting that congestive heart failure is not a primary mechanism for development of hydrops fetalis secondary to anemia. In the fetuses with low cardiac output, cardiac decompensation was a primary cause of hydrops fetalis.

Introduction

Hydrops fetalis refers to a condition characterized by pathologically increased fluid accumulations in fetal soft tissues and serous cavities. It can be caused by a wide variety of disorders. Based on the largest systematic review by Bellini et al(1), congenital heart defect is a major cause of hydrops fetalis, accounting for 21.7%. However, in Southeast Asia hemoglobin Bart's disease is responsible for majority of cases(2). In chromosomally normal fetuses, low cardiac output (congenital heart defects) and high cardiac output (anemia) hydrops fetalis account for the majority of cases(1). The prognosis, though overall is poor, is crucially dependent on the etiology, which has to be identified for proper management, assessment of prognosis, and counseling of the parents. Pathophysiology of hydrops fetalis is controversial. The most common postulated mechanism is cardiac failure secondary to either cardiac defects (low cardiac output) or fetal anemia (high cardiac output). It is believed that an increase in central venous pressure secondary to cardiac decompensation results in hydrops fetalis(3). In fetal anemia, increased cardiac output is believed to be due to a decrease in blood viscosity which, in turn, leads to increased venous return and cardiac preload resulting in cardiac failure and finally hydrops fetalis. However, though cardiomegaly is considered as a universal sign of cardiac failure as believed in adult heart, we have often observed a normal cardiac function in fetuses with frank hydrops fetalis due to hemoglobin Bart's disease, in spite of marked cardiomegaly. Additionally, many of these fetuses show reactive non-stress test, raising a question whether cardiac failure is a primary cause of hydrops fetalis due to anemia or just a consequence in the end stage of hypoxia.

Inferior vena cava (IVC) Doppler changes, which directly reflect cardiac function, may be informative for pathophysiology of hydrops fetalis. Therefore, this study focused on IVC Doppler waveforms representing central venous pressure and cardiac function, in hydrops fetalis due to congenital cardiac defects (low cardiac output) and hydrops fetalis due to fetal anemia (high cardiac output). Though several studies on arterial Doppler changes in fetal anemia have been published, to our best knowledge, there has been no study on IVC Doppler indices in differentiating hydrops fetalis due to cardiac cause from that secondary to anemia. The knowledge of IVC Doppler or central venous pressure changes may be helpful in predicting prognosis or making a choice of management. The purpose of this study was to compare fetal IVC Doppler indices in fetuses with hydrops fetalis between a group of high cardiac output (fetal anemia) and a group of low cardiac output (congenital heart defects).

Materials and Methods

A prospective cross-sectional study was conducted at Maharaj Nakorn Chiang Mai Hospital, Thailand, during the year 2006 and 2009. Pregnancies with hydrops fetalis were included to the study with informed written consent. The inclusion criteria were: 1) singleton pregnancies, and 2) hydrops fetalis was proven to be caused by either hemoglobin Bart's disease or congenital heart defects. The prenatal diagnosis of hydrops fetalis was defined by the presence of fluid collections in at least two fetal body compartments such as ascites, pleural effusion, pericardial effusion or skin edema. When hydrops fetalis was diagnosed, detailed ultrasound was thoroughly performed to identify the causes and possible associated anomalies. Fetuses with hydrops fetalis secondary to other causes such as venous / lymphatic obstruction or cystic hygroma were excluded from analysis. The diagnosis of Hb Bart's disease was based on hemoglobin typing of fetal cord blood whereas the diagnosis of congenital cardiac defects was made with detailed fetal echocardiography. Ultrasound examinations were performed using real time high-resolution machines, either Aloka alpha-10 (Tokyo, Japan), or Voluson E8 (GE Healthcare, USA). The maximal ultrasound intensity was set at 100mV/cm^2 spatial peak temporal average and the high frequency filter set at 125 Hz to remove signals from slow moving tissues. In addition to standard ultrasound and fetal echocardiography, IVC Doppler waveforms were assessed and recorded. The IVC Doppler indices were obtained by placing the sample volume over the inferior vena cava immediately distal to its widening and entrance into the right atrium, usually located between the diaphragm and the renal veins. The size of the sample volume was adapted to the IVC diameter to cover it entirely. All measurements were obtained in the absence of fetal breathing movements, and the angle between the ultrasound beam and the direction of blood flow was adjusted to be less than 30 degree. Doppler velocity focused primarily on the relationships among the three velocity components including the peak forward velocity during systole (S), peak forward velocity during diastole (D), the lowest forward velocity or peak reversed velocity during atrial contraction (a), and the time average maximum velocity (Tamx). The preload index (PLI; a/S), peak velocity for veins (PVIV; $S-a/D$), and the pulsatility index for vein (PIV; $S-a/\text{Tamx}$) were automatically calculated and recorded during the measurement. In case of more one ultrasound examinations were performed, only the last examination was used for analysis. The IVC Doppler indices derived from the study were correlated to normal reference ranges of IVC Doppler indices constructed by Baschat et al(4). The IVC Doppler indices of the two groups were compared.

Statistical analysis was performed with SPSS version 17 for Windows (Chicago, IL). Student t test or Mann-Whitney U test were used for the comparison of continuous variables. The Kruskal-Wallis nonparametric test was used to evaluate trends in z-score or mean difference. Comparison of proportion was performed with chi-square or Fisher's exact test where appropriate. $P < 0.05$ was considered statistically significant.

Results

Sixty-nine fetuses with hydrops fetalis were recruited and successfully recorded for IVC spectral Doppler waveforms. They were categorized into two groups; 1) a group of high output hydrops fetalis, consisting of 51 fetuses with hemoglobin Bart's disease, and 2) a group of low output hydrops fetalis, consisting of 18 hydropic fetuses with congenital heart defect. The causes of hydrops fetalis are summarized and presented in [Table 1](#). The mean gestational age ($\pm$) of all fetuses was 26.26 ± 5.1 weeks (range 17 to 36). Mean of gestational age for the group of high output and the group of low output were not significantly different (27.61 ± 5.2 weeks and 25.78 ± 3.5 weeks, respectively, Student's T test; $p > 0.05$).

IVC spectral Doppler recordings demonstrated that all of the three indices (preload index; PLI, peak velocity index for vein; PVIV, and pulsatility index for vein; PIV) of most fetuses in both groups were confined in the 95% interval ranges of normal values. However, most fetuses in the group of high output hydrops fetalis had IVC Doppler indices lower than 50th percentile, while most of the low output group had higher indices than 50th percentile. [Figure 1](#) shows different distribution of preload index between both groups. In the group of high output hydrops fetalis, mean ($\pm$ SD) PLI, PVIV, and PIV were 0.093 ± 0.059 , 1.203 ± 0.405 , and 1.326 ± 0.413 , respectively. In the low output group, mean ($\pm$ SD) PLI, PVIV, and PIV were 0.273 ± 0.092 , 1.9583 ± 0.343 , and 2.212 ± 0.381 . The means ($\pm$ SD) of all indices in both groups were significantly different. (Student's t test; p value < 0.001) ([Figure 2](#)). Based on normal reference ranges of IVC Doppler indices reported by Baschat et al(4), the mean differences from normal mean of the IVC Doppler indices for each gestational week in the high output group were significantly lower (Student's t test; p value < 0.001), while the mean differences of IVC Doppler indices in the low output group were significantly higher (Student's t test; p value < 0.001) ([Table 2](#)).

Table 1 Distribution of the causes of hydrops fetalis

Causes	Number
High-output hydrops fetalis	51
Hemoglobin Bart's disease	51
Low-output hydrops fetalis	18
Ebstein's anomaly	5
Hypoplasia left heart syndrome	4
Heterotaxy syndrome	3
Atrial flutter / Supraventricular tachycardia	2
Small heart syndrome	3
Tricuspid atresia	1

Table 2 Comparison of inferior vena cava Doppler indices of the fetuses with low output and high output hydrops fetalis to normal mean for gestation

	n	Mean difference	t	p-value
High-output hydrops fetalis				
Preload index	51	-0.083	-6.317	< 0.001
Peak velocity index	51	-0.379	-5.186	< 0.001
Pulsatility index	51	-0.413	-5.471	< 0.001
Low-output hydrops fetalis				
Preload index	18	0.097	4.503	< 0.001
Peak velocity index	18	0.376	3.749	< 0.001
Pulsatility index	18	0.473	4.458	< 0.001

Figure 1: Scattergram of preload index of low-output (solid circle) and high-output (opened circle) hydrops fetalis

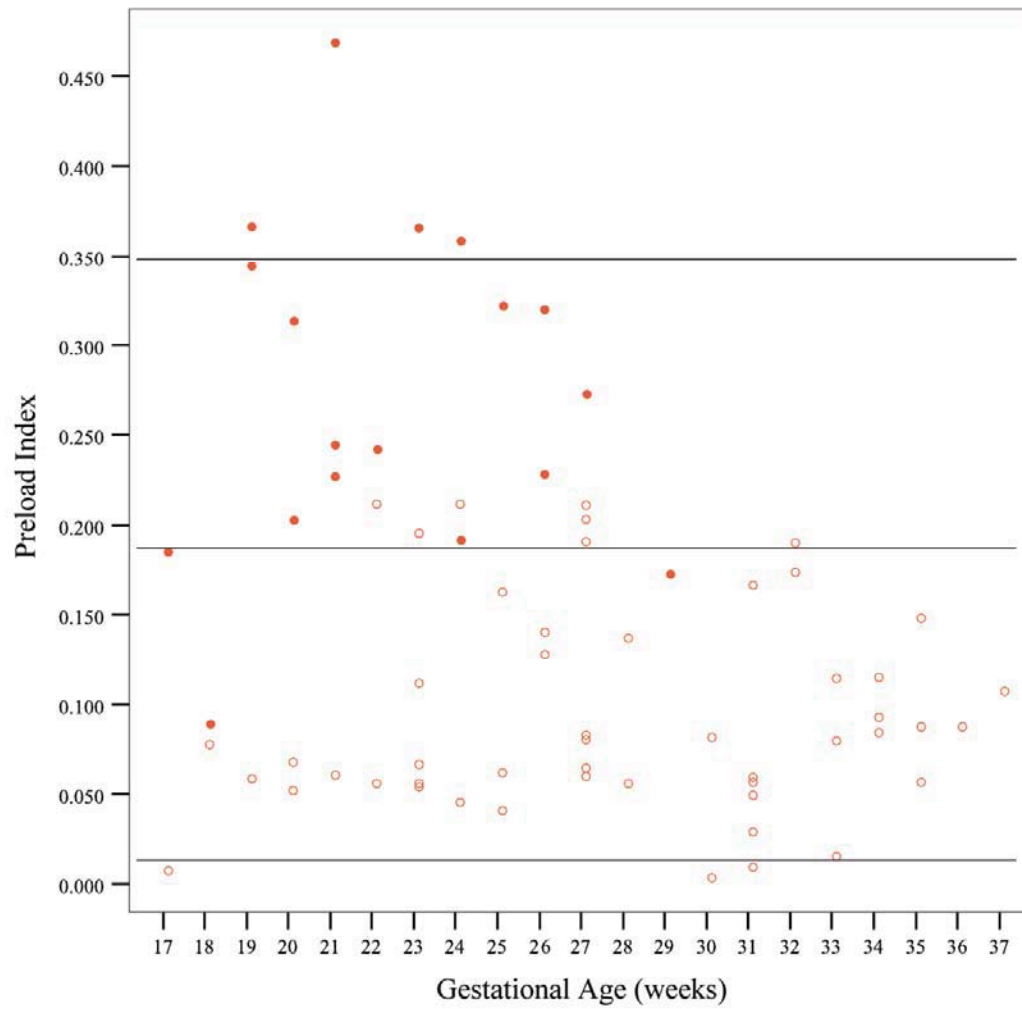
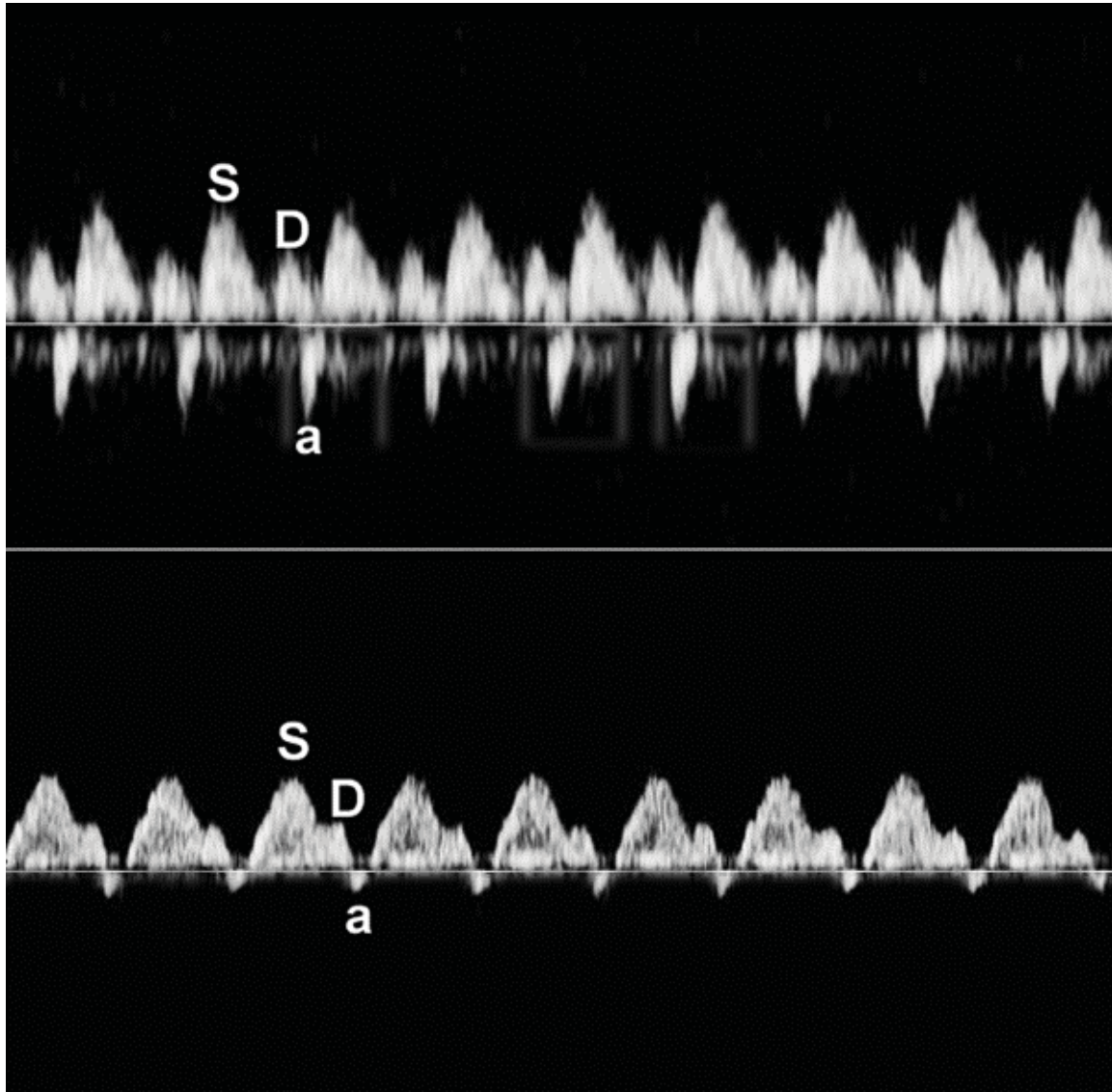


Figure 2: Example of IVC spectral Doppler waveforms of a fetus with low output hydrops fetalis; Ebstein's anomaly (upper panel) and high output hydrops fetalis (lower panel); hemoglobin Bart's disease. (*S*; ventricular systole, *D*; diastolic filling, *a*; atrial contraction) Note low backward flow during atrial contraction in the fetus with high output hydrops fetalis



Discussion

Normally, the blood flow pattern in the IVC shows a pulsating pattern synchronized with the cardiac cycle. The Doppler waveform of the IVC includes three components: an initial forward flow (S), which coincides with atrial diastole and ventricular systole; a second forward flow (D), which is seen with ventricular diastole, and reverse flow (a), which occurs during atrial contraction. The PLI, PVIV and PIV values are independent of the angle between the Doppler beam incidence and the blood flow, enabling us to evaluate central venous pressure as a surrogate marker of cardiac function.

This study confirms our hypothesis that congestive failure is not a primary cause of hydrops fetalis in fetal anemia. Though cardiomegaly is usually regarded as a universal sign of cardiac failure, as believed in adult heart, our findings suggest that cardiomegaly, which is always seen in all cases of Hb Bart's hydrops fetalis, was associated with normal cardiac function as indicated by normal or low IVC Doppler indices. This implies that cardiomegaly from fetal anemia is a consequence of cardiac adaption to anemia rather than congestive heart failure.

Several causes of hydrops fetalis can be associated with myocardial dysfunction and cardiac decompensation. In such cases, the fetuses are likely to show increased IVC Doppler indices or increased central venous pressure(5-8). Thus, IVC Doppler studies can be helpful in answering whether hydrops fetalis-associated cardiomegaly is congestive heart failure or not. This may be useful in predicting prognosis of hydropic fetuses with cardiomegaly.

