CLINICAL RESEARCH

Prenatal diagnosis of homozygous α thalassaemia by direct DNA analysis of uncultured amniotic fluid cells

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Abstract

Prenatal diagnosis of homozygous α thalassaemia was performed in eight successive patients at risk using DNA from uncultured amniotic fluid cells. The presence of α gene was determined by restriction endonuclease mapping and hybridisation with cloned α and β globin probes.

This method is reliable and may be performed at 16 weeks of gestation.

Introduction

Homozygous α thalassaemia which presents as haemoglobin Barts hydrops fetalis arises from the deletion of all four α globin structural genes.¹⁻³ This condition is more common in South East Asia owing to the higher prevalence of α thalassaemia 1 (- $\alpha\alpha$) in this area than in the Mediterranean region and Africa.⁴⁻⁶ The affected fetus usually dies in utero between 23 and 38 weeks of pregnancy or soon after birth.⁶ Despite the invariably fatal consequences of the homozygous state early diagnosis is still desirable, since these pregnancies are often complicated by pre-eclampsia, with significant maternal morbidity and mortality.⁷ Wong *et al* first employed liquid hybridisation of DNA from cultured amniotic fluid cells for prenatal diagnosis of homozygous α thalassaemia.⁸ With the development of restriction endonucleases and Southern blotting, direct DNA analysis was possible.⁹ In addition, if amniocentesis is

performed at 16 weeks of gestation enough DNA may be isolated from amniotic fluid cells without the need for culture.¹⁰

We report our preliminary experience of prenatal diagnosis for homozygous α thalassaemia using direct DNA analysis.

Subjects, materials, and methods

Over two months eight pregnant Chinese women with a history of delivery of an infant with haemoglobin Barts hydrops fetalis requested prenatal diagnosis. All except one were in the second trimester (16-23 weeks); the remaining patient (case 3; see table) presented in the 32nd week with mild hypertension. Amniocentesis was performed under ultrasound guidance and 18-26 ml amniotic fluid obtained. DNA was extracted from the cells as described¹¹ and digested with the restriction enzyme Eco RI or Bgl II. After electrophoresis and Southern transfer of the DNA fragments the filter was hybridised overnight with equal amounts of ³²P labelled α and β globin probes prepared by nick translation of the cloned DNA using the modified method of Rigby *et al*.¹² The β globin probe had previously been restricted with Bam HI and Eco RI. The hybridised filter was then washed under stringent conditions and autoradiographed from two to four days. On each occasion DNA from a normal person was digested and hybridised as control.

Results

GENE MAP

The number of amniotic fluid cells obtained by amniocentesis varied with gestational age (table). A total of 4.06×10^6 cells were obtained from a 32 week fetus, yielding 29 μ g DNA—enough for six enzyme digestions. Even the minimum number obtained from amniocentesis at 16 weeks (0.29×10^6 cells), however, gave sufficient DNA for a single enzyme digestion.

The figure shows the autoradiograph of the α and β globin DNA map. Non-thalassaemic human cellular DNA when digested with Eco RI showed a 23 kilobase (kb) α fragment and a 5.5 kb 5'- β fragment. Digestion with Bgl II yielded two normal α gene fragments, 12.5 and 7.0 kb long, and a single 5.3 kb β gene fragment. The radioactive intensities of the α and β fragments were similar. Two patients (cases 3 and 4) had no α gene but a normal β gene. This is diagnostic of homozygous α thalassaemia and was confirmed at delivery when cellulose acetate electrophoresis of cord blood haemolysates showed the

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Data on eight successive patients for prenatal diagnosis

Case No	Gestational age at diagnosis (weeks)	Amniotic fluid (ml)	Total amniotic fluid cells ($\times 10^6$)	Total DNA content (μ g)	Gene map		Outcome
					α	β	
1	21	18	1.80	17.5	Present	Present	34 weeks; Normal*
2	21	24	0.41	ND	Unsuccessful		23 weeks; induced abortion; Hb Barts 82.5%
3	32	24	4.06	29.0	Absent	Present	34 weeks; induced labour; Hb Barts 86.6%
4	23	25	1.95	14.5	Absent	Present	25 weeks; induced abortion; Hb Barts 81.5%
5	20	24	1.44	10.5	Present	Present	26 weeks; normal
6	18	24	0.50	ND	Present	Present	24 weeks; normal
7	16	26	0.29	ND	Present	Present	29 weeks; normal
8	18	26	0.62	ND	Present	Present	20 weeks; normal

ND = Not determined.
*Normal indicates no hydropic changes on ultrasound.

