Feto-maternal Hemorrhage after Cordocentesis at Maharaj Nakorn Chiang Mai Hospital

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Objective: *To evaluate the incidence and volume of feto-maternal hemorrhage following cordocentesis.*
Study Design: *Descriptive study.*
Material and Method: *One hundred and sixteen asymptomatic non-anemic pregnant women with an indication for cordocentesis at 18-22 weeks of gestation between January and June 2004 were recruited. Maternal blood samples were obtained immediately before and 30 minutes after cordocentesis. Fetal cells in the maternal blood were counted using Kleihauer Betke test. About 25,000 maternal cells per slide were scanned by the same examiner. Feto-maternal hemorrhage was considered significant if the fetal bleeding was more than 0.25 ml.*
Results: *There was a significant increase in fetal blood volume in maternal circulation after cordocentesis (Paired Student's t test, $p < 0.001$). A significant hemorrhage (> 0.25 ml) occurred in 63 from 116 women (54.7%). Only one had marked hemorrhage of more than 5.0 ml and none had massive hemorrhage (> 15 ml).*
Conclusion: *Cordocentesis at 18-22 weeks of gestation can be associated with feto-maternal hemorrhage in more than half of the cases but nearly all cases had only minimal hemorrhage and none had massive hemorrhage.*

Keywords: *Cordocentesis, Feto-maternal hemorrhage, Prenatal diagnosis*

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Fetal blood sampling with cordocentesis has been used for more than 20 years and is used with increasing frequency for prenatal diagnosis and fetal therapy. Availability of high resolution ultrasound and guiding devices have contributed to the widespread acceptance of the technique, so that nearly all centers offering targeted fetal ultrasound examination can also provide fetal blood sampling. Concomitantly, the indications for fetal blood sampling have grown considerably. In the authors' extensive experience, the most common indication is the risk of severe thalassemia syndrome and is followed by rapid karyotyping[1], other indications include identification of intrauterine infection, or evaluation of fetal acid-base status, etc.

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Widespread acceptance of cordocentesis relies on the belief that its complications are rare. In reality, cordocentesis can be associated with several complications and the procedure even by an experienced operator at midpregnancy may be related to fetal loss in about 1.4%[2]. Fetomaternal hemorrhage (FMH) following cordocentesis is one of the problems that may occur in as high as about 40% of cases[3]. Maternal risks following FMH include acute hemolytic transfusion reaction, erroneous typing of maternal blood, and isoimmunization to fetal red blood cells, white blood cells and platelets. Isoimmunization to fetal red blood cells is the most important consequence of FMH since it can cause maternal sensitization and fetal hemolytic disease[4]. Although several studies[3,5-7] have demonstrated an association between the procedure and increase in FMH, precise quantitation of FMH is difficult to conclude due to either a rather

small sample size in each study or marked variation of gestational age at the time of cordocentesis. Therefore, the authors conducted the present study with the main objective to determine the incidence and quantities of FMH associated with diagnostic cordocentesis at midpregnancy.

Material and Method

The study was conducted from January 2004 to June 2004, at Maharaj Nakorn Chiang Mai Hospital. 116 subjects were recruited with informed and written consent and met the inclusion criteria including; 1) singleton pregnancy, 2) proper indication for cordocentesis, 3) cordocentesis at 18-22 weeks of gestation, 4) healthy and no obvious known medical diseases. Cordocentesis was performed by six experienced operators, using the freehand technique under transabdominal ultrasound guidance, using the 3.5 MHz convex transducer (model prosound SSD 5000, Aloka, Tokyo, Japan). With aseptic technique, the procedure was carried out in an outpatient setting with the aid of a real-time ultrasound scanner to confirm the fetal number, viability, gestational age, normality, location of placenta and site for puncture. A 22- or 23- gauge spinal needle was used under local anesthesia without fetal paralysis. The puncture site, either near the cord insertion or a free-floating loop, was chosen based on the accessibility and quality of visualization, as well as an attempt to avoid placental penetration regardless of location. Following the procedure, the puncture site was observed for bleeding, and the fetal heart rate was assessed. Maternal blood samples for determination of the amount of FMH were obtained immediately before and 30 minutes after cordocentesis. Fetal cells in maternal blood were assessed and counted using acid elution test (Kleihauer-Betke test)[8]. The dried blood films prepared from whole blood were placed in a fetal cell-fixing solution (80% reagent ethyl alcohol) at room temperature for 3 minutes and then the slide was eluted with acid buffer for 3 minutes to remove hemoglobin A, followed by rinsing with tap water for 1 minute. All slides were prepared and scanned by the same well-trained examiner. In each slide, approximately 25,000 maternal cells were scanned on high power field. Fetal cells were quantitated by counting and determining the ratio of fetal cells (stained cells) to maternal (ghost cells) cells. The fetal whole blood volume was calculated using the formula: Number of fetal cells x 5000/Number of maternal cells count[5]. A significant hemorrhage was considered to have occurred if the fetal bleeding was more than 0.25 ml[5].