Fetuses with cardiac defects without hydrops fetalis are usually associated with normal IVC Doppler indices(3). In contrast, cardiac defects with hydrops fetalis in our study show abnormal increased IVC Doppler indices in most cases. IVC a waves corresponding to atrial contraction were found to be reversed in cases of severe congestive heart failure, probably as a result of an abnormally high atrial pressure, as seen the in low output group of this study but not in the anemic group. In fetuses with cardiac defects, abnormal IVC Doppler indices should be regarded as a sign of cardiac decompensation. Therefore, venous Doppler studies together with monitoring hydropic changes may be clinically helpful in indirectly monitoring cardiac function in fetuses with cardiac malformations.

In contrast to fetuses with low output, fetuses with high output hydrops fetalis did not show high central pressure as indicated by normal or even low PLI, PVIV and PIV in the IVC, in spite of hypervolemia and cardiomegaly, supporting a concept that hydrops fetalis

has developed before cardiac decompensation. This finding is against a hypothesis that hydrops fetalis or cardiomegaly is a consequence of congestive heart failure as a result of anemia. In fetal anemia, hypervolemia may probably be a primary cause of hydropic changes and cardiac failure is a consequence only when cardiac compensatory mechanism is exhausted. Though hypervolemia can lead to marked cardiomegaly, and finally to congestive heart failure(9), cardiomegaly reflects high competency of cardiac compensation rather than cardiac failure. In spite of hypervolemia and cardiomegaly, normal or decrease in preload suggesting effective ventricular contractility was clearly demonstrated in this study. We can conclude that the normal fetal heart is very high capable of compensation to cope with anemic hypoxia. Marked cardiomegaly due to anemia probably reflect high competency of adaptation, rather than cardiac decompensation. This is consistent with our observation that many cases of frank hydrops fetalis from Hb Bart's disease have a reactive non-stress test, indicating well-being in spite of marked cardiomegaly.

In consequence of fetal anemia, hypervolemia associated with high cardiac output may directly cause a shift of fluid to interstitial space by various compensatory mechanisms; for example, the opening of additional capillaries, which may increase the exchange area between the vascular and interstitial space, improving the extraction of oxygen and nutrients. Hypervolemia in fetuses can more easily shift fluid to interstitial space than in adults. Based on fetal lamb studies, several factors facilitate transcapillary fluid filtration into the interstitial space during fetal compared to adult life(10). The permeability of the capillary membrane for plasma proteins is 15 times higher than in adult sheep(11). Additionally, the compliance of the interstitial space of the fetus is higher than in the adult sheep. Therefore, a greater accumulation of interstitial fluid is necessary in the fetus to increase the interstitial hydrostatic pressure. Smaller changes in the hydrostatic pressure difference between capillary and interstitial space lead to a larger fluid flow across the capillary membrane(12).

However, because of the diminished preload reserve and Frank – Starling mechanism of the fetal heart, which seems to operate near the top of its ventricular function curve, it seems to be difficult for the fetus to convert any increase of myocardial filling into an appropriate augmentation of cardiac output, which limits this compensatory mechanism. It is believable that this limit is likely to occur long after development of hydrops fetalis.

One of the weak points of this study is that we did not determine the level of fetal hemoglobin. Though all of them were uniformly anemic, we did not know the degree of anemia. Thus, we could not correlate the degree of anemia and the degree of preload index.

In addition, we did not include fetuses with hydrops fetalis associated with other causes such as venous or lymphatic obstruction, thus we do not know if IVC Doppler indices are informative in such cases.

Though anemia in this study was secondary to Hb Bart's disease, a lethal cause, IVC Doppler changes in these fetuses may theoretically be applied to anemia from other less severe causes such as Rh isoimmunization or parvovirus B19. Though overall prognosis of hydrops fetalis is poor, a better prognosis may probably be expected in high output with normal IVC Doppler indices. Therefore venous Doppler assessment may be helpful in predicting the prognosis and making a choice of management.

In conclusion, in the fetuses with congenital heart defects, cardiac decompensation is a primary cause of hydrops fetalis while hypervolemia is the primary cause of hydrops due to fetal anemia. Additionally, cardiomegaly in the fetuses with high output hydrops fetalis is not a sign of cardiac failure. New knowledge provided by this study is that low output and high output hydrops fetalis have different IVC Doppler indices.

Acknowledgement

The authors wish to thank the Thailand Research Fund (TRF) for financially supporting the conduct of this research.

Conflict of interest: None

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Randomized controlled trial comparing efficacy between a vaginal misoprostol loading and non-loading dose regimen for second-trimester pregnancy termination

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Abstract

Aim: The aim of this study was to compare the efficacy of vaginal misoprostol loading dose regimen with non-loading dose regimen for termination of second-trimester pregnancy with live fetuses.

Material and Methods: A randomized controlled trial was conducted on pregnant women with a live fetus at 14–28 weeks. The patients were randomly allocated to receive either the vaginal misoprostol loading dose regimen (600 mcg, then 400 mcg every 6 h) or the non-loading dose regimen (400 mcg every 6 h). Failure to abort within 48 h was considered to be a failure.

Results: Of 157 recruited women, 77 were assigned to be in group 1 (loading group) and 80 were in group 2 (non-loading group). The median abortion time was not statistically different between the groups (14.08; 95% confidence interval: 12.45–17.77 h and 14.58; 95% confidence interval: 12.8–17.27 h, $P > 0.05$). The rates of abortion within 24 h and 48 h were also comparable between the groups. Fever and chills were more common in the loading group. No other serious complications, such as postpartum hemorrhage and uterine rupture, were found.

Conclusion: Vaginal misoprostol in the loading dose regimen had a similar efficacy to the non-loading dose regimen but was associated with more adverse maternal effects.

Key words: live fetus, loading dose, pregnancy termination, second trimester, vaginal misoprostol.

Introduction

Termination of a second-trimester pregnancy with a live fetus is a challenging procedure. Various methods,^{1,2} either invasive or non-invasive, have been introduced to manage this issue. Several techniques for termination of second-trimester pregnancy have been used, including dilation and evacuation³ and medical abortion. Medical abortion is the method of choice because most diagnoses are performed late in the second trimester and trained personnel capable of carrying out dilation and evacuation in late pregnancy are limited. Consequently, at the present time, prostaglan-

dins (especially misoprostol) are the drugs of choice because of their efficacy, ease of administration and non-invasiveness.^{4,5}

Various regimens of misoprostol use in terms of route, dosage, interval or combination of mifepristone have been tested to determine the optimal application in enhancing the efficacy of pregnancy termination. Some non-randomized controlled trials suggest that the combination of mifepristone with misoprostol have a very high efficacy for pregnancy termination.^{6–9} Although there are some randomized control trials (RCT) in the recent literature showing that the combination of mifepristone and misoprostol is more

Received: October 31 2012.

Accepted: April 4 2013.

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effective,¹⁰ mifepristone is illegal in Thailand and thus unavailable. Misoprostol is the most widely used medication for medical abortion in Thailand. Nevertheless, despite many studies on the most effective dose and timing interval, no consensus has been reached. The authors questioned whether the loading dose regimen promoted cervical change and facilitated uterine contractions better than the non-loading dose. Loading dose may have theoretical benefit, as the initial contraction may need a higher dosage than that in late labor. However, very few reports have explored this aspect. To the best of our knowledge, only a study reported by Dickinson and Evans¹¹ compared the abortion interval between a loading dose regimen and a non-loading one. The objective of this study was to compare the efficacy of the loading and non-loading regimen. However, the maintenance dose of misoprostol in this study was 400 mcg, which is more widely used, rather than 200 mcg, which was used in the study reported by Dickinson and Evans.¹¹

Methods

This study was prospectively conducted with approval of the institutional review board (the Ethics Committee), at Maharaj Nakorn Chiang Mai Hospital, Chiang Mai University, Chiang Mai, Thailand between August 2009 and May 2012. Inclusion criteria were as follows: (i) singleton pregnancies with a live fetus; (ii) indicated for pregnancy termination due to either maternal or fetal conditions, such as lethal anomalies; (iii) gestational age of 14–28 weeks; (iv) no history of uterine scar, such as previous cesarean section, hysterotomy or myomectomy; (v) Bishop score of 4 or less; and (vi) no regular spontaneous uterine contraction before drug administration. The pregnant women were randomly allocated into two groups by block randomization (loading group vs non-loading group). Participants in the loading group first received a single dose of 600 mcg vaginal misoprostol as a loading dose, followed by administration of 400 mcg vaginal misoprostol every 6 h. Participants in the non-loading group received a fixed dose of 400-mcg vaginal misoprostol every 6 h. Each dose of misoprostol in both regimens was mixed with 1 ml of 5% acetic acid to facilitate drug dissolution leading to enhancement of the efficacy as described elsewhere.¹² The cervical Bishop score status was assessed by the authors before the initiation of misoprostol. Misoprostol was repeated if adequate uterine contractions were not achieved and the cervix was still unfavorable (Bishop score of 4 or less). If a

favorable cervix was achieved but inadequate uterine contractions were found, then intravenous (i.v.) oxytocin was infused by automatic infusion pump starting from a low dose with no longer misoprostol administration. Oxytocin was started at 2 mU/min and increased as needed every 15 min to 4, 8, 12, 16, 20, 25, and 30 mU/min (maximum). Skipping a dose was defined by an omission of the next dose of misoprostol at the scheduled time interval and resuming misoprostol use at 12 h or 18 h after the previous dose. This might be used in cases of adequate contraction after 6 h of drug administration but becoming inadequate later.

Intravenous meperidine 50 mg for painful uterine contraction was given depending on the patient's need. Adverse effects of misoprostol were prospectively monitored and recorded, including fever (temperature > 38°C), chills, nausea, vomiting and diarrhea.

Successful treatment was defined as complete abortion or delivery of the fetus within 48 h after initiation of the first dose of misoprostol. In cases of failure, the next step of management depended on the cervical status, uterine contractions, physician and patient preference. Some women received only continuing i.v. oxytocin to further promote adequate uterine contraction. Some women received some additional doses of vaginal misoprostol, whereas the remainders needed more invasive procedures, such as the modified condom balloon technique or intra-amniotic hypertonic saline infusion in rare cases.

The main outcome measure included percentage of successful termination in 24 h and 48 h and median abortion/delivery time among the successful cases. The abortion/delivery time was defined as the interval from the initiation of the first dose of misoprostol to complete abortion or delivery of the fetus. According to a previous study that administered 400 mcg vaginal misoprostol every 6 h,¹³ the mean abortion time was 20 h with a standard deviation of 10 h. The authors proposed that the loading dose regimen could shorten the abortion time by 4 h. Seventy-eight women were needed in each group to gain a power of 80% and alpha error of 0.05 (two sides).

SPSS 17.0 was used for statistical analysis. The difference in the mean continuous variables was analyzed using the Student's *t*-test for normally distributed data. Differences in proportions were analyzed using the χ^2 -test or Fisher's exact test as appropriate. Mann–Whitney *U*-tests were used for determination of statistical significance of differences in non-normally

distributed variables between groups. A *P*-value of <0.05 was considered statistically significant.

Results

During the study period, a total of 175 pregnant women were eligible for the study. Of them, 18 were excluded due to various reasons, such as spontaneous contraction before allocation or refusing to join the study. Finally, 157 were available for allocation and analysis, as presented in Figure 1. Seventy-seven received the loading dose regimen (loading group) and

80 received the non-loading dose regimen (non-loading group). The baseline characteristics of the participants and indications for pregnancy termination were similar between the groups as presented in Table 1. The mean maternal age was 29 years and the mean gestational age was 20 weeks. The most common indications for pregnancy termination were fetal chromosomal abnormalities and major fetal anomaly followed by severe fetal thalassemia.

The adverse effects of misoprostol are shown in Table 2. The two most common adverse effects were fever, followed by chills. Of note, the common adverse

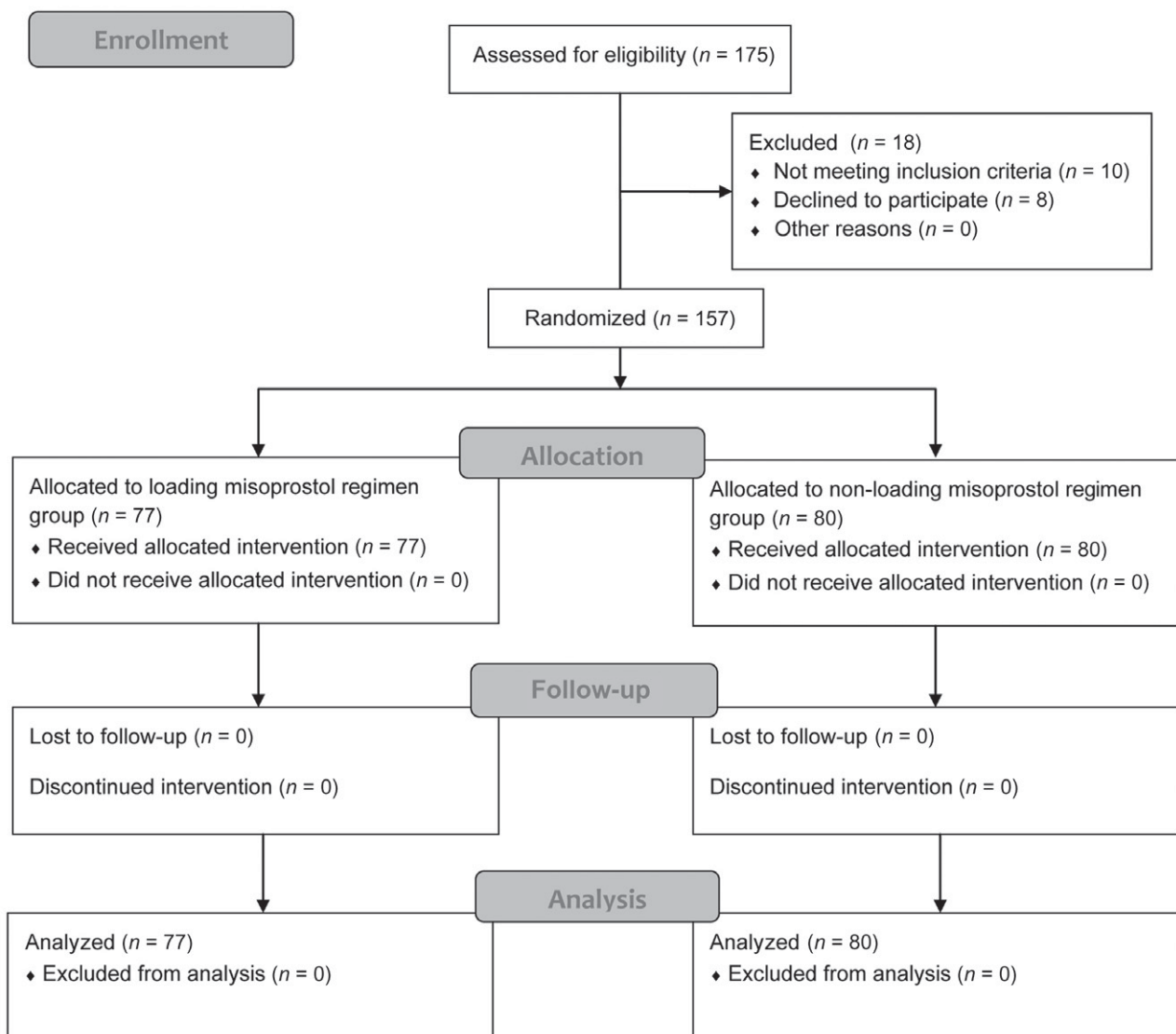


Figure 1 Flow diagram of the participants.

Table 1 Baseline characteristics and indication for pregnancy termination

Characteristics	Loading dose regimen (<i>n</i> = 77)	Non-loading dose regimen (<i>n</i> = 80)	<i>P</i> -value
Mean maternal age (years)	29.7 ± 7.0	29.2 ± 7.7	0.67
Mean gestational age (weeks)	20.6 ± 2.9	20.9 ± 3.1	0.55
Indications			
Fetal chromosomal abnormalities and major fetal anomaly	59.7%	51.3%	—
Severe fetal thalassemia	32.5%	38.7%	—
Maternal indications	3.9%	5.0%	—
Others	3.9%	5.0%	—

Table 2 Adverse effects of misoprostol

Adverse effects	Loading dose regimen (<i>n</i> = 77)	Non-loading dose regimen (<i>n</i> = 80)	<i>P</i> -value
Fever	25.5%	17.8%	0.03
Chills	24.8%	17.2%	0.03
Diarrhea	15.9%	9.6%	<0.05
Nausea	5.7%	3.2%	0.23
Vomiting	5.1%	1.9%	0.10
Postpartum hemorrhage	0%	0%	—
Uterine rupture	0%	0%	—
Other postpartum complications	0%	0%	—

Table 3 Comparison of the abortion/delivery outcomes between the groups

Abortion profile	Loading dose regimen (<i>n</i> = 77)	Non-loading dose regimen (<i>n</i> = 80)	<i>P</i> -value
Median abortion time (h)†	14.1 95%CI: 12.5–17.8	14.6 95%CI: 12.8–17.3	0.78
Median abortion time (h)† Nulliparous (<i>n</i> = 95)	14.6 95%CI: 12.5–19.9	16.6 95%CI: 13.35–19.2	0.93
Parous (<i>n</i> = 62)	12.9 95%CI: 10.1–15.9	13.9 95%CI: 11.2–20.2	0.81
Abortion			
Within 24 h	77.9%	76.3%	0.87
Within 48 h	93.5%	91.3%	0.87
Mean total dose of misoprostol (mcg)	1070.1 ± 611.1	1105 ± 785.3	0.76
Skipped dose of misoprostol	10.2%	13.4%	0.42
Single dose of misoprostol required	37.7%	23.8%	0.04
Oxytocin requirement	7.0%	10.8%	0.25
Intravenous analgesia requirement	20.4%	17.8%	0.40
Curettage	8.9%	7.0%	0.45
Estimated blood loss (mL)	100.1 ± 60.9	134.0 ± 95.4	0.01
Other method use after failure	0.6%	0.6%	0.98

†Mann–Whitney *U*-test. CI, confidence interval.

effects of misoprostol, including fever, chills and diarrhea, were significantly higher in the loading group (25.5% vs 17.8%; 24.8% vs 17.2%; and 15.9% vs 9.6%, respectively).

