Prenatal Diagnosis of α-Thalassemia-1 (SEA type) by Chorionic Villus Sampling

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Abstract

Objective : To describe the experience of prenatal diagnosis for Hb Bart's disease, by chorionic villus sampling (CVS) with DNA analysis.

Design : Descriptive study

Settings : Department of Obstetrics and Gynecology, Faculty of Medicine, Chiang Mai University.

Subjects : Sixteen high risk pregnancies at risk of Hb Bart's disease who were eligible for CVS criteria between 1 January, 1999 and May 31, 2000.

Material and Method : Fetal villi were obtained by either transcervical (TC) or transabdominal (TA) CVS route to extract DNA and detect for α-thal-1 gene deletion (SEA type) with modified Chang's method. The CVS results were confirmed by either serial ultrasound or cordocentesis or diagnosis after pregnancy termination.

Main outcome measures : The efficacy, safety and pregnancy outcomes.

Results : CVS was successfully done in all of 16 cases (5 with TC and 11 with TA), The mean gestational age was 13.25 ± 2.9 weeks. The procedure time for TA was shorter than that of TC (4.64 ± 5.4 *vs* 10.4 ± 11.3 min). The CVS result showed as follows: 3 normal fetuses, 7 α-thal-1 carriers, 4 fetal Hb Bart's, 1 misdiagnosis and 1 failure to diagnosis due to technical error. The sensitivity and specificity were 100 per cent (4/4) and 90.91 per cent (10/11), respectively. One case of Hb Bart's misdiagnosis and one failure case were later confirmed for α-thal-1 trait and α-thal-1/ Hb E trait by cordocentesis, respectively. The pregnancy outcomes included 11 livebirths, 4 terminated cases and 1 fetal loss of continuing pregnancies. No serious complications occurred.

Conclusion : This preliminary experience suggests that CVS is an effective method for early prenatal diagnosis of fetal Hb Bart's.

Key word : Prenatal Diagnosis, Chorionic Villus Sampling, Thalassemia, α-Thalassemia-1 (SEA Type), Hb Bart's, Pregnancy Outcome

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In the northern part of Thailand, the homozygote of α-thal-1 gene deletion of South East Asia (SEA) type results in severe fetal Hb Bart's. It is expected that 2 hydrops fetalis will occur for 1,000 births in this population[1]. The patient can not survive and will die soon after delivery due to severe anemia and high output heart failure. Furthermore, the mother will suffer from both psychological trauma and other obstetric complications. In our hospital, either cordocentesis or serial ultrasonography is performed to prenatally diagnose Hb Bart's disease in high risk couples at the gestational age of 18-20 weeks. The mother of an affected fetus will be counseled for pregnancy termination[2,3]. However, both methods are quite late for the anticipating mother. Currently, the chorionic villus sampling (CVS) may be more attractive to obtain fetal tissue for earlier prenatal diagnosis during the first trimester. CVS and DNA analysis have been used for several fetal diseases including β-thalassemia major. However, CVS and DNA analysis (modified Chang's method) for diagnosis of homozygous α-thalassemia-1 or Hb Bart's diseases has never been published. This study aimed to describe the authors' preliminary experience of prenatal diagnosis for Hb Bart's diseases (SEA type) in terms of efficacy, safety and pregnancy outcome by CVS.

MATERIAL AND METHOD

Sixteen α-thal-1 pregnancies at risk of fetal Hb Bart's, in which both of the couples were carriers, were recruited for prenatal diagnosis under the Chiang Mai University Strategy for thalassemia carrier screening program[2,3]. The couples at risk were counseled for the chorionic villus sampling procedure including its complications. To be eligible for the study, a woman must have a viable single fetus with a gestational age of 9 weeks or more without cervical infection or abnormal bleeding. The subjects were enrolled into the study with informed written consent.

CVS for prenatal diagnosis

After sonographic assessment of gestational age, number of fetuses, uterine position, placental site and fetal anomaly, either transcervical (TC) or transabdominal (TA) CVS approach is selected to sample fetal villi depending on placenta accessibility. In difference, TC is performed in most posterior placenta in the lithotomy position while the anterior and lateral placenta are approached by TA in the supine position. To obtain fetal villi, a 24 cm bendable catheter is used in TC, whereas, a 3.5 inch spinal needle no 22 is selected in TA. If necessary, a second attempt can be done to gain more fetal tissue. After either procedure, fetal heart beat as well as subchorion hematoma is closely observed. Dissection and cleaning of fetal villi is performed under an inverted microscope. The pure villi will be collected in a 1 ml plastic tube containing saline solution for DNA analysis.

