

# Prenatal diagnosis of $\beta$ -thalassaemia and other haemoglobinopathies in India

Chitra Thakur (Mahadik)\*, Flavian Vaz, Monisha Banerjee, Chhaya Kapadia, P. G. Natrajan, Harsha Yagnik and Sudha Gangal

*B. J. Wadia Hospital for Children and Institute of Child Health Research Society, Parel, Mumbai 400012, India*

This paper reports prenatal diagnosis of 787 fetuses of  $\beta$ -thalassaemia and other haemoglobinopathies in Indian high-risk communities. DNA based diagnosis was offered in the first, as well as the second trimester, in 489 pregnancies (with five twins) on fetal tissues such as chorionic villus (CV) and amniocytes using the amplification refractory mutation system (ARMS) and restriction fragment length polymorphism (RFLP) techniques. Two hundred and ninety-two women (with one twin), who either presented late in the second trimester or whose DNA diagnosis was not informative, were offered prenatal diagnosis using globin chain synthesis (GCS) on fetal blood cells. Maternal contamination of fetal DNA was ruled out by variable number tandem repeat (VNTR) analysis using sites in four different genes (Apo-B, D1S-80, Ig-JH and Ha-ras), while contamination of fetal blood was checked by a particle size distribution channelizer. Using both techniques we were able to offer complete diagnosis in 99.8% cases. Out of 494 fetuses tested by DNA analysis, 135 were found to be normal, 201 were carriers, whereas 146 were affected. Out of 293 fetuses analysed by GCS, 215 were unaffected and 71 were affected. In this study, both fetuses were tested in twin pregnancies, of which three required selective termination of one fetus. Because of social, religious taboos and family influences, genetic counselling was found to be an important guideline for couples selecting options for prenatal diagnosis. Our experience suggests that because of late presentation by many couples to the diagnostic centres, in developing countries like India, both the techniques of DNA analysis and GCS should be made available at major referral centres for maximum benefit to couples. Copyright © 2000 John Wiley & Sons, Ltd.

KEY WORDS: Indian communities; prenatal diagnosis; mutations; VNTR

## INTRODUCTION

Haemoglobinopathies such as  $\alpha$ - and  $\beta$ -thalassaemia and HbS, HbC, and HbE syndromes are widespread globally (Old, 1996). The Indian subcontinent has a considerably high prevalence of  $\beta$ -thalassaemia, and disorders of HbE and HbS (Marwah and Lal, 1994). It is estimated that there are about 29.7 million carriers of  $\beta$ -thalassaemia in India (Saxena *et al.*, 1998). Population screening has identified certain high-risk communities where prevalence of  $\beta$ -thalassaemia is high (Sukumaran and Master, 1973). The increase in the frequency of homozygote births is due to endogamous and consanguinous marriages in these high-risk communities (Venkatesan *et al.*, 1992). About 7000 children with  $\beta$ -thalassaemia major are expected to be born every year in India (Saxena *et al.*, 1998). Thus, India alone bears more than 10% of pathological births in Asia that are due to haemoglobinopathies (Petrou and Modell, 1995). Illiteracy, poverty, ignorance about the disease, social and religious taboos and family influences have aggravated the problem of deaths due to haemoglobinopathies. In our country, the cost of even minimum essential treatment such as blood transfusions and iron chelation therapy is unaffordable for many families with a

child with  $\beta$ -thalassaemia major. Therefore, to help with the birth of an affected child, genetic counselling, prenatal diagnosis and medical termination of pregnancies are the only options for carrier couples.

So far, depending upon the nature of the defects in DNA and haemoglobin synthesis, different molecular and biochemical laboratory methods of analysis have been recommended for prenatal diagnosis (Malavacca *et al.*, 1992; Embury, 1995; Fuchareon *et al.*, 1998; Trent *et al.*, 1998). In this paper we present our data for prenatal diagnosis of  $\beta$ -thalassaemia and other haemoglobinopathies using ARMS for mutation analysis, RFLP and GCS. We suggest a prenatal diagnosis strategy for Indian high-risk couples which we have found works well.