Table 3 presents abortion characteristics and other profiles. Median (95% confidence interval [CI]) abortion/delivery times were similar between the groups (14.08; 95%CI: 12.45–17.77 h and 14.58; 95%CI:

12.8–17.27 h, $P > 0.05$). In subgroup analysis, when separating the nulliparous and parous women, the median abortion/delivery times were also not significantly different between the regimens. Nevertheless the abortion/delivery time seemed to be shorter in parous women than that in nulliparous women, irrespective of regimen, but this was not significant. More than 90% of women aborted or delivered within 48 h after the initiation of misoprostol in both groups ($P > 0.05$).

Rates of oxytocin and analgesia requirement as well as curettage for incomplete abortion were not significantly different between the two groups. However, the estimated blood loss was significantly less in the loading group. Interestingly, the number of participants requiring a single dose of misoprostol was significantly higher in the loading group. Finally, the skipping dose rates were not significantly different.

Discussion

Our results indicate that a loading dose regimen was significantly associated with higher rates of adverse effects while the success rate was not significantly different. Nevertheless, the higher success rate with single dose and less blood loss was observed in the loading group, signifying that the loading regimen had a tendency to be more effective. However, such a high efficacy, if truly existed, would be only minimal and significance of such an outcome might only be shown with a larger sample size, and this would be unlikely to have clinical significance. Moreover, the difference in blood loss of only 30 mL in the loading group was also unlikely to have clinical impact. Therefore, our results did not support the advantage of loading dosage. In addition, there were similar rates of i.v. oxytocin use and analgesia requirements. While serious complications, such as postpartum hemorrhage or uterine rupture, were not found in the two groups, maternal side-effects, such as fever and chills, were significantly higher in the loading dose regimen. This finding is informative and may help clinicians make a decision on a regimen of choice. Though patients' satisfaction was not directly assessed in this study, the non-loading regimen with lower side-effects would certainly be a regimen of preference, while the efficacy was comparable.

Our results were contradictory to those reported by Dickinson and Evans,¹¹ who showed that the loading dose regimen (600 mcg vaginal dose followed by vaginal 200 mcg every 6 h) yielded a higher efficacy than the non-loading regimen (200 mcg vaginal every

6 h) in terms of shorter abortion time. Based on the two studies, it may be presumed that abortion/delivery time may be shorter with a loading dose of misoprostol in case of a maintenance dose of 200 mcg, but it seems unlikely to be helpful when a 400-mcg maintenance dose is used, whereas more adverse effects are significantly associated with a loading dose regimen. However, the two studies could not be compared because of the difference in gestational age, fetal viability and misoprostol maintenance dosage as mentioned above (200 vs 400 mcg). Gestational age in our study was strictly confined to 14–28 weeks of gestation but that in the study by Dickinson and Evans was extended to 30 weeks of gestation. Importantly, our study included only live fetuses while the study by Dickinson and Evans included both live and dead fetuses. As already known, higher gestational age and dead fetuses are independently associated with a higher success rate of termination of pregnancy with misoprostol.

The strengths of this study include: (i) the homogeneity of the participants in terms of gestational age and fetal viability as mentioned above; (ii) randomized controlled trial; and (iii) adequate sample size. The weakness of this study may be associated with its non-blinded nature. The assessors knew to which group the participants were allocated; however, blindness might not affect the main results as they were objectively measured. In addition, some outcomes were relatively subjective, especially blood loss estimation, which was based on the clinician's impression, not objectively measured in this study. Therefore, interpretation of such results may be less reliable. Moreover, patients' satisfaction was not assessed in this study. This study included only live fetuses in the second trimester and no obvious advantage was observed in loading regimen. However, loading regimen with lower dose for induction of labor in the last trimester should be further studied.

In conclusion, misoprostol in a dose of 400 mcg every 6 h, either with a loading dose or non-loading dose, was comparable in terms of abortion/delivery time. Higher maternal effects, such as fever and chills, were found in the loading dose regimen. Therefore, based on the present study, we recommend the non-loading dose regimen for second-trimester pregnancy termination.

Acknowledgment

The authors wish to thank the Thailand Research Fund (TRF) and the National Research University Project

under Thailand's Office of the Higher Education Commission for financial support.

Disclosure

None.

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ORIGINAL ARTICLE

Second trimester maternal serum inhibin-A in fetal anemia secondary to hemoglobin Bart's disease

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Abstract

Objective: To compare the levels of inhibin-A between pregnancies with fetal anemia secondary to Hb Bart's disease and pregnancies with normal non-anemic fetuses.

Methods: Sixty-five pregnancies at risk of fetal Hb Bart's disease scheduled for cordocentesis at 18–22 weeks were prospectively recruited into the study. Inhibin-A levels were measured from maternal blood drawn before cordocentesis. Fetal blood samples were collected for fetal Hb typing and hemoglobin (Hb) levels.

Results: Maternal serum inhibin-A was significantly higher in women with fetal Hb Bart's disease than those with unaffected fetuses (1.03 MoM (multiple of median) and 0.75 MoM, respectively, $p = 0.001$). The relationship between maternal serum inhibin-A and fetal Hb level was a quadratic equation; $\text{inhibin-A} = 5.248 - 9.415(\text{Hb}) + 4.919(\text{Hb})^2$ ($r^2 = 0.274$, $p < 0.001$). Maternal serum inhibin-A did not correlate with cardiomegaly but was significantly associated with placental thickness; $\text{inhibin-A} = 1.372 - 0.751(\text{PI}) + 0.214(\text{PI})^2$ ($r^2 = 0.237$, $p = 0.007$).

Conclusions: Maternal serum inhibin-A levels were significantly higher in pregnancies with fetal Hb Bart's disease. The elevation of inhibin-A was likely to be a consequence of fetal anemia and placentomegaly. Since inhibin-A is commonly used as a component of quadruple test; the calculated risk of Down's syndrome may be unreliable in pregnancies with fetal Hb Bart's disease or possible fetal anemia.

Keywords

Fetal anemia, hemoglobin Bart's disease, homozygous alpha-thalassemia-1 disease, inhibin-A, maternal serum marker

History

Received 24 June 2013
Revised 2 October 2013
Accepted 3 October 2013
Published online

Introduction

Quadruple test consisting of four serum markers (alpha-fetoprotein; AFP, total or free beta-human chorionic gonadotropin; hCG, unconjugated estriol; uE3 and inhibin-A) is one of the effective tests in detection of Down's syndrome in the second trimester. This set of maternal serum markers screening is well-accepted worldwide as a standard screening test of fetal Down's syndrome with a detection rate of approximately 80% at a 5% false positive rate [1]. In pregnancies with fetal anemia, some of these maternal serum markers have been reported to be abnormally altered and such changes might affect the interpretation of the fetal risk of Down's syndrome, calculated from the levels of these markers [2–4]. In pregnant women carrying anemic fetuses secondary to homozygous α -thalassemia-1 or hemoglobin (Hb) Bart's disease, AFP and hCG are significantly elevated whereas uE3 is reduced (1.94 MoM (multiple of median), 1.38 MoM and 0.81 MoM, respectively) [5]. Nevertheless, maternal serum inhibin-A levels among such a group of women have never been studied before. Maternal serum inhibin-A level was previously published to be increased in

cases of Turner syndrome with hydrops but decreased in cases without hydrops [6]. The abnormal elevation of inhibin-A may be produced from villous cytotrophoblasts with abnormal growth or differentiation in the enlarged hydropic placenta [7]. However, inhibin-A has never been studied in pregnancies with hydrops fetalis from anemic causes. Theoretically, inhibin-A, which is a placental product, may be increased in these fetuses who manifest by placentomegaly. The aim of this study was to compare the levels of inhibin-A between pregnancies with fetal anemia secondary to Hb Bart's disease and pregnancies with normal non-anemic fetuses. These data may contribute more information on the performance of Down's syndrome screening test that is influenced by fetal anemia.

Methods

A prospective study was conducted at Maharaj Nakorn Chiang Mai Hospital, Department of Obstetrics and Gynecology, Faculty of Medicine, Chiang Mai University with approval of the Research Ethical Committee. Pregnant women at risk of fetal Hb Bart's disease, scheduled for cordocentesis at 18–22 weeks between May 2011 and May 2012 were enrolled to the study. Pregnancy at risk was confirmed when both of the couple were α -thalassemia-1 carriers, diagnosed by the PCR (SEA-type) technique. Gestational age was based on a certain last menstrual

Table 1. Baseline characteristics of the pregnancies affected and unaffected by fetal Hb Bart's disease.

Characteristics	Unaffected group (n = 47)	Affected group (n = 17)	p Value
Maternal age* (yr)	26.60 ± 5.34	29.38 ± 4.53	0.937
Gestational age at sampling* (wk)	18.80 ± 0.83	19.00 ± 0.54	0.359
Maternal body weight* (kg)	55.76 ± 9.64	55.83 ± 3.92	0.633
Cardiothoracic ratio# (%)	48.00 ± 4.90	57.00 ± 7.50	<0.001
Placenta Thickness# (cm)	2.54 ± 0.37	3.32 ± 1.45	0.033
MCA-PSV# (cm/s)	23.40 ± 8.71	37.53 ± 10.10	0.040
MCA-PSV# (MoM)	1.10 ± 0.38	1.73 ± 0.40	<0.001
Hb# (gm/dL)	10.60 ± 1.38	6.30 ± 2.28	<0.001
Hb# (MoM)	0.98 ± 0.14	0.57 ± 0.20	<0.001
Hematocrit# (%)	33.05 ± 4.08	24.40 ± 8.20	<0.001

yr, Years; wk, weeks; kg, kilograms; n, number of cases; cm, centimeters; MCA-PSV, middle cerebral artery peak systolic velocity; cm/s, centimeter per second; MoM, multiples of median; gm/dL, gram per deciliter.

*Data were shown in mean ± standard deviation (SD).

#data were shown in median ± interquartile range (IQR).

period and early fetal biometry either by crown-rump length in the first trimester or biparietal diameter in the second trimester. Exclusion criteria included: (1) multiple pregnancies; (2) fetal structural or chromosomal abnormalities; (3) fetal anemia secondary to any causes other than Hb Bart's disease; (4) known maternal medical diseases and (5) failure of fetal blood sampling or contamination with maternal blood.

After informed and written consent was obtained, the demographic characteristics and medical history were recorded. Maternal blood sample was collected by venipuncture and was allowed to clot. The serum was immediately separated by centrifugation and stored at -20°C . The quantitative determination of dimeric inhibin-A levels was measured using the Diagnostic Systems Laboratories (DSL) inhibin-A enzyme-linked immunosorbent assay (DSL, Webster, TX). The measured inhibin-A level was then transformed into a MoM by dividing by the median level of inhibin-A for each gestational age based on Thai nomogram [8].

A transabdominal sonographic examination was performed for fetal biometry and fetal anatomical survey. Cordocentesis was then carried out under transabdominal real-time ultrasound guidance, using techniques as previously described [9]. Fetal blood sample was collected and tested for maternal blood contamination using a simple acid elution test. Each blood sample was divided into two sampling tubes and then immediately sent to the laboratories for (1) Hb typing using high-performance liquid chromatography and DNA analysis (if needed) for the fetal thalassemia status; (2) Hb levels and analysis of red blood cell indices using an automated complete blood count machine (Coulter STKS analyzer; Beckman, USA). The measured Hb levels were then transformed into MoM by dividing by the median levels of reference range Hb in each gestational age [10]. The degree of fetal anemia was categorized as mild (0.84–0.65 MoM), moderate (0.64–0.55 MoM) or severe (<0.55 MoM) anemia [10].

Statistical analysis

All statistical analyses were performed using SPSS 17.0 (SPSS Inc., Chicago, IL). The demographic characteristics were described and compared between the affected group

(the pregnancies with fetal Hb Bart's disease) and the unaffected group (the pregnancies with normal fetal Hb typing or α -thalassemia-1 carrier) by *t*-test for independent samples. Maternal serum inhibin-A (expressed as concentrations and MoM) was compared between both groups using the Mann–Whitney *U*-test and among the three groups of fetal anemia using the Kruskal–Wallis test. Regression analysis and the least-squares method were used to identify the relationship between maternal serum inhibin-A and the fetal Hb level. The best equation is selected based on r^2 and the adjusted r^2 criterion. $p < 0.05$ was considered statistically significant.

Results

During the study period, 65 pregnant women were recruited and maternal serum samples were collected and analyzed for inhibin-A. All of the cordocentesis procedures were successful and 65 fetal blood samples were collected for Hb typing analysis. Fetal thalassemia status showed 17 (26.2%) fetuses affected by Hb Bart's disease, 47 (72.3%) unaffected fetuses (α -thalassemia-1 carriers and normal Hb typing) and 1 (1.5%) case of fetal Hb H-Constant spring disease who was excluded from the study. The remaining 64 pregnancies were available for analysis and their baseline characteristics are shown in Table 1. No statistically significant difference in maternal age, gestational age at sampling and maternal body weight was found between the two groups ($p > 0.05$). In Hb Bart's group, sonographic markers were identified as cardiomegaly (CT ratio ≥ 0.50) in 90.9%, placentomegaly (placental thickness ≥ 3.00 cm) in 55.6%, increased middle cerebral artery peak systolic velocity (MCA-PSV) (MCA-PSV ≥ 1.50 MoM) in 80.0%, but none of the affected fetuses had the other signs of hydrops fetalis.

However, only 58 fetal blood samples had sufficient amount for Hb level analysis. In the affected group, Hb levels were classified as mild anemia in 4 (26.7%) cases, moderate anemia in 6 (40.0%) and severe anemia in 5 (33.3%). In the unaffected group, Hb levels were categorized as normal in 40 (93.0%) cases and mild anemia in 3 (7.0%) cases that were excluded from the analysis. Maternal serum inhibin-A levels were significantly higher in non-anemic group (40 cases) than in moderate to severe anemia group (11 cases) as shown in Figure 1 (1.07 MoM and 0.76 MoM, $p < 0.001$).

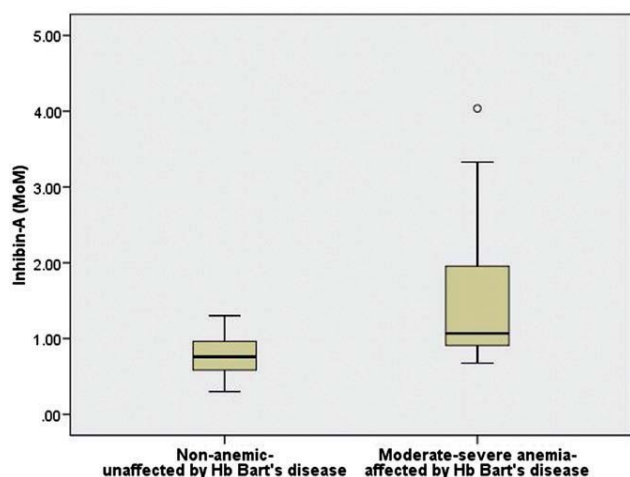


Figure 1. Boxplots of maternal serum inhibin-A (MoM) in non-anemic unaffected by fetal Hb Bart's disease and moderate-severe anemia affected by fetal Hb Bart's disease..