PCR technique for α-thal-1 (-- SEA/) gene analysis

With the PCR technique, the breakpoint area of α-thal-1 of SEA type and several parts of the α-globin gene cluster are amplified by modified Chang's method[4]. The procedure involves three primers to detect the homo-, hetrozygote of α-thal-1 and normal α-globin gene. As a result, Hb Bart's will show a pair of deletion specific 188 basepair (bp) bands while a double band of 314 bp will appear in normal DNA sequence. The heterozygote will show both 188 bp and 314 bp bands.

Three oligonucleotide primers were selected for this study.

Primer A : 5' GCG ATC TGG GCT CTG TGT TCT 3'
Primer B : 5' GTT CCC TGA GCC CCG ACA CG 3'
Primer C : 5' GCC TTG AAC TCC TGG ACT TAA 3'

The CVS results would be informed to the patient in terms of normal, α-thal-1 trait and Hb Bart's within one week. To ensure the result, serial ultrasounds seeking for hydropic change such as fetal cardiomegaly, placental thickening cordocentesis were performed in all cases. If both CVS and sonographic results were not well correlated, cordocentesis will be performed to confirm the final diagnosis before the termination of pregnancy was done. The normal and α-thal-1 trait result cases were followed-up by antenatal care until delivery. The pregnancy outcomes including complications were recorded for the final analysis.

RESULTS

Between 1 January 1999 and 31 May 2000, sixteen pregnancies at risk of fetal Hb Bart's disease were enrolled into the study. The mean maternal and gestational ages were 29.19 ± 5.7 years and 13.25 ± 2.9 weeks, respecitively. All cases, 5 with TC and 11 with TA, had satisfactory villi collection. The procedure time in the TA group was significantly less than that of TC (4.64 ± 5.35 *vs* 10.4 ± 11.33 min) the same as the number of second attempts. TA seems to be more painful than TC (11 in 11 *vs* 1 in 5) but has less bleeding (0 in 11 *vs* 2 in 5).

The CVS result showed 3 cases of normal fetus, 7 α-thal-1 carriers, 4 fetal Hb Bart's, 1 misdiagnosis and 1 failure to diagnosis due to technical error (Table 1). The pregnancy outcomes included 11 livebirths, 4 terminated cases and 1 fetal loss. Two hypertensive pregnancies were observed in this study.

It was apparent that all four affected cases presented remarkable sonographic findings such as widening of nuchal translucency, fetal cardiomegaly (cardiothoracic ratio >0.5) and placental thickening (>2 cm) but varied in gestational age and its severity. The first two cases developed frank hydrops fetalis at 14 -15 weeks while the last two were detected at 18 and 19 weeks with only placentomegaly. All of the affected fetuses were terminated by misoprostol vaginal suppository induction. It was found that the third case was associated with multiple anomalies. Unfortunately, the fetal karyotype was not performed. The only false positive case, developed no edematous change up to 18 weeks, was later diagnosed for α-thal-1 trait by cordocentesis and delivered normally at term.

There was one failure case of CVS due to failure in DNA extraction and this case was finally found to be α-thal-1/Hb E by fetal hemoglobin typing. As shown, the result of both normal and α-thal-1 trait groups were well collerated with pregnancy outcomes even though postnatal cord blood

Table 1. CVS result for α-thal-1 thalassemia.

Data	TA-CVS	TC-CVS	Total
Result			
Normal	3	-	3
Alpha thal-1 trait	2	5	7
Hb Bart's	4	-	4
Failed *	1	-	1
Misdiagnosis **	1	-	1
Pregnancy Outcomes			
Abortion	-	1	1
Termination of pregnancy	4	-	4
Normal pregnancy	7	4	11
Late Complication			
PIH	1	1	2
Fetal Anomaly ***	1	-	1

* one case of failure resulted due to DNA extraction failure, the diganosis of α-thal-1/Hb E was confirmed by cordocentesis at 17 weeks' gestation.

** one case of misdiagnosis for Hb Bart's, the final result was α-thal-1 trait confirmed by cordocentesis at 18 weeks' gestation.

*** one case of fetal anomaly was shown is have cystic hygroma, low set ears, club foot and amputation of anterior of rt. foot.

analysis did not confirm it. The sensitivity and specificity of CVS for prenatal diagnosis of fetal Hb Bart's were 100 per cent (4/4) and 90.91 per cent (10/11) without any false negative result.

DISCUSSION

Besides aminocentesis and cordocentesis, CVS is an alternative method for diagnosis of fetal Hb Bart's disease with an associated abortion rate comparable to that of other invasive procedures[5-11]. Compared to cord blood assessment, fetal DNA analysis is more attractive due to its simpler sampling technique and earlier diagnosis which provides better management and lowers parental anxiety. Route of approach depends on placental accessibility, instrument availability and operator's experience. Despite the same success, fetal loss rate in TA seems to be better than that of TC (5.12% *vs* 3.07%) which was also found in this study (6.3% *vs* 0%)[8,12], though the sample size in this preliminary experience was too small to show statistical significance. However, neither chorioamnionitis nor limb reduction were observed in this series. Even though there was one case of multiple anomalies, it would have resulted from Hb Bart's disease or abnormal chromosome rather than CVS itself. Unfortunately, post abortal genetic study of this case was not performed.

