Original Article

Prenatal Diagnosis of Homozygous Alpha-Thalassemia 1 of Southeast Asian Type by Polymerase Chain Reaction

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Abstract : *The association of hemoglobin Bart's (Hb Bart's) hydrops fetalis or homozygous α-thalssemia of Southeast Asian type is very common in Southeast Asia especially in Thailand. Becaues of the fact that the pregnancy may be complicated by toxemia, ante-or post-partum hemorrhage as well as psychological burden for the family and the mother who have to carry a nonviable fetus to term. The prenatal diagnosis of Hb Bart's hydrops is therefore justified so that the family can be given a choice of early termination of the pregnancy.*

In order to identify the presence of Hb Bart's hydrops in the fetus, the collaboration study in 18 high risk pregnancies with Hb Bart's hydrops fetalis were undertaken between February 1993 and October 1996 at Obstetric and Gynecology Department and the Hematology Division, Department of Pediatrics, Pramongkutklao Hospital, Bangkok, Thailand.

Amniocentesis was done at 16-33 weeks of gestation. DNA analysis were performed by polymerase chain reaction (PCR) using 2 techniques, 1) three nucleotide primers and 2) four nucleotide primers. After either therapeutic abortion or birth, heart blood or cord blood was drawn to confirm diagnosis by Hb electrophoresis and DNA analysis. Of 18 high risk fetuses, 6 were recognized as Hb Bart's hydrops fetalis, 7 showed the alpha-thalassemia 1 trait, 1 showed alpha-thalassemia 2 trait and 4 were normal fetuses. The technique was entirely suitable for prenatal diagnosis of Hb Bart's hydrops fetalis. This technique was rapid, simple non-radioactive method, less expensive and available in most PCR laboratories.

Key Words : ● *Hydrops fetalis* ● *Thalassemia* ● *PCR* ● *Amniocentesis*

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Hemoglobin (Hb) Bart's hydrops fetalis is a lethal condition, invariably complicated by serious maternal complications[1-3]. It is due to homozygous alpha-thalassemia 1 (- - SEA/- - SEA), in which all four alpha thalassemia genes are deleted[4]. Hb Bart's hydrops fetalis is the most common cause of late fetal loss in Southern Chinese, Filipinos, and other Southeast Asian. Alpha-thalassemia 1 carrier (trait) is common in Thailand. Its prevalence is 3.5% in Bangkok[5] and up to 14 % in Northern Thailand (Chieng Mai)[6], giving an expected homozygote incidence of 0.3 and 4.9 per thousand pregnancy in Bangkok and Chieng Mai respectively.

Materials and Methods

Amniocentesis & DNA extraction

Eighteen high risk pregnancies for Hb Bart's hydrops fetalis were included in this study. To identify the presence of Hb Bart's hydrops fetalis, amniocentesis was performed under ultrasound guidance at 16 and 33 weeks of the gestation. The amniotic fluid of 15-20 mL was collected. The sample was then centrifuged at 10,000 rpm. for 10 minutes. To identiy the defective DNA, the precipitate that contained the amniocytes was extracted by 2 methods : 1). By heating amniocytes[7] at 100°C for 2 minutes in 0.1 N NaOH 2M NaCl; 2). by phenol chloroform extraction.[8] Parents' blood was extracted by phenol chloroform. DNA analysis for α-thalassemia 1 of Southeast Asian type was performed by three primers technique[9]. DNA analysis for rapid diagnosis of PND samples was done by four primers technique described by Chehab, et al[10]. The detail of each technique was described.