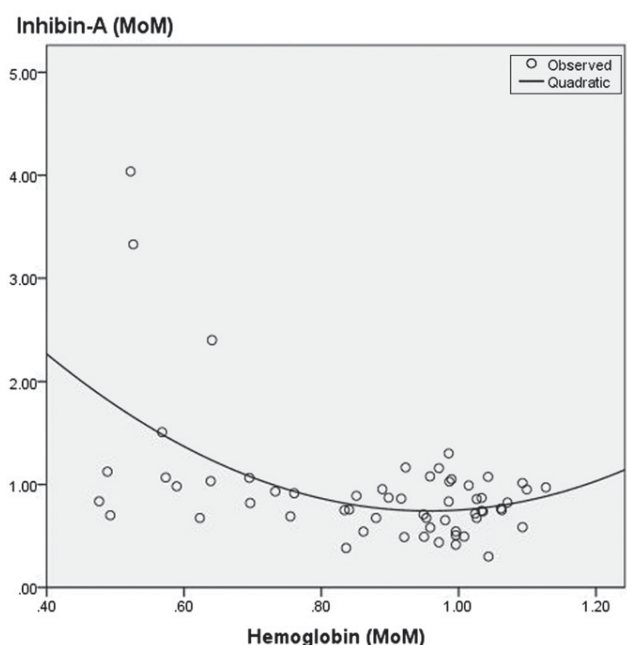
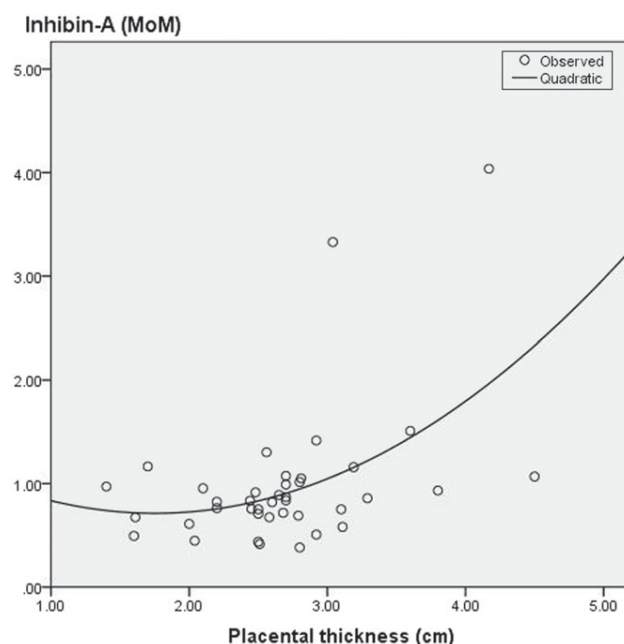


Figure 2. The relationship between maternal serum inhibin-A (MoM) and fetal hemoglobin (MoM); $\text{inhibin-A} = 5.248 - 9.415(\text{Hb}) + 4.919(\text{Hb})^2$ ($r^2 = 0.274$, $p < 0.001$).

The best-fitted model for relationship between maternal serum inhibin-A and fetal Hb level was a quadratic equation; $\text{inhibin-A} = 5.248 - 9.415(\text{Hb}) + 4.919(\text{Hb})^2$ ($r^2 = 0.274$, $p < 0.001$) as demonstrated in Figure 2. Maternal serum inhibin-A did not correlated with cardiomegaly ($r^2 = 0.106$, $p = 0.114$) but had a positive relationship with placental thickness; $\text{inhibin-A} = 1.372 - 0.751(\text{Hb}) + 0.214(\text{Hb})^2$ ($r^2 = 0.237$, $p = 0.007$) as shown in Figure 3.

Table 2 shows maternal serum inhibin-A levels as absolute concentration and MoM in pregnancies affected and unaffected by fetal Hb Bart's disease and in each group of fetal anemia. Inhibin-A levels were significantly higher in the affected pregnancies than those in unaffected pregnancies and had a tendency to increase with severity of fetal anemia.

Discussion

Maternal serum inhibin-A is a useful analyte in quadruple tests for second trimester prenatal Down's syndrome screening. The median level of inhibin-A in Down's syndrome pregnancy was 1.79 MoM [11], whereas the levels were lower in fetuses with trisomy 18 (0.84 MoM) [12] and Turner syndrome (0.64 MoM) [6]. However, second trimester inhibin-A levels have been reported to be markedly elevated in Turner syndrome with hydrops (3.91 MoM) [6]. In quadruple test, this elevation of inhibin-A resulted in a 2.6-fold increased Down's syndrome risk in cases of Turner syndrome with hydrops [6]. The same pattern of abnormally high maternal serum marker level was also observed in hCG which was increased in fetal hydrops from Turner syndrome, triploidy and other etiologies, including fetal anemia secondary to Hb Bart's disease (1.38 MoM) [5,13–16]. Similarly, these two biochemical analytes are produced from syncytiotrophoblast. Therefore, the higher levels in maternal blood can be explained by the abnormal production of placental proteins from the enlarged hydropic placenta. Besides, the elevated inhibin-A levels secondary to abnormal placentation were reported to be associated with the subsequent risk of severe preeclampsia and intrauterine growth restriction [17]. Importantly, the pathological study of placenta demonstrated that elevated second trimester maternal serum levels of inhibin-A may result from abnormal accelerated differentiation of the villous cytotrophoblasts [7].

Hb Bart's or homozygous α -thalassemia-1 disease is a common cause of lethal fetal anemia in South East Asia. Hb Bart, a combination of four gamma globin chains due to defect in alpha chain formation, binds oxygen with high affinity and causes fetal anemia and tissue hypoxia. Consequently, the compensatory mechanisms extensively develop to overcome fetal tissue hypoxia, resulting in

Table 2. Maternal serum inhibin-A levels in pregnancies affected and unaffected by fetal Hb Bart's disease and each groups of fetal anemia.

	<i>n</i>	Inhibin-A (pg/mL) median (IQR)	<i>p</i> Value	Inhibin-A (MoM) median (IQR)	<i>p</i> Value
Thalassemia status	64		0.001*		0.001*
• Unaffected fetuses	47	160.27 (88.51)		0.75 (0.41)	
• Affected fetuses	17	215.63 (247.94)		1.03 (1.13)	
Degree of fetal anemia	58		0.021#		0.028#
• Non-anemic fetuses	40	161.66 (78.75)		0.76 (0.39)	
• Mild anemia	7	171.89 (46.68)	0.729†	0.77 (0.22)	0.836†
• Moderate anemia	6	225.24 (179.95)	0.013‡	1.05 (0.83)	0.016‡
• Severe anemia	5	233.89 (616.32)	0.040¶	1.13 (2.91)	0.047¶

pg/mL, picogram per milliliter; IQR, interquartile range.

†Pairs of significant difference for non-anemic versus mild anemic group.

‡Pairs of significant difference for non-anemic versus moderate anemic group.

¶Pairs of significant difference for non-anemic versus severe anemic group.

*Mann–Whitney *U*-test.

#Kruskal–Wallis test.

hydropic changes, such as subcutaneous edema, cardiomegaly, hepatomegaly, ascites and placentomegaly.

During early second trimester, Hb Bart's fetus usually manifests by cardiomegaly, placentomegaly and increased MCA-PSV [18,19]. Hydrops fetalis, defined as two or more body spaces of fluid collection, is not common until late second trimester although moderate to severe fetal anemia is already occurred. In this study, the characteristics of the affected fetuses were homogeneous and unique for high-output fetal anemia in early stage of disease. Fetal cardiomegaly, placentomegaly and high MCA-PSV were identified in most affected cases whereas frank hydropic change was demonstrated in none of them. This may imply that the elevation of inhibin-A in Hb Bart's fetuses was rather a consequence of placentomegaly due to anemia than a result of hydropic changes in the fetus. In the previous report, inhibin-A was markedly increased in fetal Turner syndrome with hydrops [6]; however, the data on placental volume in these fetuses were not shown. Importantly, a good relationship between maternal serum inhibin-A levels and placentomegaly found in this study supported the hypothesis that inhibin-A is excessively produced from the enlarged placenta.

This is the first study demonstrating that maternal serum inhibin-A levels inversely correlated with fetal Hb levels. Anemic fetuses tended to have higher levels of maternal serum inhibin-A, as seen a significant increase among fetuses with moderate to severe anemia. Nevertheless, such an increase was not significant in mild degree of fetal anemia, this was probably due to too small sample size to show a significant difference. Since fetal anemia in this study was caused by Hb Bart' disease, a lethal condition, further studies may be investigated to explore whether maternal serum inhibin-A can predict fetal anemia secondary from other causes such as parvovirus B19 infection and rhesus alloimmunization.

In the geographical area with high prevalence of Hb Bart's disease, inhibin-A as a serum marker in quadruple test should be interpreted with high precaution for a falsely increased risk of Down's syndrome in cases of fetal Hb Bart's disease. Similarly, abnormal levels of AFP, hCG and uE3 in pregnancy with fetal anemia may result in a unreliable calculated risk of

Down's syndrome and other chromosome abnormalities [5]. Although sonographic markers such as cardiomegaly, placentomegaly and high MCA-PSV in Hb Bart's fetus are not difficult to identify, these minor signs may be overlooked with the inattention of sonographers during second trimester maternal serum marker Down's syndrome screening test at 15–18 weeks of gestation.

In conclusion, maternal serum inhibin-A levels was significantly higher in pregnancies with fetal Hb Bart's disease. The elevation of inhibin-A was likely to be a consequence of fetal anemia and placentomegaly, in spite of hydropic changes. Since inhibin-A is commonly used as a component of quadruple test; the calculated risk of Down's syndrome may be unreliable in pregnancies with fetal Hb Bart's disease or fetal anemia.

Declaration of interest

The authors wish to thank the Thailand Research Fund (TRF) for financial support in the conduct of this research. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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Cardio-STIC (spatio-temporal image correlation) as genetic ultrasound of fetal Down syndrome

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Short title: Cardio-STIC as genetic ultrasound of Down syndrome

Key Words: STIC (spatio-temporal image correlation), Down syndrome, 4D-ultrasound, Prenatal Diagnosis

Words count = 3004 (excluding abstract & references) Abstract = 249

Abstract

OBJECTIVE: To evaluate efficacy of cardio-STIC in detection of fetal Down syndrome

METHODS: Cardio-STIC volume datasets (VDS) were prospectively collected from women during 16-22 weeks, consisting of 40 VDS acquired from fetuses with Down syndrome and 400 VDS from normal fetuses. All VDS were blindly analyzed.

RESULTS: Between both groups, most dimensions were comparable but the right-sided dimensions were significantly greater in fetuses with Down syndrome. Interestingly, shortening fraction was also significantly higher in affected fetuses. Right-to-left disproportion and shortening fraction were used as cardiac markers as well as other 8 structural markers to predict fetal Down syndrome. Tricuspid regurgitation had highest sensitivity (30%); followed by VSD (27.5%), right-to-left disproportion (20.0%), and echogenic intra-cardiac foci (17.5%). If the test was considered positive in case of the presence of any cardiac marker, cardio-STIC had a detection rate of 72.5% and false positive rate of 19.5%. Likelihood ratio of each marker for modifying priori risk was also provided.

CONCLUSION: Cardio-STIC as genetic ultrasound for Down syndrome had a detection rate of about 70% and false positive rate 20%. Cardio-STIC analysis can be helpful in estimation of fetal risk for Down syndrome and counseling when the prenatal diagnosis of the syndrome is made.

Introduction

Though cardiac defects are the most common structural abnormalities in Down syndrome, assessment of the cardiac markers has not been consistent (1-3). In the study of genetic sonogram, cardiac defects were often excluded from analysis. This may probably be due to the fact that cardiac markers are too complex for general practitioners to assess with confidence. In addition, most studies focusing on cardiac markers reported only individual markers such as aberrant right subclavian artery (ARSA), tricuspid regurgitation (TR), or specific anomalies as a single parameter. The most comprehensive study on cardiac markers as genetic ultrasound of fetal Down syndrome is that reported by DeVore et al(4;5), who explored cardiac markers based on 2D-ultrasound, together with non-cardiovascular markers. Nevertheless, they did not include some significant markers like ARSA or cardiac biometry. Additionally, because of the difficulty in detecting structural and functional cardiovascular abnormalities, fetal echocardiography as a component of the genetic sonogram may be difficult to apply as a screening test for Down syndrome.

Only echogenic intra-cardiac focus (EIF), a weak soft cardiac marker, is often included in genetic sonogram system for fetal Down syndrome. Other potential cardiac makers have not been included because of its difficulty for implementation as mentioned above. In fact, the presence or absence of cardiac markers should be greatly impact on modifying risk, since congenital heart defects (CHD) are common among fetuses with Down syndrome, 40-60% of liveborn infants with Down syndrome(6). Therefore, if feasible, cardiac markers should be included in genetic sonogram system.

Fortunately, with modern cardio-STIC, cardiac markers will be available for general practice, either by subsequent off-line analysis in their own convenience without time limit or online remote consultation with the experts. Therefore, with availability of cardio-STIC, cardiac markers may become more important role in genetic sonogram or even in screening congenital heart defects in near future. The aim of this study was to evaluate the efficacy of second trimester sonographic cardiac markers for fetal Down syndrome, by thorough exploration of the volumes of virtual cardiac cycles derived from fetuses with Down syndrome compared to those of normal fetuses. This is one of the first studies regarding fetal heart survey to uncover the genetic secret from the virtual cardiac cycles without direct real-time examination on the patients. We hope that online-service on analysis of cardio-STIC, which can simply be acquired by any trained practitioners and anywhere, will become more available in fetal centers for widely use of cardiac markers in near future.

Materials and Methods

A prospective study of diagnostic test was conducted at a tertiary center, medical school hospital, with approval of the Institute Review Boards, Ethics Committee. High risk pregnancies for fetal Down syndrome indicated by maternal serum markers, pregnancies scheduled for fetal anomaly screening and pregnancies with fetal Down syndrome diagnosed by chorionic villous sampling or amniocentesis were enrolled into the study with written informed consent. Inclusion criteria consisted of: 1) singleton pregnancies, 2) gestational age of 16-22 weeks, relied on a certain last menstrual period and early fetal biometry either by crown-rump length in the first trimester or biparietal diameter in the second trimester, and 3) known chromosome study either by invasive prenatal diagnosis or postnatal study by neonatologists. Exclusion criteria included: 1) multiple pregnancies; 2) fetal structural abnormalities other than isolated cardiac defects; 3) abnormal chromosome other than Down syndrome, and 4) poor quality volume datasets.

The recruited participants underwent standard ultrasound examination for fetal biometry as well as anomaly screening and cardio-STIC acquisition. All ultrasound examinations were performed by maternal-fetal-medicine staffs, using real-time equipment with 4D-STIC, (Voluson E8; GE Healthcare, USA) with transabdominal 2-4 MHz curvilinear transducers. During examination, the acquired volume datasets were stored for subsequent analysis with offline cardio-STIC using 4D Views software (Version 10; GE Medical Systems, Zipf, Austria). On cardio-STIC acquisition, volume datasets (VDSs) were obtained by transverse sweeping after the best four-chamber view was visualized. Both transverse and apical FCV were acquired in both black/white and color flow mapping (CFM) volumes. The volumes were acquired in the absence of fetal movement while the women were asked to momentarily suspend breathing during the volume acquisition (lasting 7.5-12.5 seconds). The VDSs with patients' identification code without chromosome study results were recorded in the ultrasound hard drive. The VDSs were exported to another separate external hard disk for anonymous analysis by one of the authors who had no any information of the patient. The results of VDSs analysis were recorded in a separate database file for subsequent merging with the file of patients' baseline data as well as fetal outcomes (Down syndrome and normal fetuses).

On off-line cardio-STIC analysis, volume processing, navigation through the volume and measurement of various cardiac dimensions using both conventional and STIC-M-mode were performed as standard techniques described elsewhere(7-10). Cardiac biometry included; 1)

cardiac circumference (cm), 2) left ventricular inner width (cm), 3) right ventricular inner width (cm), 4) right/left ventricular inner width ratio, 5) interventricular septum thickness (cm), 6) left ventricular wall thickness (cm), 7) right ventricular wall thickness (cm), 8) biventricular outer diameter (cm), 9) left ventricular shortening fraction (%), (shortening fraction = dimension at end-diastole – dimension at end-systole / dimension at end diastole), 10) right ventricular shortening fraction (%), 11) aortic valve diameter (cm), 12) pulmonary valve diameter (cm), 13) distal aortic isthmus diameter (cm), 14) transverse aortic arch diameter (cm), 15) ductus arteriosus diameter (cm), 16) left atrial inner width (cm), 17) right atrial inner width (cm), 18) right/left atrial inner width ratio. The dimensions / functions which were significantly different between fetuses with Down syndrome and normal fetuses were considered as cardiac markers which would be further tested for diagnostic accuracy. These biometric cardiac markers were considered abnormal (positive cardiac markers) when greater than 95th centile or less than 5th centile for each gestational weeks, based on our own normal reference ranges(7-10).

The structural cardiac markers used for differentiating normal and affected fetuses included; 1) ventricular septum defect (VSD), 2) endocardial cushion defects or atrio-ventricular canal (AVC), 3) pericardial effusion, 4) tricuspid regurgitation (TR) lasting more than half of systole, 5) mitral regurgitation (MR), 6) aberrant right subclavian artery (ARSA), checked by visualization of the transverse 3-vessel trachea view of the upper thorax as described by Chaoui et al(11), using color flow mapping volumes instead, 7) echogenic intracardiac foci (EIF), 8) specific structural cardiac defects (other than AVC and VSD) and 10) abnormal biometric cardiac markers mentioned above. The examples of cardiac markers are shown in **Figure 1-5**.

Statistical analysis: Comparisons of various cardiac dimensions between both groups were performed using Student's T test. The diagnostic performance of each marker was assessed for detection rate (sensitivity), false-positive rate (1–specificity), positive likelihood ratio (LR; sensitivity / (1–specificity)) and negative LR ((1–sensitivity) / specificity) were assessed. The analyses were performed using statistical software package SPSS 20.0 (SPSS Inc., Chicago, IL, USA).