Descriptive statistics were analyzed with SPSS program version 11. Change in fetal blood volume in the maternal circulation was tested for significance with paired t test. Frequency of FMH in each interval was determined. The present study was approved by the Research Ethics Committee of the Faculty of Medicine, Chiang Mai University.

Results

One hundred and sixteen asymptomatic non-anemic pregnant women at 18-22 weeks of gestation were recruited. The mean maternal age ($\pm$ SD) was 30.3 $\pm$ 6.7 years (range 17-45). The most common indication for cordocentesis was fetal risk of severe thalassemia syndrome (71.6%), followed by genetic risk (18.1%) and combined risk of both (10.3%). The mean time used per procedure was 4.10 $\pm$ 5.6 minutes (0.5-35 minutes). The procedures were transplacental in 64 patients (55.2%), and not in 49 patients (42.2%). Nearly all procedures could be successfully performed with only one attempt 107 cases (92.2%), the remainder was performed after 2, 3 and 4 attempts (6.0%, 0.9% and 0.9%, respectively). The puncture site was free loop in 92 procedures (79.3%) and 23 (19.8%) were performed at the cord insertion. Seven procedures (6.1%) were complicated by transient fetal bradycardia, not longer than 1 minute. There was a significant increase in the whole fetal blood volume in maternal circulation from the level before to that after cordocentesis (paired Student's t test, $p < 0.001$). The mean increased fetal blood volume was 0.74 $\pm$ 1.2 ml (range 0-10 ml). A significant hemorrhage (> 0.25 ml) occurred in 63 out of 116 patients (54.7%) as shown in Table 1. Only one had hemorrhage more than 5.0 ml and none had massive hemorrhage (>15 ml). Transplacental penetration, puncture site, and time used for each procedure were not significantly related to the volume of fetal bleeding.

Table 1. Frequency of fetomaternal hemorrhage associated cordocentesis as determined by fetal cell counts (Kleihauer-Betke test)

Fetomaternal hemorrhage (ml)	No. (%) of cases
Non-significant (< 0.25 ml)	53 (45.7)
Significant (> 0.25 ml)	63 (54.3)
Minimal (0.26-1.0 ml)	35 (30.2)
Moderate (1.1-10.0 ml)	27 (23.3)
Marked (10.1-15 ml)	1 (0.9)
Massive (> 15 ml)	0 (0.0)
Total	116 (100.0)

Discussion

Detection or empirical treatment of FMH is particularly crucial for Rh-negative mothers delivering Rh-positive infants. Detection and quantitation of FMH can also be important in the diagnosis and management of the various pregnancy and fetal complications. Several tests are available to identify FMH. Qualitative tests are usually performed for screening and the quantitative tests are necessary if the qualitative tests are positive. Although various methods have been developed to quantitate FMH[9], the most commonly employed technique is still acid elution. In contrast to hemoglobin A, hemoglobin F resists elution when blood films are exposed to an acid buffer. Subsequent staining causes cells with hemoglobin F to stand out from a background of ghost cells. Measurement of maternal serum alpha fetoprotein (AFP) has been reported as a method for detection as well as quantitatification of FMH. Some studies have suggested that AFP determinations may be more sensitive and reliable than acid elution technique[10]. However, van Selm et al reported that both techniques were comparable for assessment in the second trimester, while in the third trimester the Kleihauer-Betke technique appears to be more sensitive in detecting FMH after cordocentesis[5]. Whereas, the best technique is yet to be elucidated, the authors chose to use Kleihauer-Betke test in the present study since it is more relevant to the real practice. In general practice, to prevent isoimmunization following cordocentesis in non-sensitized Rh-negative pregnant women, they are advised to receive Rh Immunoglobulin. The amount of Rh Immunoglobulin to be given can be determined from the Kleihauer-Betke test done within 24 hours of the procedure. It has been shown experimentally that one prophylactic dose of 300 micrograms of Rh Immunoglobulin can prevent Rh D isoimmunization after an exposure up to 30 ml of Rh D-positive blood or 15 ml of fetal cells[11].

Kleihauer-Betke test in the present study was performed with extreme precaution and quality control of the test was continuously monitored to reassure the results. This is necessary since improper technique can readily lead to false interpretation. Although this procedure appears relatively straightforward, several problems may arise. The differential hemoglobin elution is sensitive to pH, time, and temperature; technique is, therefore, important. Moreover, actual quantitation relies on a relatively subjective interpretation. First, subjectivity is introduced in the determination of what represents a positive cell, and second significant human error may be introduced by the approach used in counting. It is not uncommon to encounter a sample in which intermediate staining of cells occurs.