## MATERIALS AND METHODS

### Subjects

During the period May 1990–May 1999, 787 fetuses of 656 mothers underwent prenatal diagnosis. The pregnancies were at high-risk for homozygous  $\beta$ -thalassaemia and compound heterozygosity for other haemoglobinopathies and  $\beta$ -thalassaemia; few had risk of homozygous HbS disease. The couples originated from different parts of India and neighbouring countries such as Bangladesh, Kuwait, Maldives, and Sri Lanka. The prerequisites for prenatal diagnosis

\*Correspondence to: C. Thakur, B. J. Wadia Hospital for Children, Institute of Child Health Research Society, Acharya Donde Marg, Parel, Mumbai 400012, India.

were: (a) thalassaemic or haemoglobinopathy status of both parents (quantitation of HbA<sub>2</sub> and/or abnormal haemoglobin values) to identify the haemoglobinopathy to be tested in the fetus; (b) blood group of the mother to avoid the complications due to Rh incompatibility; (c) mutation analysis of both parents (carried out in our laboratory) so that only these mutations can be looked for in the fetal DNA; and (d) written consent of the couple.

### Collection of fetal samples

Chorionic villus samples (423) were collected at 10–12 weeks of gestation, confirmed by an ultrasound (Natrajan, 1996). For mothers presenting late, amniotic fluid (71) was collected at 15–16 weeks, while fetal blood (293) was collected at 18–20 weeks. The procedures were conducted under ultrasound guidance using a 21-gauge sterile disposable needle with stylette without anaesthesia. In the majority of cases chorionic villus sampling was done transabdominally in the outpatient centre. The fetal blood was collected mostly through the ventricle of the heart, umbilical cord and hepatic vein (Nicolaidis *et al.*, 1985). Fetal loss was seen in 0.9% of the total procedures restricted to fetal blood samplings conducted through the umbilical cord (Table 1).

### DNA Analysis

Chorionic villus samples were dissected carefully to remove maternal decidua, presence of which would lead to misdiagnosis (Old and Fitches, 1993). Amniocytes and fetal nucleated cells were separated by centrifugation and washed. DNA was extracted from the tissue/cells by the phenol-chloroform extraction method (Old and Higgs, 1982). PCR amplification was done using ARMS primers as described by Old (Old *et al.*, 1990). The DNA from peripheral blood leukocytes of parents was screened for 23  $\beta$ -thalassaemia mutations known to occur in the Indian sub-continent (Baysal, 1998). The primers used to detect the common mutations (i.e. IVS I-5 (G→C), IVS I-1 (G→T), FS 41/42 (-TCTT), FS8/9 (+G), 619 bpd (base pair deletion)); the rare mutations (i.e. C15 (G→A), FS16 (-C), Cap + 1 (A→C), C30 (G→C), C30 (G→A), FS47/48 (+ATCT), IVS I-1 (G→A), C88 (+T), IVS I-25 bpd, C5 (-CT), IVS II-837 (T→G), IVS II-1 (G→A), -88 (C-T)); and very rare mutations such as IVS I-110 (G→A), IVS I-130 (G→C)\*, IVS I-130 (G→A)\*\*\*, C44 (-C)\*, IVS I-128 (T→G). (The primers for mutations denoted by \* and \*\* were

included as they were identified earlier in our series of patients by Drs M. Petrou and J. Old respectively). The other abnormal haemoglobins tested by ARMS primers were HbE and HbS, while HbD was detected by RFLP. Subsequently, fetal DNA was tested only for mutations present in the parents. In fetuses, where the parents had identical mutations, amplification of normal gene sequences was carried out to differentiate between heterozygotes and homozygotes. The chances of misdiagnosis due to maternal DNA contamination in fetal DNA was ruled out by performing VNTR analysis (Decorte *et al.*, 1990).