Results

A total of 492 cardio-STIC volume datasets (VDS) were prospectively collected between 16-22 weeks of gestation, 52 were excluded because of poor quality volumes (28), unavailable final fetal outcomes (13), structural anomalies (8), and other chromosome abnormalities (3). Of them, 440 VDS, which were acquired, were included to the study, consisting of 40 VDS acquired from fetuses with Down syndrome and 400 VDS from normal fetuses meeting the inclusion criteria of normal chromosomes and no other structural abnormality.

Mean gestational age of the two groups was comparable (19.30 ± 2.03 vs 19.07 ± 2.19 weeks; $p=0.504$, in unaffected and affected group, respectively). Various dimensions of the heart and great arteries were measured and compared between affected and unaffected group as presented in Table 1. Most structural dimensions were not significantly different between both groups. Nevertheless, the right-sided dimensions (right ventricular inner width, right atrial inner width, right-to-left ratio) were significantly greater in fetuses with Down syndrome whereas cardiac size (circumference, biventricular outer diameter) and right ventricular wall thickness tended to be greater in fetuses with Down syndrome but not statistically significant. Interestingly, shortening fraction of both ventricles was significantly higher in the affected fetuses.

Based on the difference in cardiac dimensions between both groups as mentioned above, right-to-left disproportion was considered a potential cardiac marker of fetal Down syndrome. The chamber disproportion as a cardiac marker was diagnosed if right/left atrial inner width ratio or right/left ventricular inner width ratio greater than the mean +2SD for each gestational week. Likewise, increased shortening fraction of left or right ventricle was considered a positive cardiac marker for fetal Down syndrome if one or both of them were greater than the mean +2SD for each gestational week. Therefore, based on the results of cardiac biometry in Table 1 together with structural markers, ten cardiac markers were tested for performance in predicting fetal Down syndrome as follows: 1) right-to-left disproportion, 2) increased shortening fraction of the ventricles, 3) isolated ventricular septum defect, 4) atrio-ventricular canal (endocardial cushion defect), 5) pericardial effusion, 6) tricuspid regurgitation, 7) mitral regurgitation, 8) aberrant right subclavian artery, 9) echogenic intra-cardiac foci, 10) other specific cardiac anomalies (atrial septal defect; ASD, tetralogy of Fallot; TOF, double-outlet right ventricle; DORV, etc.)

Of all cardiac markers, TR had the highest sensitivity (30%); followed by VSD (27.5%), right-to-left disproportion (20.0%), and echogenic intra-cardiac foci (17.5%). Several

affected cases had more than one marker. If the test was considered positive in case of the presence of any cardiac marker, cardio-STIC had a detection rate of 72.5% and false positive rate of 19.5%. Other specific congenital heart defects other than VSD and AVC were found only in 4 cases consisting of TOF with right aortic arch (2) ASD (1) and DORV (1).

Table 1: Comparison of cardio-biometry between normal and Down syndrome fetuses

Group	Normal (N = 400)		Down syndrome (N = 40)		P value
	Mean	SD	Mean	SD	
Cardiac circumference (cm)	6.01	1.11	6.22	1.28	0.251
Left ventricular inner width (cm)	0.66	0.15	0.67	0.18	0.883
Right ventricular inner width (cm)	0.68	0.15	0.75	0.18	0.030
Right/Left ventricular inner width ratio	1.02	0.01	1.13	0.03	0.000
Interventricular septum thickness (cm)	0.16	0.03	0.15	0.03	0.135
Left ventricular wall thickness (cm)	0.17	0.04	0.18	0.04	0.240
Right ventricular wall thickness (cm)	0.17	0.04	0.19	0.05	0.095
Biventricular outer diameter (cm)	1.84	0.36	1.96	0.43	0.057
Left ventricular shortening fraction (%)	0.34	0.05	0.36	0.06	0.005
Right ventricular shortening fraction (%)	0.32	0.05	0.35	0.04	0.001
Aortic valve diameter (cm)	0.28	0.07	0.27	0.09	0.709
Pulmonary valve diameter (cm)	0.32	0.07	0.32	0.09	0.904
Distal aortic isthmus diameter (cm)	0.21	0.04	0.21	0.04	0.953
Transverse aortic arch diameter (cm)	0.23	0.04	0.23	0.05	0.801
Ductus arteriosus diameter (cm)	0.22	0.04	0.22	0.05	0.864
Left atrial inner width (cm)	0.67	0.26	0.67	0.27	0.921
Right atrial inner width (cm)	0.68	0.27	0.78	0.26	0.025
Right/left atrial inner width ratio	1.02	0.06	1.20	0.15	0.000

Table 2 Diagnostic indices of each cardiac marker in predicting fetal Down syndrome

Cardiac Markers	Down (n = 40)	Unaffected (n = 400)	P-value	Sensitivity (95% CI)	Specificity (95% CI)	LR + (95% CI)	LR – (95% CI)
Right-to-left disproportion	8 (20.0%)	17 (4.3%)	0.001	20.0 (7.6-32.4)	95.8 (93.8-97.7)	4.71 (2.17-10.21)	0.84 (0.72-0.98)
Increased SF of ventricles	4 (10.0%)	4 (1.0%)	0.001	7.5 (-0.7-15.7)	99.0 (98.0-100.0)	7.5 (1.74-32.34)	0.93 (0.86-1.02)
Ventricular septum defect	11 (27.5%)	3 (0.8%)	< 0.001	27.5 (13.7-41.3)	99.3 (98.4-100)	36.70 (10.67-126.02)	0.73 (0.63-0.88)
Atro-ventricular canal	4 (10.0%)	0 (0.0%)	< 0.001	10.0 (0.7-19.3)	100.0 (100.0-100.0)		0.90 (0.81-1.00)
Pericardial effusion	7 (17.5%)	16 (4.0%)	0.001	17.5 (5.7-29.3)	95.5 (93.5-97.5)	3.89 (1.73-8.74)	0.86 (0.75-0.99)
Tricuspid regurgitation	12 (30.0%)	27 (6.8%)	< 0.001	30.0 (15.8-44.2)	93.2 (90.8-95.7)	4.44 (2.45-8.08)	0.75 (0.61-0.92)
Mitral regurgitation	2 (5.0%)	1 (0.25%)	< 0.001	5.0 (-0.2-11.8)	99.8 (99.3-100.2)	20.00 (1.85-215.75)	0.95 (0.89-1.02)
Aberrant right subclavian artery	4 (10.0%)	2 (0.5%)	< 0.001	10.0 (0.7-19.3)	99.5 (98.8-100.2)	20.00 (3.78-105.82)	0.91 (0.82-1.00)
Echogenic intracardiac foci	7 (17.5%)	14 (3.5%)	< 0.001	17.5 (5.7-29.3)	96.5 (94.7-98.3)	5.00 (2.14-11.66)	0.86 (0.74-0.99)
Other specific heart anomalies	4 (10.0%)	4 (1.0%)	< 0.001	10.0 (0.7-19.3)	99.0 (98.0-100.0)	10.00 (2.60-38.47)	0.91 (0.82-1.01)
Any cardiac sonomarker	29 (72.5%)	77 (19.3%)	< 0.001	72.5 (58.7-86.3)	80.5 (76.9-84.6)	3.76 (2.85-4.97)	0.34 (0.21-0.57)

Figure 1 Cardio-STIC-M depicts right-to-left disproportion (IVS: interventricular septum; LA: left atrium; RA: right atrium; RV right ventricle)

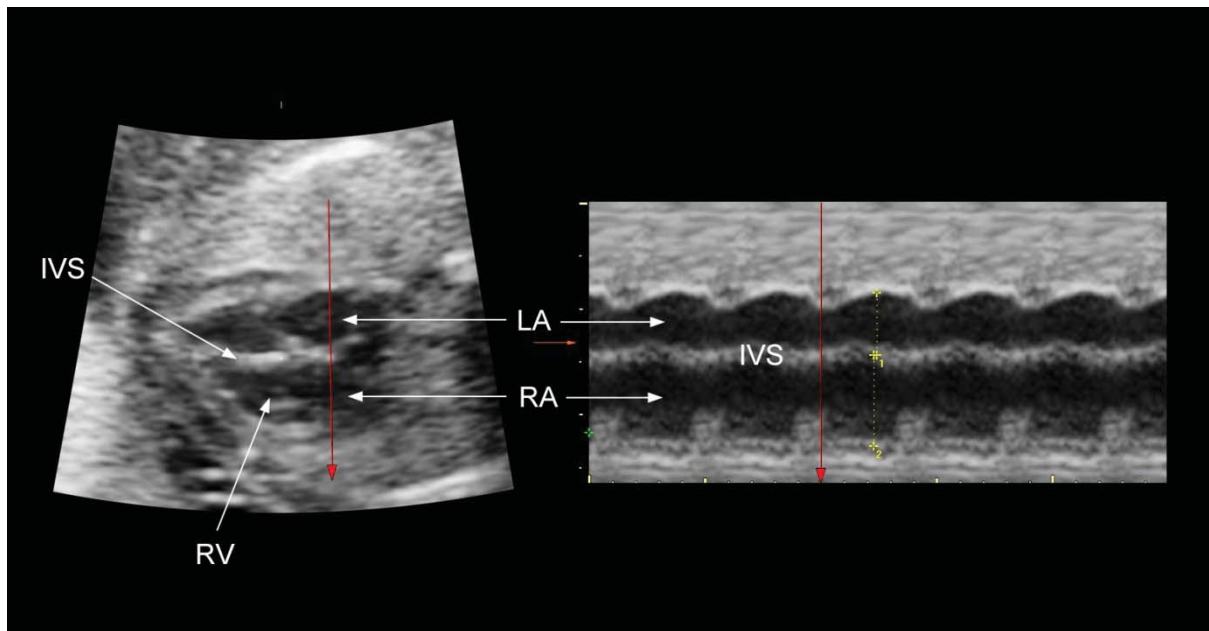


Figure 2 Left: pericardial effusion and thickened right ventricular wall (*) (RV; right ventricle); Right: aberrant right subclavian artery (ARSA), running behind the trachea (T)

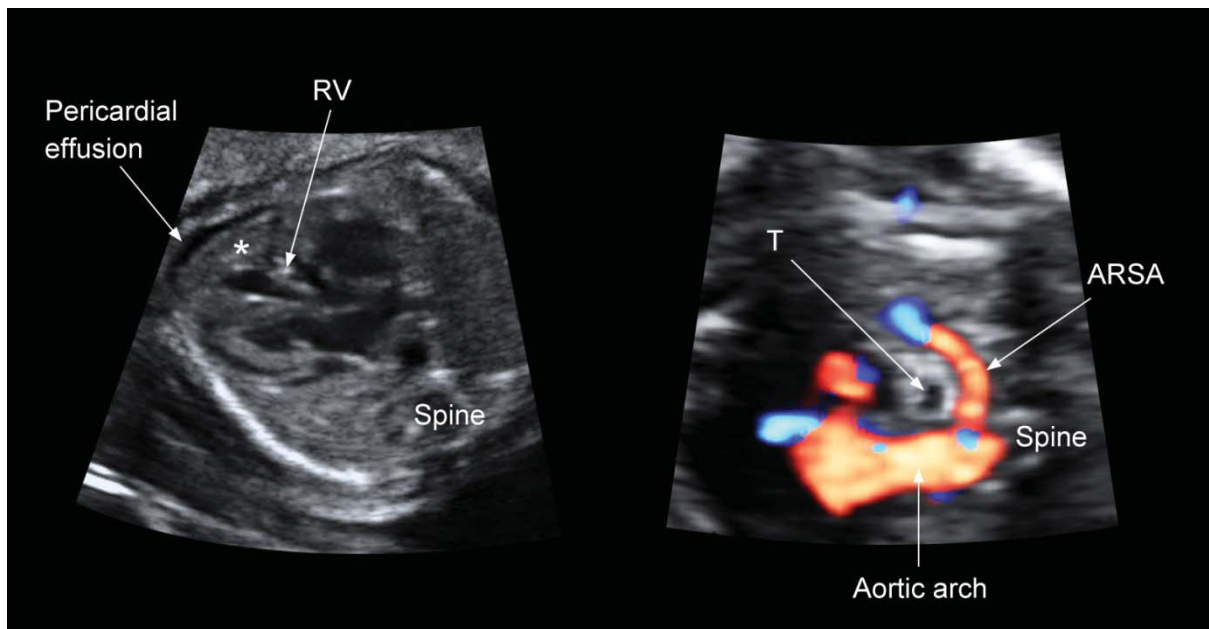


Figure 3 *Left:* tricuspid regurgitation (TR); *Right:* mitral regurgitation (MR) (LV: left ventricle; RV: right ventricle)

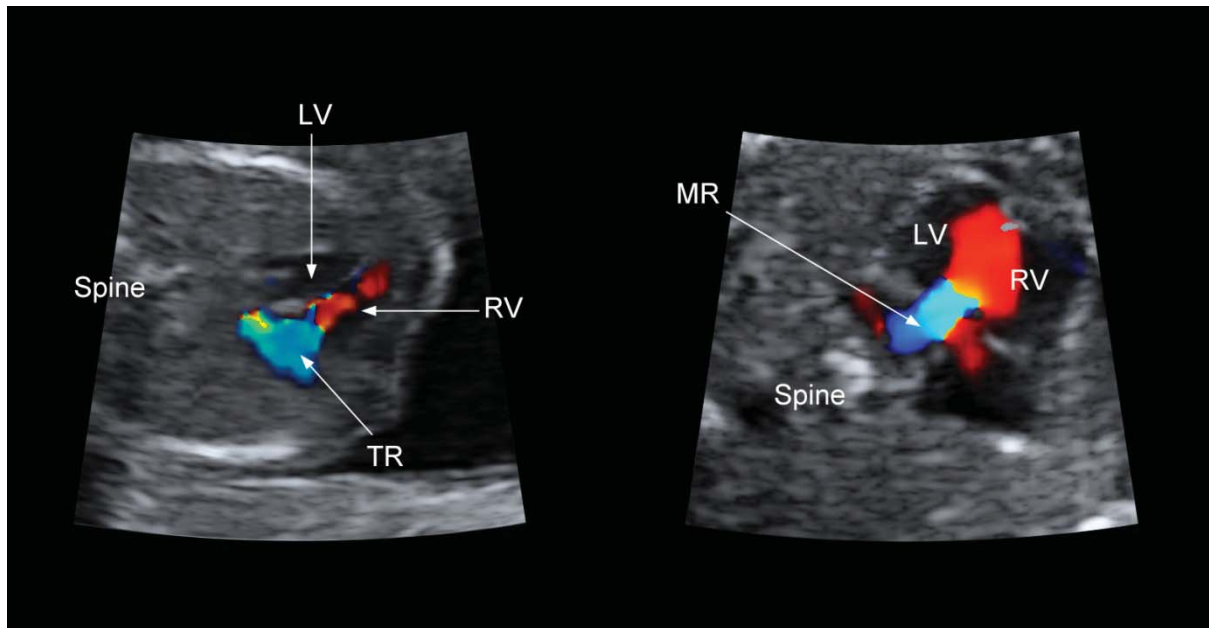


Figure 4 Echogenic intracardiac focus (EIF) and Atrio-ventricular canal during systole (*left*) and diastole (*right*)