Autoradiograph of filter containing human DNA digested with either Eco RI (ER) or Bgl II (Bg) and hybridised with equal amounts of ³²P labelled α and β globin DNA. Arrows indicate size of gene fragments in kilobases (kb). α Gene fragments are 23.0, 12.5, and 7.0 kb; β fragments are 5.5 and 5.3 kb. Lanes 1, 2 show samples from non-thalassaemic control. Lanes 3, 4, 5 show samples from fetuses with α thalassaemia trait. Lane 6 shows sample from fetus with homozygous α thalassaemia. presence of haemoglobin Barts (86.6% and 81.5% respectively) and haemoglobin Portland (13.4% and 18.5% respectively). Five other patients (cases 1, 5, 6, 7, and 8) showed α and β globin genes in the normal position, but the radioactive intensity of the α fragments was less than of the β fragment, compatible with the diagnosis of α thalassaemia 1 (-/- $\alpha\alpha$). In case 2 we failed to achieve complete restriction digestion with Eco RI and no distinguishable gene pattern was observed. All the parents showed the characteristic gene map pattern of α thalassaemia 1.

ULTRASOUND FINDINGS

None of the fetuses studied showed abnormalities at the time of amniocentesis. When case 2 was recalled for a possible second amniocentesis at 23 weeks ultrasound showed evidence of ascites in the fetus; the mother, who had had two previous pregnancies complicated by

hydrops fetalis, requested termination. Subsequent cord blood studies showed that this fetus also was affected with homozygous α thalassaemia. None of the other fetuses showed any abnormality at subsequent follow up by fortnightly ultrasonography up to the time of this report (table).

Discussion

Haemoglobin Barts hydrops fetalis is common in South East Asia¹³ owing to the high prevalence (3-5%) of α thalassaemia 1 in the population.¹⁴ Thus parents with a history of haemoglobin Barts hydrops fetalis should be counselled about prenatal diagnosis. In addition, couples of South East Asian origin who have microcytic hypochromic red cells, a normal haemoglobin A₂ concentration, and no iron deficiency should be referred for investigation, and if diagnosed as α thalassaemia 1 they should also be counselled. Amniocentesis carries a very low risk to both the mother and fetus.¹⁵ The enhanced sensitivity of direct DNA analysis avoids the need for amniotic fluid cell culture. We found that it was possible to obtain a gene map with DNA from 0.29×10^6 cells obtained by amniocentesis at 16 weeks of gestation. This was probably due to the higher specific activity and affinity of the cloned α and β globin probes used, whereas other workers have used DNA synthesised with reverse transcriptase from globin mRNA.^{6,7} Results can be obtained within nine to 10 days, so that if amniocentesis is attempted at 16 weeks, termination, if indicated, may be done well before 24 weeks. In this study we attempted prenatal diagnosis even in a third trimester pregnancy. This is not usual, but the patient was already in advanced gestation when the laboratory procedure for DNA analysis was established. She developed mild hypertension and hydramnios and we considered that prenatal diagnosis even at that late stage was desirable, since it would influence her obstetric management. Chorionic villi may be used as a source of fetal DNA and biopsy done between eight and 10 weeks of gestation,¹⁶ thus allowing earlier prenatal diagnosis. While there are considerable advantages of diagnosis in the first trimester, further experience from a larger biopsy series is required to assess the safety of the procedure compared with the risk of spontaneous abortion during early pregnancy. The limited amount of DNA extractable from amniotic fluid cells makes the choice of restriction enzyme important. Complete digestion with Eco RI and the subsequent Southern transfer of the larger α fragment are difficult, so that Bgl II is preferable. The additional use of β DNA probe in the hybridisation served as an internal control to indicate efficient extraction of DNA from the amniotic fluid cells. Prenatal diagnosis of homozygous α thalassaemia is still not available in most parts of South East Asia, and for these regions ultrasonography may be used as an alternative to detect hydrops fetalis.¹⁷ Although it is sometimes possible to detect ascites and hydropic changes before the 24th week, however, these are not always evident: in our case 3 no ultrasound abnormalities were observed even at 34 weeks. Since amniotic fluid cells may be frozen and shipped to laboratories, amniocentesis and prenatal diagnosis should be offered to couples at risk.