### VNTR analysis

The sites used for VNTR analysis were within Apo-B, D1S-80, Ig-JH and Ha-ras genes. Until 1991, Apo-B, D1S-80, Ig-JH and Ha-ras sites were known to exist in 12, 16, 6 and 18 allelic forms respectively (Decorte *et al.*, 1990; Budowle *et al.*, 1991). VNTR analysis was conducted when: (a) the fetus was diagnosed as a carrier, with both parents having the same mutations; and (b) the fetus was diagnosed as being affected, with both parents having different mutations. The ARMS and VNTR analysis could provide definitive diagnosis of the fetus being normal, a carrier and/or homozygous. In cases where mutations of one or both parents were unidentified and when affected/normal siblings or grandparents were available for testing, RFLP analysis was conducted for diagnosis (Old *et al.*, 1984).

### RFLP analysis

RFLP analysis was mostly conducted during 1993–1994 when DNA based diagnosis was carried out using primers only for five common mutations. The dimorphic sites used to assess the polymorphisms were within the  $\beta$ -globin gene cluster. The sites and the restriction enzymes used were: G $\gamma$ , A $\gamma$ /(Hind III), 5' $\psi$  $\beta$ , 3' $\psi$  $\beta$ /(Hind II),  $\beta$ /(Rsa I, Ava II and Hinf I) (Varawalla *et al.*, 1992). Since 1995, ARMS primers for many more mutations have been introduced, and very few cases had one or both parents mutations unidentified. In such cases, the couples were advised that the diagnosis of the fetus would be carrier or normal if one parent's known mutation was absent or it may be affected or a carrier if one parent's known mutation was present. If this option was not favoured, and in cases where the mutations of both parents were unidentifiable, GCS was conducted at 18–20 weeks' gestation.

### Globin chain synthesis

The fetal blood samples were tested for purity by passing through a particle size distribution channelizer (ZM 256 Coulter electronics, USA). Reticulocytes from fetal blood were allowed to mix with 0.2 mCi of <sup>3</sup>H leucine (TRK 170 — Amersham, UK) in freshly synthesized globin chains *in vitro*. The globin chains

Table 1—Information on the fetuses tested for prenatal diagnosis

Information factors	DNA analysis (n=494)	Globin chain synthesis (n=293)
Homozygotes	480 (97.1%)	282 (95.9%)
Misdiagnoses	2 (0.4%)	1 (0.3%)
Fetal loss	0 (0.0%)	8 (2.8%)

were extracted and the amount of radioactivity incorporated in  $\alpha$ ,  $\beta$  and  $\gamma$  chains was measured chromatographically using a CM-23 cellulose column (Clegg *et al.*, 1968). The  $\beta/\gamma$  chain synthesis ratio was calculated from the plots. A cut-off value of  $\beta/\gamma > 0.025$  was used for diagnosis (Alter, 1984). Diagnosis using GCS differentiated between affected and unaffected status of the fetus. It was not possible to discriminate between normal or carrier status of the unaffected fetuses. In borderline results ( $\beta/\gamma$  ratio  $\leq 0.025$ ), resampling was done after two weeks to confirm the diagnosis.

## RESULTS

### Sequence of methods used for prenatal diagnosis

As seen from Figure 1, during the years 1990–1992, the only method available for prenatal diagnosis at our centre was GCS, therefore all diagnoses were offered using this method. The DNA laboratory was established in 1992. During 1993–1994, primers for five common Indian mutations (see ‘Material and methods’) were available; therefore, DNA based prenatal diagnosis was given to those fetuses at risk for these mutations. During this period, first trimester prenatal diagnosis was given by RFLP analysis, where these mutations were absent. GCS was also continued where these mutations were absent and for cases referred in the late second trimester. Subsequently, primers for an additional 18 mutations were introduced; therefore GCS was reduced to few cases of late presentations and for cases non-informative by DNA analysis. During initial stages, available post-termination of pregnancy samples were retested to confirm the affected status diagnosed by GCS, while parents of unaffected babies rarely reported to the centre as they were not symptomatic. Those who reported were given results on their normal or carrier status.