DNA analysis (three primers technique)

Amplification was achieved using the primers described by Winichagoon, et al.[11] The three primers were A4: 5'-GGGGCGCCTTGG GGAGGTTC-3', A9: 5'-ATATATGGGTCTGGA AGTGTATC-3' and A1B: 5'-GTTCCCTGAG CCCCGACACG-3'. The 20 μL reaction mixture contains 0.2 μg of DNA,10 pmole of each primer (A4, A1B, A9), 40 μmole of dNTPs and 0.5 unit Ampli Taq DNA polymerase in PCR buffer (10 mM Tris,pH 8.3/50 mM KCl/2.5 mM $MgCl_2$ 0.01%(w/v) gelatin). The 35 cycle reaction was performed in DNA Thermal Cycle (Perkin-Elmer Cetus). Before starting PCR reaction the DNA samples was denatured at 95°C for 5 minutes. In the subsequent cycle the samples was denatured at was 95°C for 1minute, then cooled to 63°C for 2 minutes for annealing, and incubated at 72°C for 2 minutesfor extension. The last cycle was 72°C for 7 minutes and 8 μL of amplified DNA were electrophoreses in a 2.5% agarose gel at 100 volts then stained in ethidium bromide, and visualized on UV transilluminator .(Figure 1)

DNA analysis (four primers technique)

The four primers methods described by Chehab, et al[10]. The first pairs primer (A1: 5'-TACTGTAGATACCCGTGTACAA-3' and A2: 5'-ATCATGATGGAAACATAG TAAT-3') amplified 136 base pair (bp) region of α globin gene cluster. The second pair (P3: 5'-ACACA

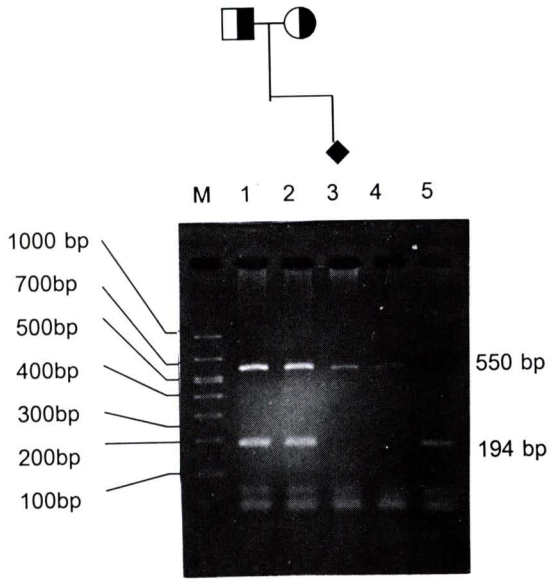

Figure 1. Photograph of ethidium bromides stain agarose gel electrophoresis of amplified DNA from a family at risk for Hb Bart's hydrops fetalis. Lane 1, and 2 represent father, and mother with α-thal 1 trait, lane 3 is PND fetus, lane 4 and 5 are Hb Bart's hydrops fetalis and normal control. M= DNA marker (GelMarkerTM), bp=base pair

ACTGTGTTCACTAGC-3' and P4: 5'-CAACTTC ATCCACGTT CACC-3') was added to the mixture to amplify a 110 bp segment of β-globin gene cluster which served as a control. The 100 μL mixtures contained 0.5 μg of DNA, 50 pmole of each primer, 200 μM of each dNTP s, 2.5 units AmpliTaq DNA polymerase in PCR buffer (10 mM Tris, pH 8.3/50 mMKCl/2.5 mMMgCl$_2$ 0.01% (w/v) gelatin). The first cycle was 92°C for 5 minutes 49°C for 1 minute 30 second, and 72°C for 1 minute, 30 seconds. The second to thirty sixth cycle were 92°C, 49°C and 72°C for 1 minute, 1 minute 30 seconds, and 1 minute 30 seconds respectively. The last extension cycle was 72°C, 10 minutes. The 8 μL of amplified DNA was electrophoresed in a 2.5% agarose gel at 150 volts, stained in ethidium bromide, and visualized on UV transilluminator.

Results

Of 18 high risk fetuses, 6 were diagnosed as Hb Bart's hydrops fetalis that justified therapeutic abortion. Seven of them were α-thalassemia 1 trait, 1 case was α-thalassemia 2 trait and 4 were normal fetuses. The maternal gestation age which amniocentesis performed and hematologic data were described in table 1. The diagnoses were all confirmed by hemoglobin electrophoresis and DNA analyses using 3 primers technique, after either therapeutic abortion or birth. There was no fetal loss nor complications in our study.