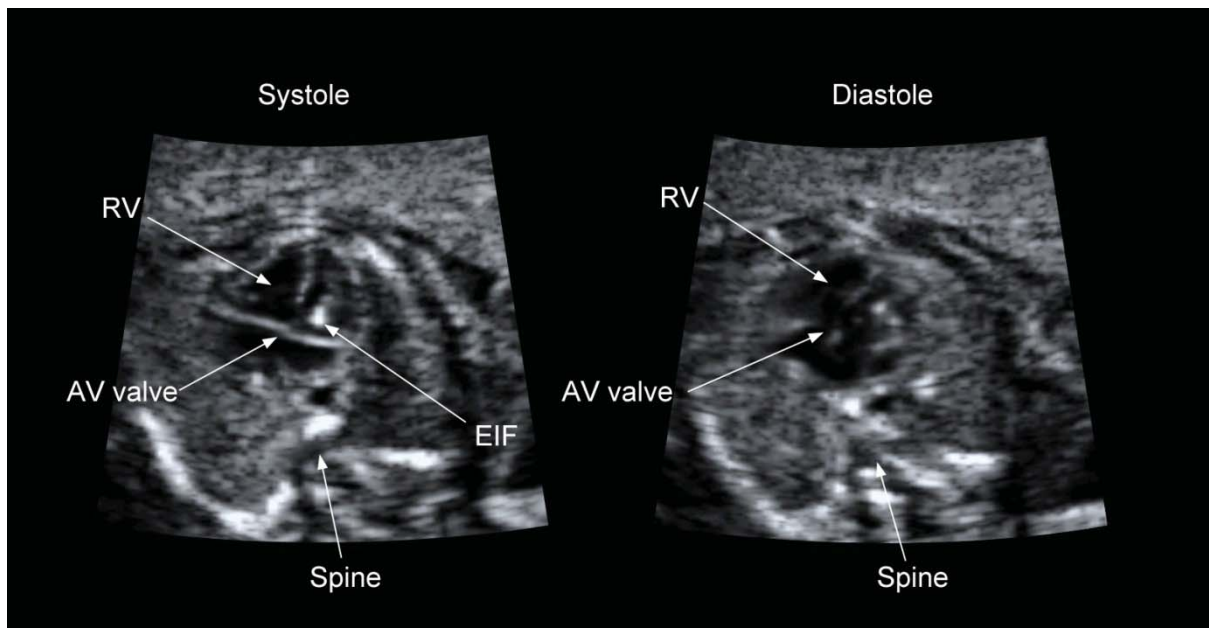
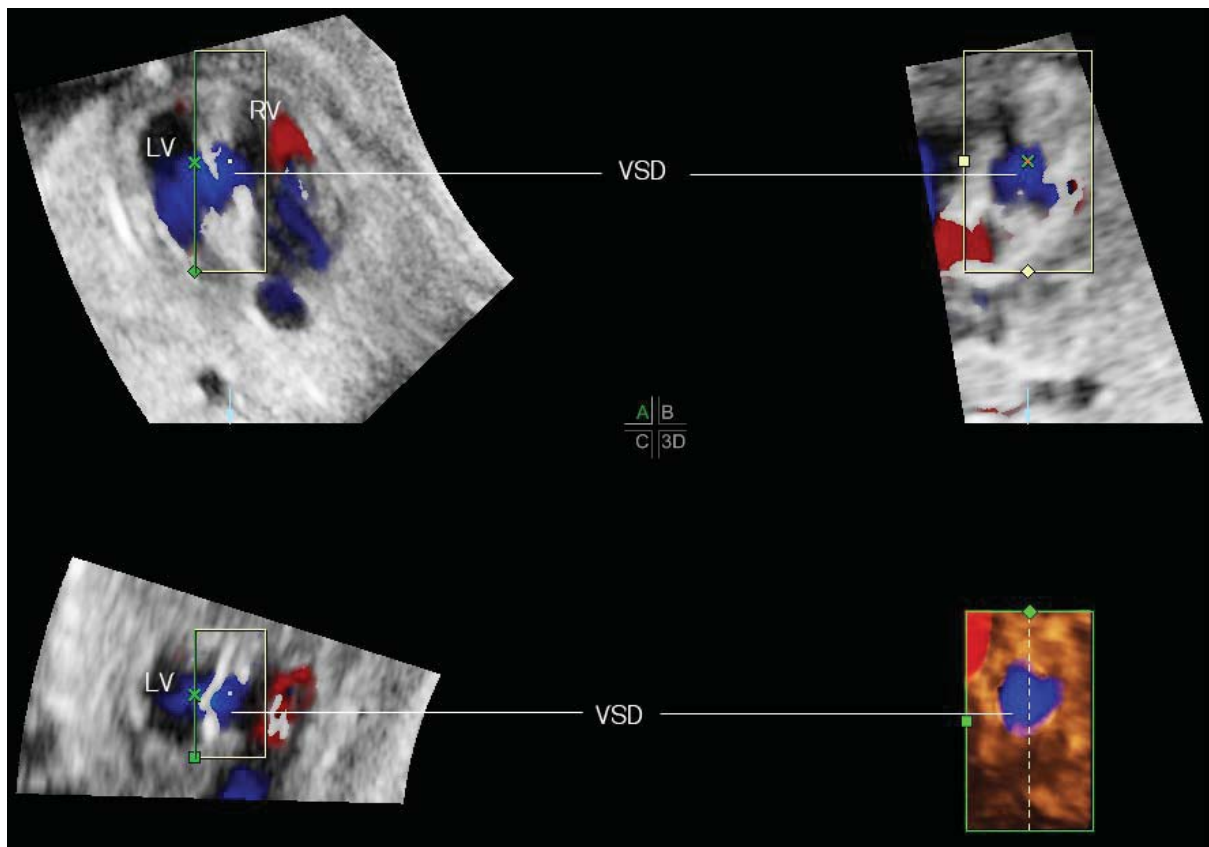


Figure 5 Render mode shows interventricular septal defect (VSD)



Discussion

Prevalence of cardiac markers among fetuses with Down syndrome has been varied from study to study. For examples, Nyberg et al(2), Vergani et al(3) and Paladini et al(12) reported the prenatal prevalence of congenital heart defect among fetuses with Down syndrome to be 10.6%, 23% and 56% respectively. The differences may be associated with degree of an attempt to look for, resolution of the ultrasound machine, and familiarity to fetal echocardiography. Additionally, most studies regarding genetic sonogram did not include cardiac makers other than echogenic foci or TR in some studies. The detection rate may be associated with degree of awareness of these markers. For example, ARSA is simple to diagnose if we are vigilant to look for. To look for all of the ten cardiac markers presented here as a systematic checklist may enhance the detection rate of fetal Down syndrome. Unlike other previous studies on genetic cardiac markers which reported only one or two markers, we took advantage of off-line cardio-STIC analysis without time limit to navigate thoroughly all over the VDSs to see any possible markers helping detect Down syndrome. Additionally, all cardiac dimensions were measured to achieve more objective markers. For

example, the measurement of cardiac chamber to identify chamber disproportion rather than subjective assessment as used in previous report(5). Likewise, the measurement of shortening fraction, which is relatively difficult using real-time 2D ultrasound, is simple and easy to obtain the correct planes with cardio-STIC-M.

Once a VDS is acquired, this can remotely be accessed and analyzed and may address the problem of lack of expertise especially with a view to making more technicians available to screen the fetal cardiac markers or even other congenital heart diseases. This experience signifies that it is possible for practitioners or technicians, who are not familiar to fetal echocardiography, to acquire cardio-STIC for later thorough off-line analysis in their own convenience without time limit, or online consultation with the experts for remote analysis. With innovative Cardio-STIC, the volumes of the fetal heart can be simply manipulated to obtain the correct cardiac plane exactly in the three orthogonal planes and all three axes. For example, we can easily control the volume to get the interventricular septum (IVS) to be exactly horizontal in all panels. This is critical for measurement of cardiac dimension especially in measuring chamber disproportion. With 2D ultrasound, it is very vulnerable to obtain an improper plane of the FCV, even ultrasound beam exactly perpendicular to the IVS, since it may be tilted toward or outward the examiners on the z-axis, resulting in asymmetry of both sides of the heart and inaccurate measurement. With 3D ultrasound, IVS can simply be maneuvered to be in the exact horizontal line in panel A and B and total en-face view of the IVS be displayed in the panel C. Correct specific anatomical landmarks for fetal cardiac measurements can be meticulously adjusted and controlled by manipulating the three axes on the multiplanar view in no time.

In screening test, detection rate, false positive rate and OAPR (odds of being affected, given a positive result or a ratio of affected positive: unaffected positive) are most of concern(13). With careful analysis of the cardio-STIC VDSs, these cardiac markers can modify the priori risk of fetal Down syndrome derived from maternal age and / or serum biochemical markers. The post-test odds or OAPR can be determined by multiplying the priori risk by the positive LR of each cardiac marker which is present and the negative LR of each marker searched for but not found(14). In addition to conventional genetic sonogram, cardiac markers may also add some value to modify the priori risk.

The prevalence of Down syndrome in this study was very high, 40 in 440 instead of 1 out of 600-1000 as commonly quoted. This was due to the fact that most patients with known diagnosis of fetal Down syndrome (from CVS or amniocentesis) were more likely to be invited to the study than those with unaffected fetuses. Nevertheless, the prevalence of the

disease typically affects the predictive value but not affect sensitivity and specificity. The sensitivity and specificity as well as likelihood ratio of cardiac markers were still reliable and could be used to calculate the post-test odds.

With cardiac markers, considered positive when any of the ten markers was present, about 70% of fetuses with Down syndrome were detected, with a false-positive rate of about 20%. Certainly, this unacceptably high false-positive rate may preclude cardiac markers being used as a primary screening tool. However, it can be helpful when used as an adjunct to other non-cardiac markers, especially used in women at high risk determined by their priori risk. Additionally, cardio-STIC genetic sonogram could be used for pregnant women who present late for prenatal care; i.e. after mid-pregnancy, and are therefore not eligible for serum screening. Cardio-STIC may be used as an adjunct to serum screening for Down syndrome to increase the detection rate. This may be advantageous for couples who desire the highest detection rate before considering invasive testing, though this issue needs to be elucidated by further studies. Using these markers to define the post-test risk in this group of pregnant women may decrease a number of invasive diagnostic procedures, while maintaining a high detection rate for Down syndrome.

Considering individual markers, VSD, cardiac disproportion and TR were sensitive markers (27-30% of cases) while AVC and ARSA had low sensitivity but relatively high specificity. The prevalence of atrioventricular canal (AVC) in fetuses with Down syndrome was relatively low when compared to postnatal studies. It was found in 10% of the affected fetuses, of which three were isolated findings. However, it had high specificity since no any case was found in normal fetuses. Except for ASD, the main specific CHDs (VSD, AVC, TOF etc) in this study were consistent with postnatal series. According to the large postnatal study of infants with Down syndrome, Cleves et al(15) reported that ASD was the most common CHD in affected infants, found in 17.36%, while it was identified in only 1 case (2.5%) in this study. It is reasonable to presume that ASD tends to be missed for diagnosis with cardio-STIC analysis, though final diagnosis of CHD was not confirmed in all our cases. Thus, it is a limitation of prenatal cardio-STIC. We hope that 4D ultrasound with better resolution in the future innovation will improve the diagnosis of ASD. Of note, shortening fraction of the ventricles, which has never been used as genetic sonogram before, can add significant detection rate. Surprisingly, shortening fraction of both ventricles seems to be better in fetuses with Down syndrome, suggesting improved ventricular performance. This finding was consistent with that in postnatal series reported by Russo MG, et al(16), who found that shortening fraction of left ventricle, reflecting systolic function, in 22 children

with Down syndrome was significant higher than that in normal control. It was unclear what mechanism was responsible for such an observation in these fetuses but it is hypothesized that the better performance of the left ventricle in adults with Down syndrome is associated with the reduction in afterload(16).

The limitations may include some technical limits of STIC, such as TR assessment, which was relied on only CFM, not coupled with spectral pulsed Doppler analysis in cardio-STIC. This study focused on only cardiac markers derived from cardio-STIC VDS, therefore how much they added diagnostic value to conventional genetic sonogram could not be determined. However, it could theoretically enhance the sensitivity, as seen in the study reported by DeVore(5), who found cardiac markers significantly improved the detection rate. Moreover, the problem commonly encountered with cardio-STIC is that low quality volumes lead to difficulty in analyses in some VDSs. Thus the principles of careful acquisition to obtain high quality volume must be strictly followed. Additionally, cardiac markers in this study were not confirmed by autopsy or postnatal life. Therefore, sonographic markers might have not been consistent with the real pathology. Finally, though the cardio-STIC VDS were acquired several performers, the volume analyses were based on a single experienced operator. The study does not address the inter- and intra-observer variability of the assessment. Further studies are needed to address the outstanding issues as well as the potential value of this assessment in screening Down syndrome.

The strength of this study may include: 1) the advantages of cardio-STIC itself, especially ability to manipulate VDSs to get proper planes for measurement, 2) blinded interpretation of the VDS by the author who had no any information of the patients could make a comparison more reliable, 3) homogeneity of gestational age, confined to 16-22 weeks of gestation, and 4) inclusion of all cardiac markers which have never described elsewhere before.

In conclusion, cardio-STIC analysis can detect about 70% of fetal Down syndrome with a false positive rate 20%. It can be helpful in estimation of fetal risk for Down syndrome and counseling when the prenatal diagnosis of the syndrome is made.

Acknowledgement

The authors wish to thank the Thailand Research Fund and the National Research University Project under Thailand's Office of the Higher Education Commission for financial support.

Conflict of Interest: None

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Reference Ranges of Myocardial Performance Index from 12-40 Weeks of Gestation

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Words count = 2064 (excluding abstract & references)

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Short title: Fetal MPI from 14-40 weeks

Key Words: Fetus, myocardial performance index, Prenatal diagnosis, Reference range, Tei index, Z-scores

Abstract

Objective: To establish reference ranges of fetal myocardial performance index (MPI) in normal singleton pregnancies from 12 to 40 weeks gestation.

Design: Prospective descriptive study

Setting: A tertiary care teaching center in the Northern part of Thailand

Participants: A total of 562 normal singleton pregnancies at 12-40 weeks of gestation

Intervention: Fetal echocardiography was performed for isovolumetric contraction time (ICT), isovolumetric relaxation time (IRT), ejection time (ET) and MPI by one experienced sonographer, using strict criteria for measurement. The measured MPI values were regressed against gestational age (GA) and biparietal diameter (BPD) to determine the best-fitted model.

Primary outcome measures: MPI for each gestational week and each BPD point (cm)

Results: A total of 562 measurements were successfully obtained with good quality Doppler tracings and complete data for analysis. Predicted mean of MPI for GA and BPD are as follows: Predicted mean MPI = $0.404 + 0.004 \times \text{GA (weeks)}$ ($r^2 = 0.143$, $p < 0.001$) and $= 0.419 + 0.015 \times \text{BPD (cm)}$ ($r^2 = 0.139$, $p < 0.001$) as functions of GA and BPD, respectively. The predicted SD of MPI for GA and BPD are constant throughout pregnancy, 0.071348 and 0.073440, respectively.

Conclusion: MPI are significantly increased with gestational age. The reference ranges of MPI according to gestational age and BPD, as a marker in evaluating global cardiac function between 12 -40 weeks of gestation was constructed.

Key Words: *Fetus, myocardial performance index (MPI), prenatal diagnosis, Reference range, Tei index, Z-scores*

Article summary

1) Article Focus

- MPI (myocardial performance index of the fetues) values at each gestational week

2) Key Messages

- MPI are significantly increased with gestational age.
- The reference ranges of MPI according to gestational age and BPD were constructed.
- These normative data may be used for evaluating global cardiac function

3) Strengths: Adequate overall sample size, only one operator (no interobserver variation)

Limitations: small sample size in some weeks of gestation, poor quality images in some cases

Introduction

Fetal echocardiography has been developed for evaluating cardiac abnormality in both structure and function, especially pulsed Doppler examination in assessment of fetal hemodynamic[1]. In 1995, Tei et al[2] presented myocardial performance index (MPI) or Tei index for evaluating cardiac function in adult. MPI, as a noninvasive Doppler-derived tool, incorporates both systolic and diastolic time intervals in expressing global systolic and diastolic ventricular function. MPI has been reported in the literature in assessment of myocardial performance in a variety of clinical conditions in adults including myocardial infarction dilated cardiomyopathy and amyloidosis[3]. Tsutsumi et al[4] were the first to report using MPI to assess fetal global myocardial function, followed by several studies[5-8]. In 2005, Ichizuka K[9] found that MPI may be a useful tool for the assessment of fetal cardiac status in a variety of sick fetuses. Recipient fetuses in twin-to-twin transfusion syndrome or large fetuses of diabetic mothers and fetuses with hydrops may show abnormal MPI in early phase of such conditions. In 2013, we also reported the association of abnormal MPI and fetus with hemoglobin Bart's disease[10] and MPI can be used as an effective marker for fetal cardiac dysfunction. However, in evaluation of the effectiveness of MPI in predicting fetal cardiac abnormality, it is essential that normal reference ranges of the marker must first be established and we should also have our own normative data for evaluation of pregnancies in our own population. Furthermore, normal reference ranges provided by the previous studies are very controversial and confusing. It was very difficult to clinically use previous published reference ranges because of markedly different values among those studies caused by different techniques in measurement and small sample size in most reports. Therefore, this study aimed to establish normal reference ranges of fetal MPI from 12-40 weeks of gestation using with well-defined criteria of measurement.

Patients and Methods

This prospective study was conducted at Maharaj Nakorn Chiang Mai hospital, Chiang Mai University between October 2010 and March 2013, with approval by the Ethic Committee. Normal singleton pregnancies were recruited with written informed consents. Inclusion criteria were 1) accurate gestational age based on well-defined regular menstrual period and consistent with sonographic fetal biometry in the first half of pregnancies, 2) gestational age of 12–40 weeks, 3) singleton pregnancies, and 4) no obstetric and medical complications. Exclusion criteria were 1) fetal anomalies, 2) fetal growth restriction or macrosomia, and 3) poor quality imaging. Fetal echocardiography was performed using a real-time scanner with GE Voluson E8 (GE Medical Systems, Zipf, Austria), equipped with a transabdominal curvilinear transducer of frequency 2 to 5 MHz. The Doppler tracings were recorded during fetal quiescence over 4-5 cardiac cycles. In each examination, a standard ultrasound examination, including fetal anomaly screening and fetal biometry, was also performed.