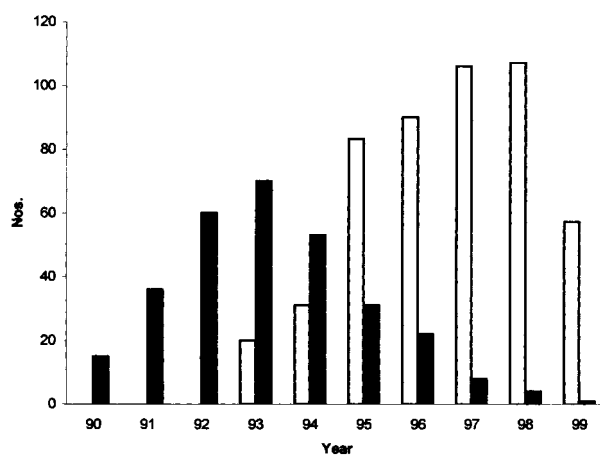


Figure 1—Number of prenatal diagnosis tests performed during 1990–1999. ■ GCS, □ DNA analysis

### Statewise and countrywise distributions of couples referred for prenatal diagnosis

Most cases were referred from Maharashtra and Gujrat (West India, Table 2). The second largest group was Sindhis (migrated from Sind, Pakistan at the time of partition) mostly settled in north west India. We investigated few cases from East, Central and South India. Eleven cases were referred from neighbouring countries.

### Prenatal diagnosis

Out of 787 fetuses, 494 were diagnosed using DNA analysis. Their analysis showed 135 (27.3%) normal, 201 (40.6%) carriers and 146 (29.5%) affected status (Figure 2), while from amongst 293 fetuses tested by GCS, 215 (73.3%) were unaffected and 71 (24.2%) were affected. The fetuses were at risk for homozygous  $\beta$ -thalassaemia (750), compound heterozygous conditions of haemoglobinopathies and/or HbS, HbE, HbD with  $\beta$ -thalassaemia (31) and sickle cell anaemia (6) (Tables 1 and 3). Of the 494 samples analysed by DNA methods, 12 had inconclusive results. In four cases, amplification using normal primer was not satisfactory and/or VNTR analysis results were non-informative. In eight cases only one parent's mutation could be identified, which was present in the fetuses. After counselling, nine of the twelve couples opted to go for GCS. Seven fetuses were diagnosed as unaffected, two were affected. Of the 293 diagnoses given using GCS, seven cases had inconclusive results either due to borderline  $\beta/\gamma$  synthesis ratio (four cases) or bad elution patterns due to poor globin synthesis (three cases). In the former group, the results in two cases were confirmed by GCS after two weeks, one couple opted to continue the pregnancy without retesting (this baby showed thalassaemia major status after birth) and one case was lost to follow-up. In the latter group, one case was confirmed by DNA analysis, one couple opted to continue the pregnancy, where the baby was born as a carrier, while the third case was lost to follow-up.

### Mutation analysis of $\beta$ -thalassaemia

As per the parents' mutations, fetuses were grouped as at risk for homozygosity (both parents having the same mutations) or heterozygosity (both parents having different mutations) for  $\beta$ -thalassaemia mutations (Tables 4 and 5). Since the Indian population shows a clear division in several communities which mostly practice endogamy, the distribution pattern of mutations was studied in different communities. Homozygosity was shown by 265 out of 479 (55.3%) fetuses. From amongst these, homozygosity for IVS I-5 (G→C) mutation was most common in Maharashtra Marathas, Muslims and Gujrati Bhanushalis, while homozygosity for 619 bpd and IVS I-1 (G→T) was common in Punjabis and also in Sindhis and Gujrati Lohanas, who are genetically closer (Their