False negative results can occur due to a faulty technique. For example; hemoglobin F elutes if left too long in the eluting solution[12]. Additionally, fetuses do produce some hemoglobin A, so those cells are missed. False positive results can also occur because of several problems including maternal genetic hemoglobinopathies with persistence of maternal hemoglobin F, such as thalassemia trait or disease[13]. Furthermore, 10-25% of pregnant women may produce elevated levels of hemoglobin F between 10 and 32 weeks of gestation[14,15] and some women still have elevated levels at term. Although the authors were not able to quantify the absolute number of fetal cells in maternal circulation before and after cordocentesis because of aforementioned false positive cells or the presence of maternal hemoglobin F cells in cases of thalassemia carrier, the amount changed after cordocentesis could still represent the cordocentesis-related FMH. Therefore, maternal hemoglobin F cells from any cause were unlikely to have an impact on the authors' interpretation.

The current results suggest that cordocentesis at 18-22 weeks of gestation is significantly associated with FMH and places the patient at risk for isoimmunization. Nevertheless, the amount of hemorrhage was only minimal in nearly all cases and massive hemorrhage was not observed at all. These data indicate that any non-sensitized Rh-negative mothers should be given Rh immunoglobulin (Rhogam) to prevent isoimmunization but routine determination of the exact amount of FMH after uncomplicated cordocentesis to identify massive bleeding (> 30 ml of fetal whole blood), which needs an extra dose of Rhogam, is probably not necessary.

Unlike previous studies, the present study focussed only on cordocentesis at 18-22 weeks of gestation. Since the gestational age can have an impact on the amount of cordocentesis-related FMH[5], the incidence of FMH in the present study may, therefore, represent only for mid-pregnancy cordocentesis. Surprisingly, the authors found no significant association between FMH and transplacental technique, or time used for each procedure. This may possibly be due to the fact that such an effect is too small to be detected with the present sample size.

In conclusion, cordocentesis at 18-22 weeks of gestation can be associated with significant feto-

maternal hemorrhage in more than half of the cases but nearly all cases had only minimal hemorrhage and none had massive hemorrhage.

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การเจาะเลือดจากสายสะดือทารกกับภาวะเลือดออกจากทารกสู่เลือดมารดา ประสบการณ์ที่โรงพยาบาลมหาราชนครเชียงใหม่

จิตติมา รุจิเวชพงศธร, ธีระ ทองสง, ชเนนทร์ วนาภิรักษ์, วีรวิทย์ ปิยะมงคล, สุพัตรา ศิริโชติยะกุล, พฤหัส จันทร์ประภาพ, เฟื่องลดา ทองประเสริฐ

วัตถุประสงค์: *เพื่อประเมินอุบัติการณ์และปริมาณการมีเลือดออกจากทารกสู่มารดาเนื่องจากการเจาะเลือดสายสะดือทารก*

ชนิดของการวิจัย: *การวิจัยเชิงพรรณนา*

วัสดุและวิธีการ: *สตรีตั้งครรภ์ปกติที่ไม่มีภาวะแทรกซ้อนทางอายุกรรม 116 ราย ซึ่งมีข้อบ่งชี้สำหรับการเจาะเลือดสายสะดือทารกขณะอายุครรภ์ 18-22 สัปดาห์ถูกเชื้อเชิญเข้าร่วมวิจัยโดยสมัครใจ ทำการเจาะเลือดมารดาช่วงสั้น ๆ ก่อน และ 30 นาทีหลังเจาะเลือดสายสะดือ และนำไปตรวจหาเซลล์ลูกโดยวิธีทดสอบ Kleihauer-Betke ซึ่งในแต่ละสไลด์จะทำการตรวจหาจากเซลล์มารดาประมาณ 25,000 เซลล์ โดยผู้ตรวจคนเดียวกัน และถือว่าทารกมีเลือดออกสู่มารดาอย่างมีนัยสำคัญถ้ามากเกิน 0.25 มล.*

ผลการศึกษา: *มีการเพิ่มขึ้นอย่างมีนัยสำคัญของปริมาณเลือดทารกในมารดาภายหลังการเจาะเลือดสายสะดือทารก (paired t test, $p < 0.001$) การมีเลือดออกอย่างมีนัยสำคัญ (> 0.25 มล.) พบใน 63 รายจาก 116 ราย (ร้อยละ 54.7) เกือบทั้งหมดมีเลือดออกเพียงปริมาณน้อย มีเพียงรายเดียวเท่านั้นที่เลือดออกมากกว่า 5 มล. และไม่มีรายใดเลยที่เลือดออกเกิน 15 มล.*

สรุป: *การเจาะเลือดสายสะดือทารกขณะอายุครรภ์18-22 สัปดาห์มีความสัมพันธ์กับการมีเลือดออกจากทารกสู่มารดาอย่างมีนัยสำคัญ แต่ปริมาณเลือดที่ออกเกือบทั้งหมดมีเพียงปริมาณเล็กน้อย*