MPI measurements

At the level of apical four-chamber view of the heart, the Doppler sample volume (2-4 mm) was placed to include both the lateral wall of the ascending aorta and the mitral valve where the clicks corresponding to the opening and closing of the two valves were clearly visualized. Images that use for measurements of MPI were obtained by placing the sample volume at the outflow tract of the left ventricle to obtain the aortic and mitral flow simultaneously. Gain was usually reduced substantially to get the valve clicks more echogenic than the EA and aortic waveforms. The EA waveforms were displayed as a positive flow. The maximum speed of Doppler sweep velocity was used. To be more precise measurement in large zoomed images, all parameters were measured with off-line, using Adobe photoshop software by only one sonographer to increase accuracy and to reduce inter-observer variation. All valve clicks were used to identify the closure and opening of the atrio-ventricular and semilunar valves during the measurement of the time intervals. The ICT, ET, and IRT were calculated using the beginning of the mitral and aortic valve clicks as markers and expressed in milliseconds, (four decimals). The time cursor was placed just before the echo of each valve click. The cursor was placed as close as possible to the echo of the click, but not including the white echo, as described by Hernandez-Andrade et al[7]. At least three consecutive waveforms with high quality were measured. MPI (Tei index) is defined as the sum of the isovolumetric

contraction time (ICT) and the isovolumetric relaxation time (IRT), divided by the ventricular ejection time (ET): $(ICT+IRT)/ET$. (Figure 1)

Statistical analysis: The collected data were analyzed using the Statistical Package for the Social Sciences (SPSS), version 17.0 (Chicago, IL). Regression analysis was used to express the correlation between MPI and gestational ages. The best-fit regression equation was determined using technique suggested by Royston and Wright[11] The regression models were fitted separately to the mean and SD of the DA diameter as functions of gestational age and biparietal diameter (BPD) to generate the best-fitted regression equations. The assumption of normality and the goodness of fit of each regression model were checked by examination of the scatter patterns of points relative to fitted means and SDs, expressed as Z-scores. Z-scores were assessed for normality by Q-Q plots and the Shapiro-Francia W' test. The nomograms of MPI was constructed for the 2.5th, 5th, 10th, 50th, 90th , 95th and 97.5th percentile and Z score for each gestational week and BPD. Intra-observer variability was identified with intraclass correlation coefficient (ICC) technique.

Results:

Of 577 examinations, 15 were excluded due to incomplete outcomes, 562 were available for analysis. Of them, mean ($\pm$ SD) maternal age was 27.15 ± 5.87 years. Mean ($\pm$ SD) gestational age at delivery was 38.82 ± 1.39 weeks. Mean ($\pm$ SD) birth weight was 2999.72 ± 409.34 grams. Of them, 47.5% were nulliparous and 52.5 were parous women.

Predicted mean of MPI, based on gestational weeks and BPD, was best described by linear regression model for both gestational age (GA) and BPD. The models are as follows: Predicted mean MPI = $0.404 + 0.004 \times \text{GA (weeks)}$ ($r^2 = 0.143$, $p < 0.001$) (Figure 2) with predicted SD of MPI = 0.071348 (relatively constant, irrespective of gestational age) (Figure 3) and predicted mean MPI = $0.419 + 0.015 \times \text{BPD (cm)}$ ($r^2 = 0.139$, $p < 0.001$) (Figure 4) with predicted SD of MPI = 0.073440 (relatively constant, irrespective of BPD). The distribution of calculated Z-scores of MPI against gestational age or BPD indicating adequate level of fit (Figure 5). Intra-observer was analyzed and found good agreement with intra-class correlation coefficient of 0.834 (95% CI 0.822-0.866, $p < 0.001$).

From the models, Z-scores can readily be calculated using the formula: $\text{Z-score} = [\text{Measured MPI} - \text{Predicted mean MPI}] / \text{Predicted SD of MPI}$. For example, Z-score for the measured MPI of 0.66 of a fetus at gestational age of 21 weeks can be calculated as follows: Predicted mean MPI = $0.404 + 0.004 \times \text{GA (weeks)} = 0.488$; Predicted SD of MPI = 0.071348; $\text{Z-score} = (0.62 - 0.488) / 0.071348 = 2.41$. Therefore, the fetus has an abnormal MPI, at 2.41 SD above the predicted mean fetal MPI for a fetus whose gestational age of 21 weeks; out of normal range.

Table 1: Fetal myocardial performance index (MPI) as a function of gestational age*(N represents the sample size for each gestational weeks)*

GA wk	N	Myocardial performance index (MPI)						
		2.5th	5th	10th	50th	90th	95th	97.5th
12	15	0.31	0.34	0.36	0.45	0.55	0.57	0.59
13	15	0.32	0.34	0.37	0.46	0.55	0.58	0.60
14	16	0.32	0.35	0.37	0.46	0.55	0.58	0.60
15	14	0.33	0.35	0.38	0.47	0.56	0.58	0.61
16	20	0.33	0.35	0.38	0.47	0.56	0.59	0.61
17	23	0.34	0.36	0.38	0.48	0.57	0.59	0.62
18	41	0.34	0.36	0.39	0.48	0.57	0.60	0.62
19	40	0.34	0.37	0.39	0.48	0.57	0.60	0.62
20	41	0.35	0.37	0.40	0.49	0.58	0.61	0.63
21	37	0.35	0.37	0.40	0.49	0.58	0.61	0.63
22	23	0.36	0.38	0.40	0.50	0.59	0.61	0.64
23	15	0.36	0.38	0.41	0.50	0.59	0.62	0.64
24	22	0.36	0.39	0.41	0.50	0.60	0.62	0.64
25	17	0.37	0.39	0.42	0.51	0.60	0.63	0.65
26	15	0.37	0.40	0.42	0.51	0.60	0.63	0.65
27	12	0.38	0.40	0.43	0.52	0.61	0.63	0.66
28	20	0.38	0.40	0.43	0.52	0.61	0.64	0.66
29	18	0.39	0.41	0.43	0.53	0.62	0.64	0.67
30	19	0.39	0.41	0.44	0.53	0.62	0.65	0.67
31	20	0.39	0.42	0.44	0.53	0.63	0.65	0.67
32	23	0.40	0.42	0.45	0.54	0.63	0.66	0.68
33	16	0.40	0.43	0.45	0.54	0.63	0.66	0.68
34	12	0.41	0.43	0.46	0.55	0.64	0.66	0.69
35	16	0.41	0.43	0.46	0.55	0.64	0.67	0.69
36	13	0.42	0.44	0.46	0.56	0.65	0.67	0.70
37	14	0.42	0.44	0.47	0.56	0.65	0.68	0.70
38	14	0.42	0.45	0.47	0.56	0.65	0.68	0.70
39	7	0.43	0.45	0.48	0.57	0.66	0.69	0.71
40	3	0.43	0.45	0.48	0.57	0.66	0.69	0.71

Table 2: Fetal myocardial performance index (MPI) as a function of biparietal diameter (cm)

BPD	2.5 th	5 th	10 th	50 th	90 th	95 th	97.5 th	BPD	2.5 th	5 th	10 th	50 th	90 th	95 th	97.5 th
2.5	0.31	0.34	0.36	0.46	0.55	0.58	0.60	6.0	0.37	0.39	0.42	0.51	0.60	0.63	0.65
2.6	0.31	0.34	0.36	0.46	0.55	0.58	0.60	6.1	0.37	0.39	0.42	0.51	0.60	0.63	0.66
2.7	0.32	0.34	0.37	0.46	0.55	0.58	0.60	6.2	0.37	0.39	0.42	0.51	0.60	0.63	0.66
2.8	0.32	0.34	0.37	0.46	0.55	0.58	0.61	6.3	0.37	0.39	0.42	0.51	0.60	0.63	0.66
2.9	0.32	0.34	0.37	0.46	0.56	0.58	0.61	6.4	0.37	0.40	0.42	0.52	0.60	0.63	0.66
3.0	0.32	0.34	0.37	0.46	0.56	0.58	0.61	6.5	0.37	0.40	0.42	0.52	0.61	0.63	0.66
3.1	0.32	0.35	0.37	0.47	0.56	0.58	0.61	6.6	0.38	0.40	0.43	0.52	0.61	0.63	0.66
3.2	0.32	0.35	0.37	0.47	0.56	0.59	0.61	6.7	0.38	0.40	0.43	0.52	0.61	0.63	0.66
3.3	0.33	0.35	0.37	0.47	0.56	0.59	0.61	6.8	0.38	0.40	0.43	0.52	0.61	0.64	0.67
3.4	0.33	0.35	0.38	0.47	0.56	0.59	0.61	6.9	0.38	0.40	0.43	0.52	0.61	0.64	0.67
3.5	0.33	0.35	0.38	0.47	0.56	0.59	0.62	7.0	0.38	0.40	0.43	0.53	0.61	0.64	0.67
3.6	0.33	0.35	0.38	0.47	0.57	0.59	0.62	7.1	0.38	0.41	0.43	0.53	0.62	0.64	0.67
3.7	0.33	0.35	0.38	0.48	0.57	0.59	0.62	7.2	0.38	0.41	0.43	0.53	0.62	0.64	0.67
3.8	0.33	0.36	0.38	0.48	0.57	0.60	0.62	7.3	0.39	0.41	0.44	0.53	0.62	0.65	0.67
3.9	0.33	0.36	0.38	0.48	0.57	0.60	0.62	7.4	0.39	0.41	0.44	0.53	0.62	0.65	0.68
4.0	0.34	0.36	0.39	0.48	0.57	0.60	0.62	7.5	0.39	0.41	0.44	0.53	0.62	0.65	0.68
4.1	0.34	0.36	0.39	0.48	0.57	0.60	0.63	7.6	0.39	0.41	0.44	0.53	0.63	0.65	0.68
4.2	0.34	0.36	0.39	0.48	0.57	0.60	0.63	7.7	0.39	0.42	0.44	0.54	0.63	0.65	0.68
4.3	0.34	0.36	0.39	0.48	0.57	0.60	0.63	7.8	0.39	0.42	0.44	0.54	0.63	0.65	0.68
4.4	0.34	0.36	0.39	0.49	0.57	0.60	0.63	7.9	0.39	0.42	0.44	0.54	0.63	0.66	0.68
4.5	0.34	0.37	0.39	0.49	0.58	0.60	0.63	8.0	0.40	0.42	0.45	0.54	0.63	0.66	0.68
4.6	0.34	0.37	0.39	0.49	0.58	0.61	0.63	8.1	0.40	0.42	0.45	0.54	0.63	0.66	0.69
4.7	0.35	0.37	0.40	0.49	0.58	0.61	0.63	8.2	0.40	0.42	0.45	0.54	0.63	0.66	0.69
4.8	0.35	0.37	0.40	0.49	0.58	0.61	0.64	8.3	0.40	0.42	0.45	0.54	0.64	0.66	0.69
4.9	0.35	0.37	0.40	0.49	0.58	0.61	0.64	8.4	0.40	0.43	0.45	0.55	0.64	0.66	0.69
5.0	0.35	0.37	0.40	0.49	0.58	0.61	0.64	8.5	0.40	0.43	0.45	0.55	0.64	0.67	0.69
5.1	0.35	0.38	0.40	0.50	0.59	0.61	0.64	8.6	0.41	0.43	0.46	0.55	0.64	0.67	0.69
5.2	0.35	0.38	0.40	0.50	0.59	0.61	0.64	8.7	0.41	0.43	0.46	0.55	0.65	0.67	0.70
5.3	0.36	0.38	0.41	0.50	0.59	0.61	0.64	8.8	0.41	0.43	0.46	0.55	0.65	0.67	0.70
5.4	0.36	0.38	0.41	0.50	0.59	0.62	0.64	8.9	0.41	0.43	0.46	0.55	0.65	0.68	0.70
5.5	0.36	0.38	0.41	0.50	0.59	0.62	0.65	9.0	0.41	0.43	0.46	0.56	0.65	0.68	0.70
5.6	0.36	0.38	0.41	0.50	0.59	0.62	0.65	9.1	0.41	0.44	0.46	0.56	0.65	0.68	0.70
5.7	0.36	0.38	0.41	0.51	0.59	0.62	0.65	9.2	0.41	0.44	0.46	0.56	0.66	0.68	0.70
5.8	0.36	0.39	0.41	0.51	0.60	0.62	0.65	9.3	0.42	0.44	0.47	0.56	0.66	0.68	0.70
5.9	0.36	0.39	0.41	0.51	0.60	0.62	0.65	9.4	0.42	0.44	0.47	0.56	0.66	0.69	0.71

Figure 1: Examples of MPI measurement; 1) Isovolumetric Contraction Time (ICT), 2) Ejection Time (ET), 3) Isovolumetric Relaxation Time (IRT)

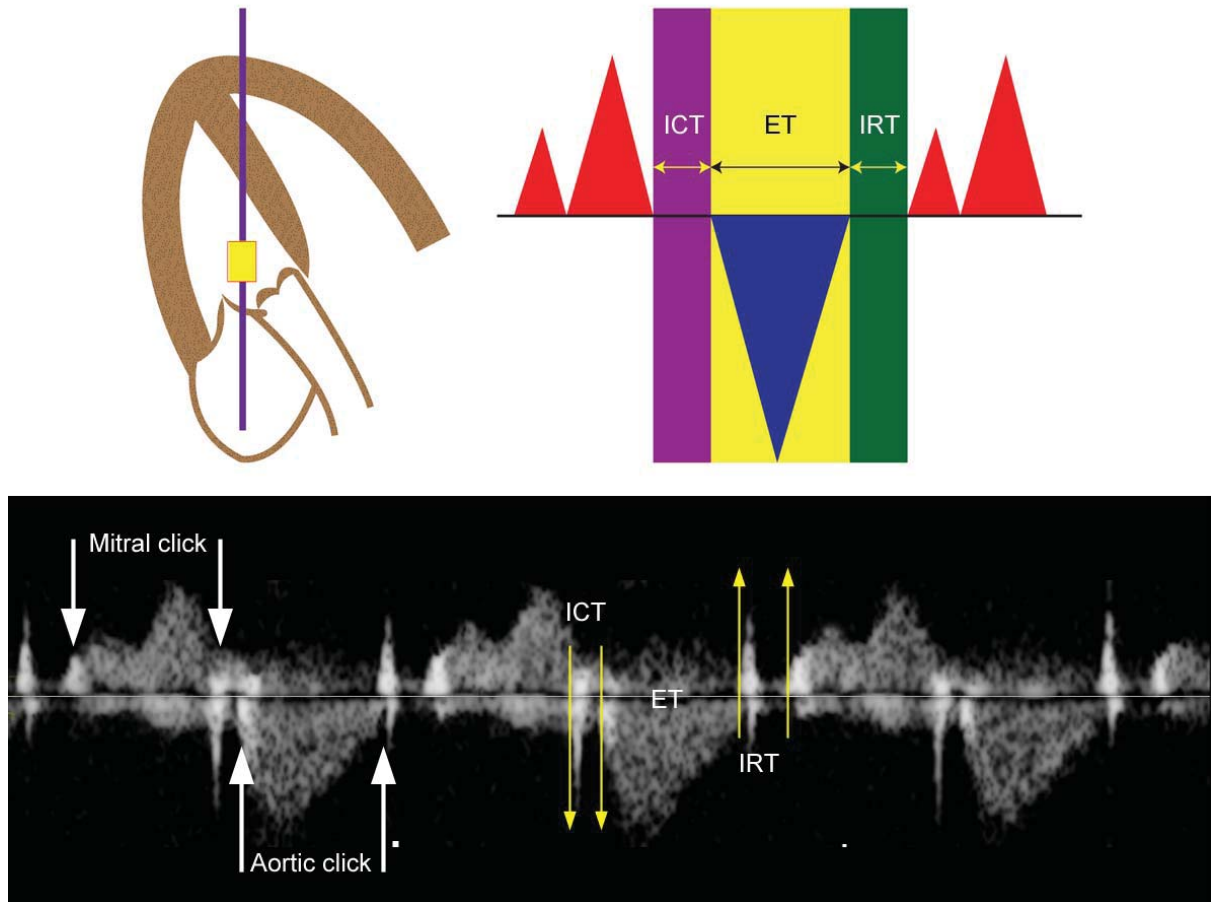


Figure 2: Linear function relationship between gestational age in weeks and MPI (The lines represent 2.5th, 5th, 10th, 50th, 90th, 95th, and 97.5th percentile)