Table 2—Origin of families referred and methods used for prenatal diagnosis

Origin	DNA analysis	GCS	Total (%)
India			
1. West (Gujrat, Maharashtra, Rajasthan)	206	155	361 (51.9)
2. East (Assam, West Bengal, Orissa)	9	6	15 (2.1)
3. Central (Madhya Pradesh)	16	12	28 (4.0)
4. South (Andhra Pradesh, Karnataka, Tamilnadu)	6	7	13 (2.0)
5. North (Bihar, Haryana, Himachal Pradesh, New Delhi, Punjab, Uttar Pradesh)	33	36	69 (10.0)
6. Sindhi (Pakistan)	136	63	199 (28.6)
Other countries	9	1	10 (1.43)
1. Bangladesh	3	—	3
2. Dubai	1	—	1
3. Maldives	4	1	5
4. Sri Lanka	1	—	1
Total	415	280	695 <sup>a</sup>

<sup>a</sup>Excludes cases tested in more than one pregnancy using the same technique.

*et al.*, 1984). Homozygosity for rare mutations C-15 (G→A), IVS II-837 (T→G), FS16 (–C), C30 (G→A), IVS I-25 bpd was shown by 14 out of 204 fetuses. When fetuses at risk for heterozygosity of  $\beta$ -thalassaemia mutations were examined (Table 5), most of them showed combinations of mutations commonly occurring in these populations, as listed by Madan (Madan *et al.*, 1998).

The 20 fetuses where either one or both parent's mutations were unidentifiable mainly belonged to communities from Maharashtra and West Bengal. Twelve of these in whom one parent's mutation was known, favoured the diagnosis based on absence or presence of the known mutation in fetal tissue. Four fetuses were diagnosed as unaffected as they did not carry the known mutation, while eight fetuses who carried the known mutation were advised to be tested by GCS. Five opted for GCS (four had an unaffected and one had an affected fetus) and three were lost to follow-up. Eight couples opted to have results only by GCS as they didn't want to undergo repeated fetal

samplings. Except one all were diagnosed to be unaffected.

### VNTR analysis

Maternal contamination in fetal DNA was checked by VNTR analysis. When one of the polymorphic alleles of the mother was absent in fetal DNA, the fetal DNA was considered free from maternal contamination (Figure 3). VNTR analysis was performed in 112 fetal DNA samples of which 89 fetuses were carriers and 23 were compound heterozygotes. The four sites, when tested sequentially, were informative in 46/112, 28/67, 18/39 and 5/20 cases respectively (Table 6). In two cases, fetal DNA was not sufficient for completing the VNTR analysis using all four sites. In 12 cases VNTR analysis results were not informative in spite of testing for all the four sites. In all the non-informative cases, the parents were counselled accordingly. Most of them opted to depend upon the diagnosis given on the basis of the mutation analysis results. None of the parents reported back with an adverse diagnosis. In 98/98 informative VNTR analysis, maternal contamination was not detected.

### Globin chain synthesis

Out of 293 fetuses diagnosed by this method, clear results were obtained in 286 fetuses, where 215 were unaffected and 71 were affected (Table 3). The  $\beta/\gamma$ -globin chain synthesis ratio in all the fetuses diagnosed as being affected was zero, while those diagnosed as being unaffected had a ratio of  $>0.025$ .

### Prenatal diagnosis of twins

During the course of this study six twin pregnancies were referred for prenatal diagnosis, being at risk for  $\beta$ -thalassaemia major. Except one, which was studied using GCS, diagnosis of the rest was based on mutation analysis. In two of the six twins both babies were either normals or carriers and the pregnancies were continued. In one pregnancy, both