We thank Professor Y W Kan for advice and encouragement; Dr B Forget and Dr T Maniatis for α and β globin probes; S H Ho Foundation Ltd, Wideland Foundation Ltd, and Wu Chung Traveling Fellowship for financial support; and Messrs A Wong and C S Kwan for skilful technical work.

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(Accepted 8 February 1984)

Fetal circulation during epidural analgesia for caesarean section

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Abstract

Fetal blood flow was examined during epidural analgesia in six women with uncomplicated pregnancies undergoing elective caesarean section. A non-invasive, ultrasonic technique was used to measure blood flow in the fetal descending aorta and intra-abdominal part of the umbilical vein before induction of analgesia with etidocaine and bupivacaine and 15 and 30 minutes afterwards. No appreciable change in fetal blood flow was observed.

Introduction

Lumbar epidural analgesia is being increasingly used for elective caesarean section. Among its advantages over general anaesthesia are the lack of drug induced respiratory depression of the newborn and the better preserved placental blood flow.¹ Until now the fetal heart rate has been the only fetal circulatory variable to be controlled under obstetric analgesia. Recently, a non-invasive method of measuring fetal blood flow became available.² We used this new method to evaluate possible effects of epidural analgesia on fetal circulation.

Patients and methods

Fetal blood flow was examined in six women with uncomplicated pregnancies who were to be delivered by elective caesarean section. The indications for caesarean section were cephalopelvic disproportion in five cases and the age of the mother in one (she was a 42 year old primigravida). All of the women gave their informed consent.

The blood flow in the fetal descending aorta and the intra-abdominal part of the umbilical vein was examined with a 2 MHz pulsed Doppler technique combined with real time B mode ultrasonography.² The mean blood flow was calculated and the waveform of the maximum blood velocity analysed. The waveform was characterised by the pulsatility index $(= (\text{peak velocity} - \text{minimum velocity}) / \text{mean velocity})$.³ The measurements were performed with the pregnant woman tilted slightly to the left (15°) to avoid hypotension. Fetal heart rate and the maternal pulse rate and blood pressure were also measured. Fetal blood flow was recorded before the induction of epidural analgesia and 15 and 30 minutes thereafter.

After preloading with 0.5 l of a balanced electrolyte solution analgesia was induced at the L3-4 interspace using 10 ml etidocaine 1.5% and 10-12 ml bupivacaine 0.5% both containing adrenaline (5 $\mu\text{g/ml}$). Cutaneous analgesia up to the level of T7 and T5 ensued after 15 and 30 minutes, respectively.

The women were then delivered by caesarean section. The infants had a mean gestational age of 38.2 (SEM 0.3) weeks, a mean birth weight of 3388 (194) g, a mean umbilical arterial pH of 7.22 (0.02), and a mean umbilical venous pH of 7.28 (0.02). The one minute Apgar score was ≥ 8 in all of them.

Results

The table gives the mean values of the maternal and fetal circulatory variables. In no instance was the maternal systolic blood pressure lower than 100 mm Hg. The mean blood velocity and the diameters of the fetal aorta and umbilical vein were within the normal range before analgesia in all six fetuses and did not change during the study period. The figure shows the fetal aortic and umbilical blood flows; no appreciable change in fetal blood flow was observed.

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