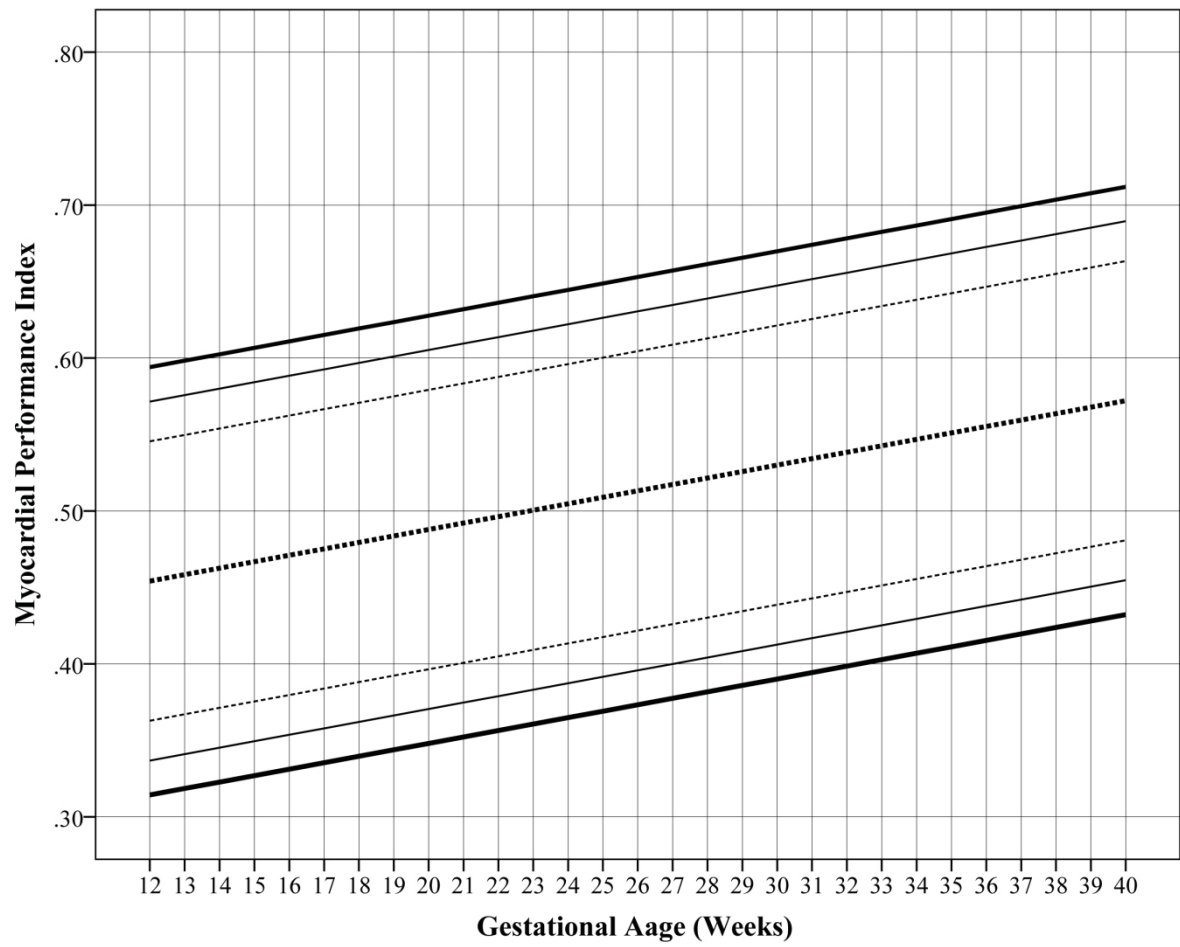


Figure 3: Scaled absolute residuals of MPA appear to show no trend with gestational age
(dotted line representing the mean of scaled absolute residuals)

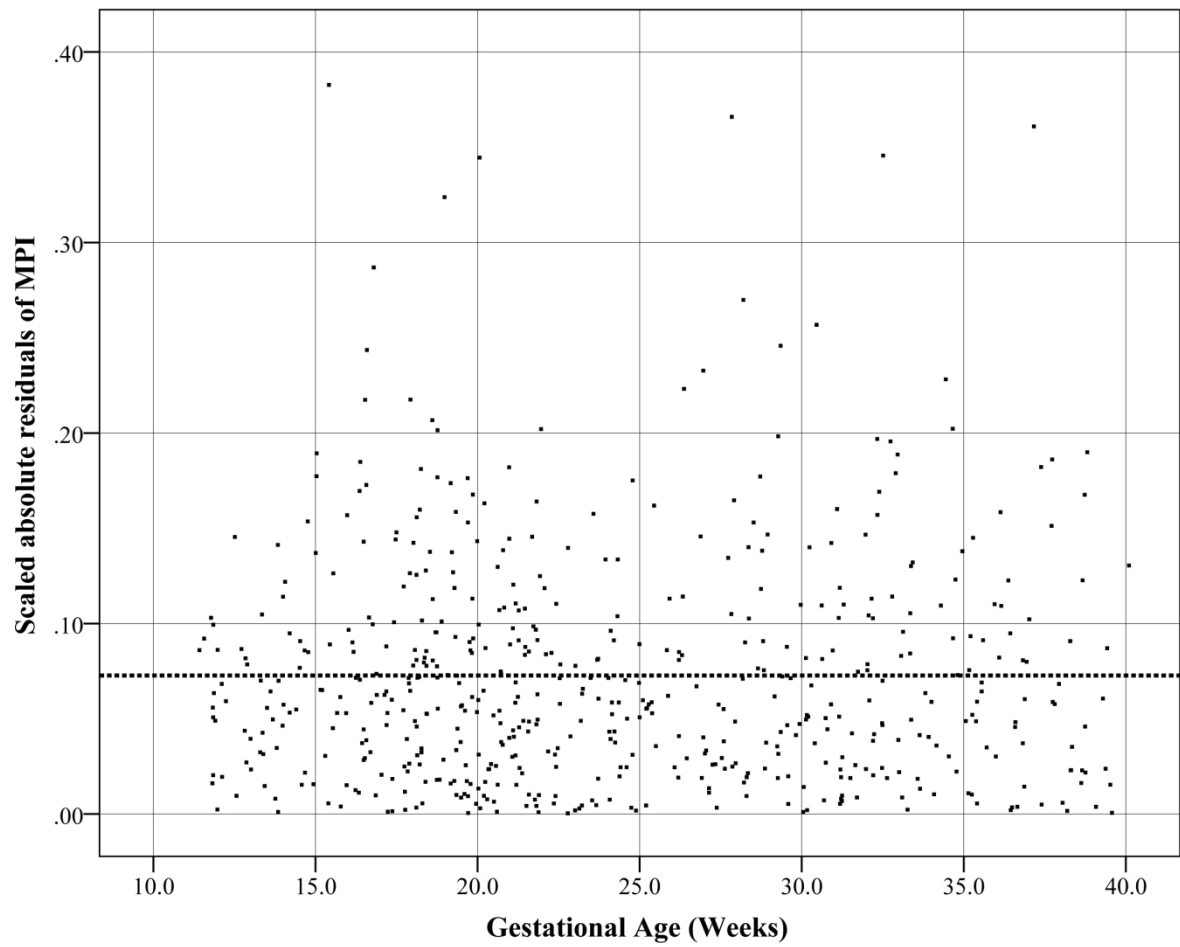


Figure 4: Linear relationship between biparietal diameter (cm) and MPI (The lines represent 2.5th, 5th, 10th, 50th, 90th, 95th, and 97.5th percentile)

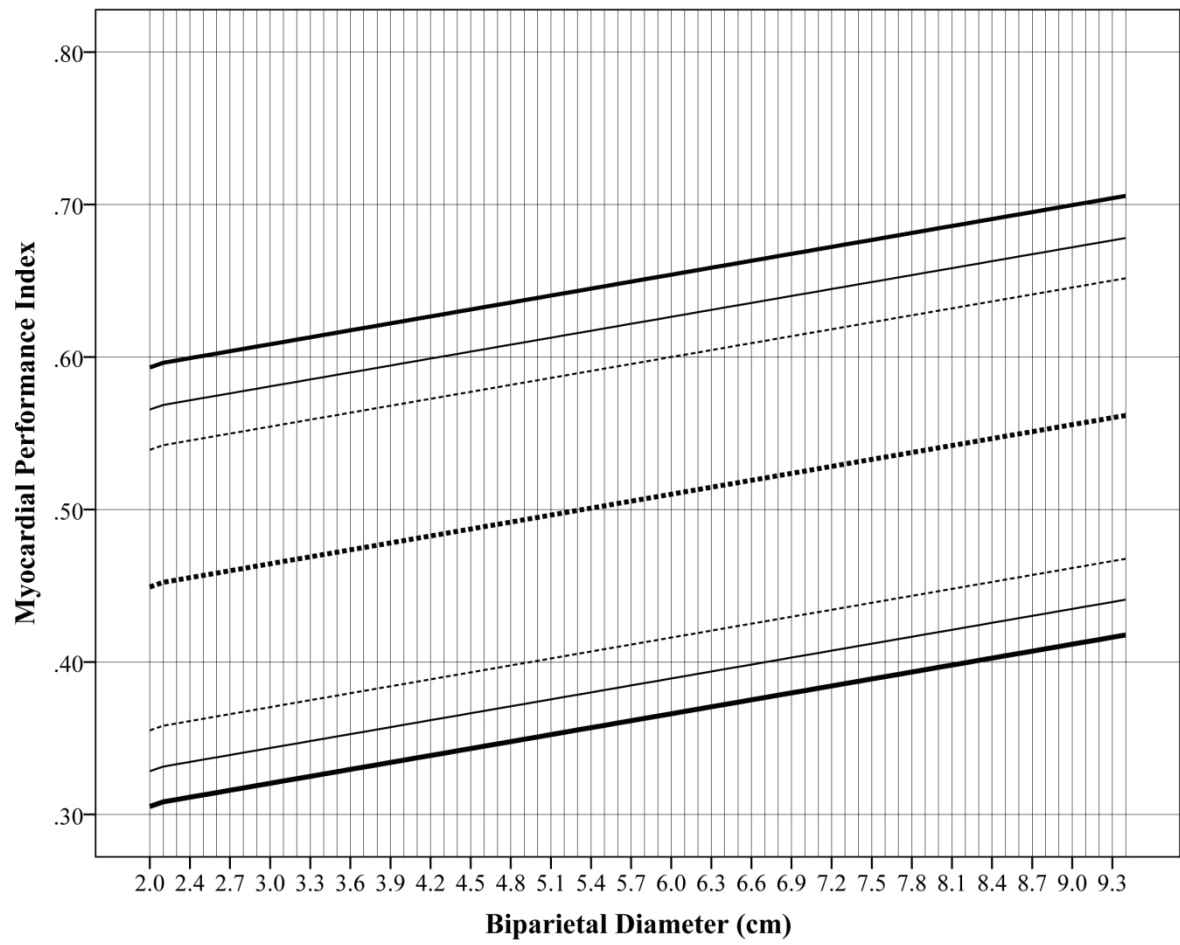
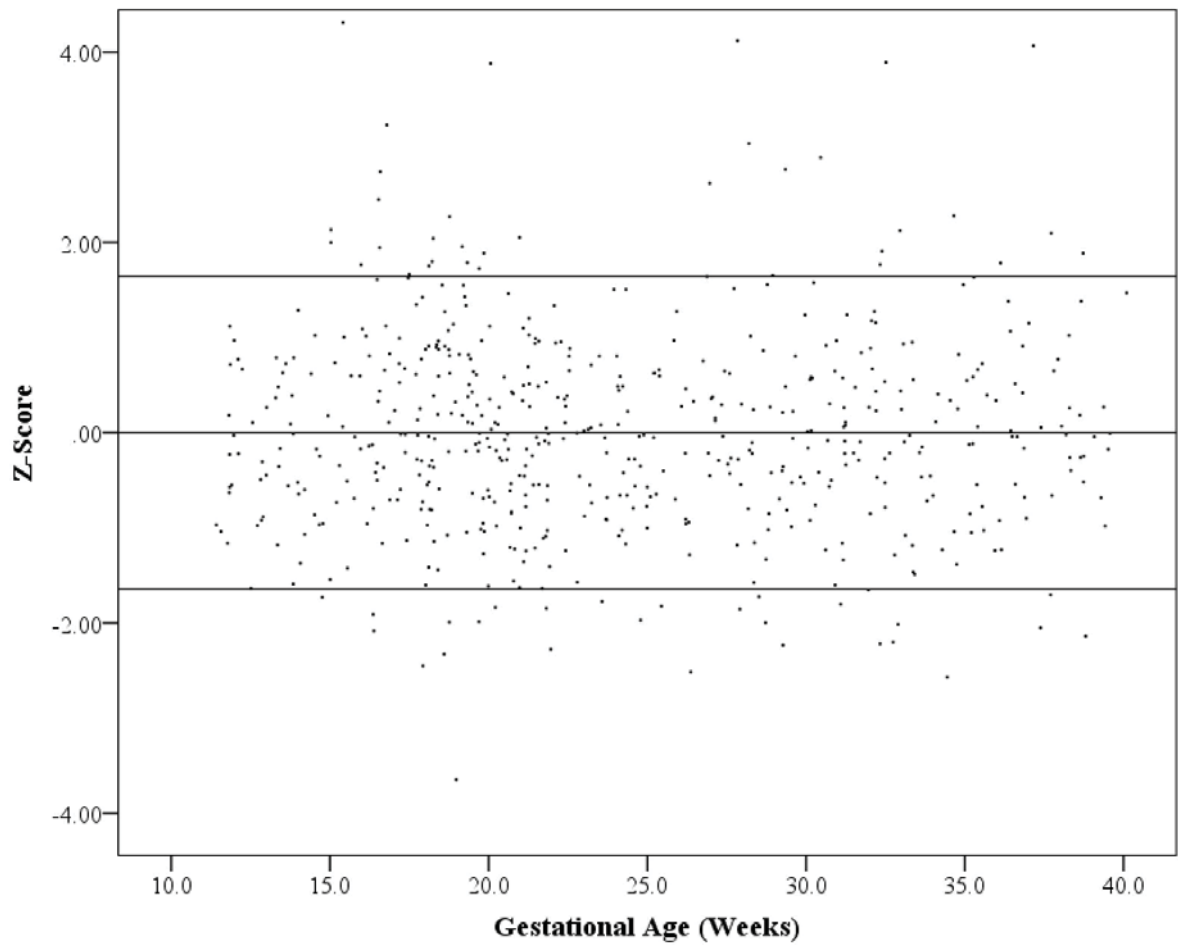


Figure 5: Distribution of calculated Z-scores of MPI against gestational age indicating adequate level of fit



Discussion

Fetal cardiac function can be assessed by many techniques such as 2D real time, M-mode, color Doppler, pulse Doppler and recently STIC technique. Over the last decade, MPI or Tei index has been more commonly used to evaluate fetal global cardiac function, both systolic and diastolic ventricular contraction. For example, systolic dysfunction prolongs pre-ejection (isovolumetric contraction time, ICT) and a shortening of the ejection time (ET), whereas both systolic and diastolic dysfunctions result in abnormality in myocardial relaxation which prolongs the relaxation period (IRT). We found the MPI values were significantly increased with gestational age and BPD. Compared to other previous studies, our results are most consistent with those reported by Cruz-Martinez et al[12], though the MPI values in our study were slightly greater and the best fitted models were somewhat different. Simple linear equation was our best model for both gestational age and BPD, while Cruz-Martinez et al[12] a third degree cubic polynomial model after a natural logarithmic transformation was the best. Moreover, we found that the predicted mean SD of MPI was relatively constant for both gestational age and BPD (Figure 4), no need for regression of SD to determine the predicted mean SD. Our simple model was associated with high reliability as evidenced by distribution of calculated Z-scores of MPI against gestational age and BPD indicating adequate level of fit, less than 10% confined out of 95% range as seen in Figure 5.

Previous studies showed that the normal reference ranges of mean MPI have wide variations. Some studies found that the mean MPI is relatively constant throughout pregnancy, between 0.35 to 0.6[4,6,13,14], while other studies demonstrated that the MPI values are gestational age-dependent[4,5,7,15]. Moreover, some studies showed that the values progressively decrease with gestational age[4,5]. The discrepancies may probably be due to the lack of agreement in the technique measuring the time periods of the MPI and more importantly, small sample size in several previous studies could not show a small age-dependent change. Of note, the series with large sample size[8,12], including this study, showed consistently increasing values of MPI with gestational age. Though controversial, based on the most large and recent studies it is very convincing that MPI values are age-dependent. The measurement of MPI should be performed in a stringent manner with well-defined criteria. Note that several criteria for time period measurement of MPI have been used in previous studies, especially the point for placing the cursor. For examples, placing the cursor within the echo of the valve clicks[16], or from the end to the beginning of the valve clicks[1], or just before

the echo of the valve click[7]. Additionally, some studies did not define the method used to delimitate the time periods has not been defined[16,17].

Unlike all previous studies, in which the MPI measurements have been compared only with gestational age, our study also provided reference ranges of MPI values as a function of BPD. As expected, MPI values were significantly increased with BPD. Though, most reference ranges of cardiac dimension are usually presented as a function of gestational age. Cardiac parameters based on gestational age can often be problematic since gestational age is a derived value from regression analysis of measured values of the fetal head or femur that introduces an additional error. In the postnatal period, cardiac measurements are compared with the size of the individual and not his or her age. For these reasons, it is preferable to directly compare MPI measurements with the fetal biparietal diameter. This allows for the comparison of the cardiac parameters with the size of the fetus independent of its age.

This study established the normal reference ranges of mean MPI with wide range of gestational age, between 12-40 weeks that included late first trimester until term. Mean MPI was calculated only from left ventricle because it was easy for practical use and more reproducible. Additionally, left MPI can represent the global function, thus right MPI is not necessary. The technique of valve click was used to decrease the intra-observer variation in measurement. The result showed a significant correlation of mean MPI and gestational ages. The mean MPI increased with gestational age, from 0.45 at 12 weeks of gestation to 0.57 at 40 week of gestation.

The strength of this study included 1) clear criteria for the measurement and calculation of MPI, specifying placement of the calipers; 2) the measurements were performed by only one of the authors, no bias introduced by inter-observer variability; 3) larger sample size, compared to some other previous study[4,5,8,15] and wide range of gestational age from 12 to 40 weeks of gestation, though the number of cases per week was varied, ranging 3 to 41. Another weakness should be mentioned was that many cases were excluded due to poor quality of images. This may reflect a limitation of clinical application of MPI in some cases.

In conclusion, left MPI values were significantly increased with gestational age and BPD as a linear model. The tables of nomogram as well as regression model for Z-score calculation are provided. This is the first study of reference ranges of MPI measurements as a function of

both gestational age and BPD and can be clinically used as baseline data for comparison in assessment of fetal cardiac function under various pathological conditions.

Acknowledgement

The authors wish to thank the Thailand Research Fund (Grant number: RTA5380008) and the National Research University Project under Thailand's Office of the Higher Education Commission for financial support.

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