Despite the small sample size, CVS and DNA analysis by modified Chang's method showed a high sensitivity and specificity of 100 per cent and 90.91 per cent, respectively. Maternal DNA contamination is the common error which leads to a false negative result. By careful fetal villi dissection, this problem can be reduced even though DNA finger printing was not applied in this study. However, the follow-up scanning results confirmed no hydropic change in unaffected cases. On the other hand, preferential amplification if occurring in normal allele, will lead to false positive result[13]. For accurate prenatal diagnosis, sonographic edematous changes should be evaluated to confirm the CVS result before pregnancy termination[14]. To avoid a second invasive procedure, cordocentesis should be preserved for a discordant result between ultrasonography and CVS.

In this study, a quarter of Hb Bart's high risk pregnancies could be identified and terminated as early as 13 weeks' gestation which supported Mendelian's law and showed the effectiveness of the Chiang Mai Strategy for risk couple identification[2,3]. It can be concluded that CVS with DNA analysis is an effective prenatal diagnosis method for fetal Hb Bart's in early pregnancy, though more attention must be paid to overcome some technical errors.

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การวินิจฉัยก่อนคลอดของแอลฟ่าธาลัสซีเมีย-1 โดยวิเคราะห์ดีเอ็นเอจากการตัดชิ้นเนื้อรก

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การศึกษานี้มีวัตถุประสงค์บรรยายประสบการณ์การวินิจฉัยก่อนคลอดของโรคฮีโมโกลบินบาร์ทด้วยวิธีตัดชิ้นเนื้อรกตรวจวิเคราะห์ดีเอ็นเอ (ด้วยวิธีของ Chang's) ในรายงานนี้ประกอบด้วยสตรีตั้งครรภ์ที่มีความเสี่ยงต่อการมีบุตรเป็นโรคฮีโมโกลบินบาร์ทจำนวน 16 ราย ซึ่งยินยอมและเลือกที่จะทำการวินิจฉัยก่อนคลอดด้วยวิธีตัดชิ้นเนื้อรก ซึ่งอาจทำโดยวิธีผ่านทางปากมดลูกหรือผ่านทางหน้าท้อง ผลการวินิจฉัยของการวิเคราะห์ดีเอ็นเอจากชิ้นเนื้อรกนี้จะได้รับการยืนยันจากการวิเคราะห์เลือดทารกที่เก็บจากสายสะดือ หรือการวินิจฉัยหลังยุติการตั้งครรภ์อีกครั้งหนึ่ง การตัดชิ้นเนื้อรกสามารถกระทำได้สำเร็จในทุกราย (16 ราย) โดยวิธีผ่านทางปากมดลูก 5 ราย และผ่านทางหน้าท้อง 11 ราย เวลาสำหรับหัตถการตัดชิ้นเนื้อรกแบบผ่านทางหน้าท้องสั้นกว่าตัดผ่านทางปากมดลูกอย่างมีนัยสำคัญ (6.64±5.4 เทียบกับ 10.4±11.3 นาที) ผลการวิเคราะห์ดีเอ็นเอจากชิ้นเนื้อรกเป็นดังนี้คือ ทารกปกติ 3 ราย, ทารกเป็นพาหะแอลฟ่าธาลัสซีเมีย-1 7 ราย เป็นโรคฮีโมโกลบินบาร์ท 4 ราย วินิจฉัยผิดพลาด 1 ราย ไม่สามารถวินิจฉัยได้เนื่องจากความผิดพลาดทางด้านเทคนิค 1 ราย ความไวและความจำเพาะในการวินิจฉัยมีค่าร้อยละ 100 (4/4) และ 90.9 (10/11) ตามลำดับ ผลลัพธ์การตั้งครรภ์มีดังนี้คือ ทารกรอดชีวิต 11 ราย ยุติการตั้งครรภ์ 4 ราย และแท้งเอง 1 ราย (ในกลุ่มที่ดำเนินการตั้งครรภ์ต่อไป) ไม่พบภาวะแทรกซ้อนรุนแรงอื่น ๆ โดยสรุปจากประสบการณ์เบื้องต้นนี้บ่งชี้ว่าการตัดชิ้นเนื้อรกเพื่อวิเคราะห์ดีเอ็นเอน่าจะเป็นทางเลือกที่มีประสิทธิภาพในการวินิจฉัยก่อนคลอดโรคฮีโมโกลบินบาร์ท

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