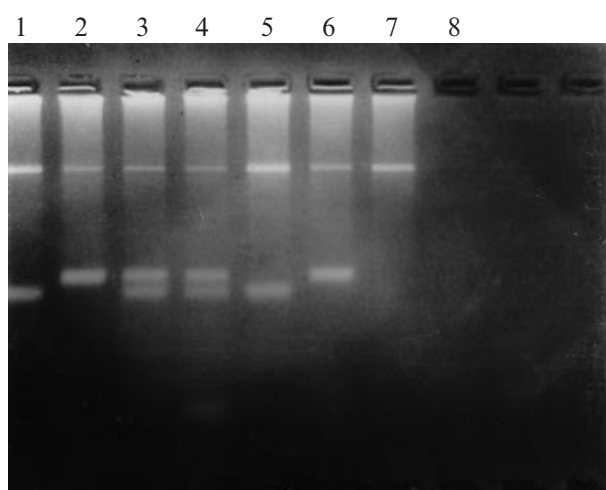


Figure 2—ARMS-PCR amplification for prenatal diagnosis. Lane 1: father, 619 bpd; Lane 2: mother, IVS I-1 (G→T); Lanes 3 and 4:  $\beta$ -thalassaemia major fetus, 619 bpd and IVS I-1 (G→T); Lane 5: positive control for 619 bpd; Lane 6: positive control for IVS I-1 (G→T); Lane 7: negative control; Lane 8: blank

Table 3—Prenatal diagnosis of  $\beta$ -thalassaemia and other haemoglobinopathies

Fetuses at risk for:	DNA analysis			GCS		
	Total	Unaffected	Affected	Total	Unaffected	Affected
$\beta$ -thal./ $\beta$ -thal.	478 <sup>a</sup>	327	140	277 <sup>b</sup>	205	65
$\beta$ -thal./HbE	8	6	2	1	—	1
$\beta$ -thal./ $\delta\beta$ -thal <sup>c</sup>	2 <sup>a</sup>	1	—	6	5	1
$\beta$ -thal./HbS	3	1	2	2	1	1
$\beta$ -thal./HbD thal.	—	—	—	3	1	2
HbS/HbS	2	1	1	4	3	1
HbS/HbD <sup>c</sup>	1	—	1	—	—	—
Total	494	336	146	293	215	71

<sup>a</sup>12 inconclusive diagnoses<sup>b</sup>7 inconclusive diagnoses<sup>c</sup>First reports of prenatal diagnoses done using PCR in India.

fetuses were affected and the parents opted to terminate the pregnancy. In two pregnancies one fetus was a carrier and another had  $\beta$ -thalassaemia major. Both these couples opted to continue the pregnancy; the babies were born as diagnosed. In one case, where one fetus was normal and another was major, selective termination was tried, but since the fetuses had changed their positions in the interim period the normal fetus was terminated inadvertently. On rechecking, the affected fetus was also terminated.

## DISCUSSION

Worldwide, control of thalassaemia and other haemoglobinopathies is achieved through population screening, counselling of carriers and prenatal diagnosis (Petrou and Modell, 1995; Old, 1996). Until the mid 1980's prenatal diagnosis of haemoglobinopathies was not carried out in India and therefore samples were sent abroad. During 1988–1993, with the help of the Overseas Development Administration Programme of the UK, we started the three-pronged approach of control of thalassaemia and other

haemoglobinopathies in India (Petrou and Modell, 1995). Screening programmes conducted by our group and others in India had already identified Sindhi, Bhanushali, Lohana, Khoja, Sikh and Bengali communities as high risk for  $\beta$ -thalassaemia (Sukumaran and Master, 1973; Mahadik *et al.*, 1993; Gangal, 1996).

In the beginning we restricted our studies to GCS, which is a direct method of diagnosis. Later we switched to analysis of five common mutations using the ARMS technique. For couples whose mutations did not fit this group, and those who reported to us in the late second trimester, prenatal diagnosis was offered using GCS or by RFLP. Subsequently, primers for more rare and very rare Indian mutations were included in the programme (see 'Material and methods'). Some primers needed to be modified from published sequences to get the desired specificity (manuscript under preparation). We have now included primers for 23 mutations in our study, reducing the percentage of couples with one or both unidentified mutations to 2.7%. We are also attempting to identify these mutations by DGGE and sequencing.