Table 1. Prenatal diagnosis of hemoglobin Bart's hydrops fetalis

Family	G.A.	Hb	MCV	Hb type(%)				Genotype	PND
	(wk)	(g/dl)	(fl)	A	A2(E)	H	B	(parent)	
1. mother	20	10.1	54	90.7	1.5	7.8	-	α-thal1/ α-thal2	α-thal2 trait
father		14.3	77	97.6	2.4	-	-	α-thal1/ αα	
2. mother	N.A.	9.6	62	98.2	1.8	-	-	α-thal1/ αα	α-thal1 trait
father		14.3	64	97.8	2.2	-	-	α-thal1/ αα	
3. mother	16	11.8	70	96.6	3.4	-	-	α-thal1/ αα	α-thal1 trait
father		14.5	76	97.2	2.8	-	-	α-thal1/ αα	
4. mother	18	10.8	68	97.0	3.0	-	-	α-thal1/ αα	Hb Bart's hydrops
father		15	62	97.4	2.6	-	-	α-thal1/ αα	
5. mother	18	10.1	72	71.4	28.6	-	-	α-thal1/ αα/Hb E	normal
father		12.8	68	96.8	3.2	-	-	α-thal1/ αα	
6. mother	27	10.2	71	97.8	2.2	-	-	α-thal1/ αα	Hb Bart's hydrops
father		13.3	71	97.5	2.5	-	-	α-thal1/ αα	
7. mother	33	10.3	68	80.9	19.1	-	-	α-thal1/ αα/Hb E	Hb Bart's hydrops*
father		12.7	62	97.7	2.3	-	-	α-thal1/ αα	
8. mother	18	14.6	75	97.2	2.8	-	-	α-thal1/ αα	α-thal1trait
father		14.0	68	98.0	2.0	-	-	α-thal1/ αα	
9. mother	17	11.5	71	97.7	2.3	-	-	α-thal1/ αα	α-thal1 trait
father		13.6	70	98.0	2.0	-	-	α-thal1/ αα	
10. mother	23	8.9	55	82.6	14.6	-	2.8	α-thal1/ α-thal2/HbE	α-thal2 trait
father		14.2	69	79.5	20.5	-	-	α-thal1/ αα/Hb E	
11. mother	19	9.0	73	87.3	3.3	9.4	-	α-thal1/ α-thal2	Hb Bart's hydrops
father		14.2	66	98.2	1.8	-	-	α-thal1/ αα	
12. mother	20	10.4	73	77.7	22.3	-	-	α-thal1/ αα/Hb E	α-thal1 trait
father		14.0	69	69.8	3.2	-	-	α-thal1/ αα	
13. mother	16	10.0	73	98.3	-	-	-	α-thal1/ αα	normal
father		14.4	68	97.2	-	-	-	α-thal1/ αα	
14. mother	N.A.	10.7	70	79.2	20.8	-	-	α-thal1/ αα/Hb E	Hb Bart's hydrops*
father		14.7	67	78.8	21.2	-	-	α-thal1/ αα/Hb E	
15. mother	21	14.6	75	97.2	2.8	-	-	α-thal1/ αα	normal
father		14.0	68	98.0	2.0	-	-	α-thal1/ αα	
16. mother	18	12.7	71	97.4	2.6	-	-	α-thal1/ αα	normal
father		14.6	66	97.7	2.3	-	-	α-thal1/ αα	
17. mother	18	11.2	61	69.9	2.8	-	-	α-thal1/ αα	α-thal1 trait
father		12.7	65	97.7	2.3	-	-	α-thal1/ αα	
18. mother	17	10.7	63	97.2	2.8	-	-	α-thal1/ αα	Hb Bart's hydrops
father		11.4	57	82.6	17	-	-	α-thal1/ αα/Hb E	

B=Hb Bart's, N.A.=not available,*=DNA was obtained from aminotic fluid and ascites of the fetus

Discussion

Hb Barts' hydrops fetalis is a lethal condition with 100 percent fetal loss or still birth. The pregnancies are invariably complicated by serious pre or postnatal conditions such as pre or post partum hemorrhage, toxemia of pregnancy[1-3]. The prenatal diagnosis of Hb Bart's hydrops fetalis can be obtained from chorionic

villi biopsy and hybridization with α- and ζ-globin gene probe[12,13]. However, the technique is tedious, time consuming and it involves the use of radioactive labeling probe. The application of a polymerase chain reaction (PCR) base protocol[11,14,15] that can specifically detects the - -SEA deletion may soon supersede that of gene mapping.