Considering the social, cultural and religious taboos, influence of elderly family members and general ignorance about the disease, the number of couples reporting late to the prenatal diagnosis laboratory is still considerably high in India. From amongst 656 women who came once or more than once for testing, 648 had earlier affected children and had experienced the trauma of raising such children. Only eight had come to the centre during their first pregnancy, while 99, 18 and six women had appeared for prenatal diagnosis before once, twice and thrice, respectively. Knowledge of the parent's mutations was very helpful in quick diagnosis of the fetus, especially when women came repeatedly for testing. Along with early termination of pregnancy, the additional advantage of using DNA analysis is that the carrier status of the fetus is also identifiable. Hence, postnatal reconfirmation of haemoglobinopathy status is not required as is the case with GCS. In India, though carrier detection and counselling is an economical and

Table 4—Community-wise distribution of fetuses at risk for homozygous mutations

Mutations	No. tested (265)	Communities
IVS I-5 (G→C)	149	MM, Muslim <sup>a</sup> , GB, Muslim <sup>b</sup>
619 bpd	64	Sindhi, Punjabi, Muslim <sup>a</sup> , GL
IVS I-1 (G→T)	20	Punjabi, Sindhi, GL, GBR
FS41/42 (-TCTT)	11	Muslim <sup>a</sup> , GV, GJ
FS8/9 (+G)	7	GJ, GSC, Sindhi,
C15 (G→A)	7	MSC, GP
IVS II-837 (T→G)	2	Karnataka BR
FS16 (-C)	2	Uttar Pradesh SC
C30 (G→A)	2	GSC
IVS 1-25 bpd	1	GSC

Note: Maharashtra—Maharashtrians Maratha (MM), scheduled caste (MSC); Gujrat—Gujratis Bhanushali (GB), Lohana (GL), Jain (GJ), Vania (GV), Patel (GP), Brahmin (GBR), scheduled caste (GSC).

Muslim<sup>a</sup>: mostly Maharashtrian and Gujrati.

Muslim<sup>b</sup>: exclusively from the Maldives.

Table 5—Community-wise distribution of fetuses at risk for heterozygous mutations

Communities	IVS I-5/ 619 bpd	IVS I-5/IVS I-1 <sup>a</sup> ,		IVS I-5/C15	IVS I-5/HbE	IVS I-5/ unidentified	619 bpd/ IVS I-1 <sup>a</sup>	619 bpd/ FS 8-9	FS8-9/ IVS I-1 <sup>a</sup>
		FS41-42, FS8-9							
1. Sindhi	10	9	—	—	—	1	39	20	16
2. Punjabi	4	2	—	—	—	1	6	—	1
3. Gujrati									
Lohana	4	2	—	—	—	—	6	2	1
Bhanushali	2	1	—	—	—	1	—	—	—
Jain	—	3	—	—	—	—	—	—	—
Patel/SC	—	1	2	—	—	—	—	—	—
Vania	—	1	—	—	—	—	—	—	—
Muslim	—	2	—	—	—	—	1	1	—
4. Maharashtraian									
Maratha	—	—	3	—	—	1	—	—	—
Brahmin	—	2	1	—	—	—	—	—	—
Agri/SC	—	—	1	—	—	2	—	—	—
Muslim	—	—	1	—	—	—	—	—	—
5. West Bengal									
Muslim/SC	—	—	1	6	—	1	—	—	—

<sup>a</sup>IVS I-1 (G→T).

effective strategy for prevention, marriages between carriers are not avoidable (Yagnik, 1997). Therefore only prenatal diagnosis would help in controlling births of affected children. Indian parents of  $\beta$ -thalassaemia major children appear to be quite willing to undergo testing and will terminate the pregnancy if needed (Sangani *et al.*, 1990).