All cases in this study were diagnosed by amniocentesis. Seven of them had previous hydrops fetuses caused by homozygous α-thalassemia 1(patients 1,4,5,8,15,16 and 17), whereas the others 9 cases (patients 2,3,6,9,10,11,12,13 and 18) previously had hydrops fetuses of unknown causes. The 7 th and 14 th patients came in at late gestation age of 33 weeks in the former while the gestation age of the latter was undetermined. However the ultrasound showed hydropic with ascites and pericardial effusion. Amniocentesis was successfully performed. The amniotic and ascitic fluid was analysed for defective DNA. The diagnoses of homozygous α-thalassemia 1 were obtained from these samples.

We recommend that parents should be identified for α-thalassemia 1 carrier by three primers technique. The prenatal diagnosis should be obtained first by four primers technique because it is very rapid and the DNA extraction from amniotic fluid by heating at 100°C for 2 minutes in 0.1N NaCl 2M NaCl. Whereas the phenol chloroform extraction and the three primers technique should be performed for confirmation.

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การวินิจฉัยทารกในครรภ์ก่อนคลอดชนิดโฮโมซัยกัสแอลฟ่า-ธาลัสซีเมีย 1 โดยวิธีเพิ่มปริมาณดีเอ็นเอ

กิตติ ต่อจรัส, อาภรณ์ภิรมย์ เกตุปัญญา*, ทิพย์ ศรีไพศาล, ไตรโรจน์ ครุธเวโช, รัชฎะ ลำกูล และ ชไมพร สุวรรณะโสภณ

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บทคัดย่อ : *ฮีโมโกลบินบาร์ทฮัยดร็อปฟีตัลลิสพบได้บ่อยในภูมิภาคเอเชียตะวันออกเฉลียงใต้ โดยเฉพาะประเทศไทย มารดาที่ตั้งครรภ์จะพบภาวะแทรกซ้อนที่สำคัญได้แก่ ภาวะครรภ์เป็นพิษ ตกเลือดก่อนและหลังคลอด และสภาพจิตใจที่เป็นทุกข์ที่คลอดบุตรออกมาเสียชีวิต การวินิจฉัยทารกในครรภ์ก่อนคลอดและการยุติการตั้งครรภ์สามารถป้องกันโรคนี้ได้ คณะผู้รายงานได้วินิจฉัยทารกในครรภ์ก่อนคลอดในหญิงตั้งครรภ์ที่เสี่ยงต่อโรคฮีโมโกลบินบาร์ทฮัยดร็อปฟีตัลลิสจำนวน 18 รายอายุครรภ์ ระหว่าง 16-33 สัปดาห์โดยการตรวจน้ำคร่ำและวิเคราะห์ดีเอ็นเอของทารกโดยวิธีเพิ่มปริมาณยีน (โพลีเมอร์เรสเซ็นรีแอกชัน) ผลการวินิจฉัยพบว่า เป็นโรคฮีโมโกบินบาร์ทฮัยดร็อปฟีตัลลิส 6 ราย ปกติ 4 ราย เป็นพาหนะ ของแอลฟ่า-ธาลัสซีเมีย 1 และแอลฟ่า-ธาลัสซีเมีย 2 เท่ากับ 7 และ 1 รายตามลำดับ เทคนิคที่ใช้สามารถนำมาประยุกต์ใช้ได้ในห้องปฏิบัติการดีเอ็นเอทั่วไปเนื่องจากรวดเร็ว ประหยัด ง่าย และปลอดภัยเพราะไม่ต้องใช้สารกัมมันตภาพรังสี*

Key Words : ● *Hydrops fetalis* ● *Thalassemia* ● *PCR* ● *Amniocentesis*

วารสารโลหิตวิทยาและเวชศาสตร์บริการโลหิต 2541;8:33-8.