In the 794 procedures conducted, fetal loss due to the procedures used was seen in eight cases (0.9%),

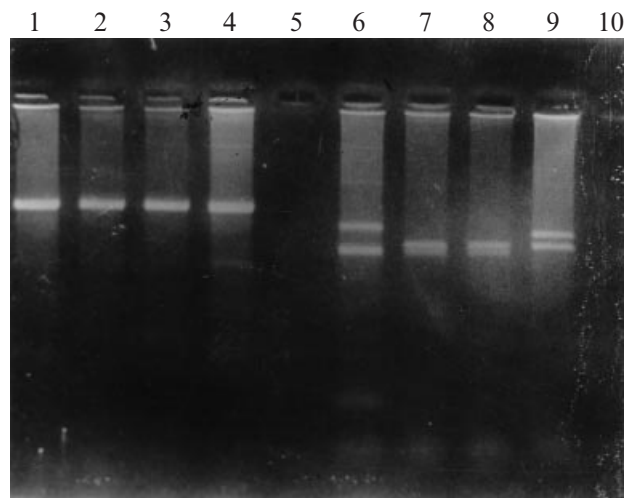


Figure 3—VNTR analysis of hypervariable sites in Apo-B and D1S-80 genes for maternal contamination. Lanes 1-4: 3'Apo-B—father, fetus, fetus, mother; Lanes 6-9: D1S-80—father, fetus, fetus, mother; Lanes 5 and 10: blank

Table 6—VNTR analysis

Sites	No. of tests performed	Informative (%)
Apo-B	112	46 (41)
D1S-80	67	28 (41.7)
Ig-JH	39	19 (47.3)
Ha-ras	20	5 (20)

which is much less than that reported previously (Alter, 1990; Colah *et al.*, 1991). Three affected fetuses were misdiagnosed as carriers (Table 1). This was perhaps due to switching of samples in DNA analysis (two cases) and due to a mild  $\beta$ + mutation in the GCS series (one case), as also reported by Petrou (Petrou *et al.*, 1990). The  $\beta/\gamma$  globin chain synthesis ratio in this case was close to low normal and was found to be due to a mild mutation, i.e. Cap+1 (A→C). This experience suggests that, although a  $\beta/\gamma$  globin chain synthesis ratio of 0.025 is a safe limit to diagnose  $\beta$ -thalassaemia major, the fetuses with a low normal synthesis ratio between 0.025–0.030 need to be retested.

Besides the ARMS technique many PCR based DNA test procedures, such as DGGE, dot blot and reverse dot blot hybridization analysis, sequencing, capillary electrophoresis, etc., have been successfully used for mutation detection as well as for prenatal diagnosis of thalassaemia and haemoglobinopathies. We have found that ARMS works well, with a high level of reliability and reproducibility of results. Similarly, GCS was found to be the only useful diagnostic test in a few cases.

Depending upon gestational age, availability of family members and laboratory procedures, different laboratory strategies of prenatal diagnosis have been suggested for India (Modell and Petrou, 1983; Colah *et al.*, 1991;1997; Varawalla, 1992; Rao *et al.*, 1997; Gorakshakar *et al.*, 1997; Saxena *et al.*, 1998). Our experience suggests the following strategy:

1. Screening of high risk couples for haemoglobinopathies and counselling
2. Conducting mutation analysis (ARMS) of parents
3. If both parents' mutations are identifiable, depending upon gestational age conduct DNA analysis of the fetus
4. If only one parent's mutation is identified, offer testing based on the presence or absence of the known mutation in fetal DNA

5. If (4) is not acceptable, and if the affected/normal child and/or grandparents are available for testing, conduct RFLP analysis
6. As option for (4) and (5), and if the mother presents late in the second trimester, conduct GCS.

Since we now test for large number of mutations, in our centre, options (4) onwards are very rarely required. During 1993–1994, 5 out of 40 fetuses were diagnosed using RFLP, while from 1997 onwards only 12 GCS tests were performed.

The pattern of mutations in different communities conducted on a large number of  $\beta$ -thalassaemia carriers has simplified the prenatal diagnosis analysis for us (manuscript under preparation). Though IVS I-5 (G→C) and 619 bpd are most prevalent, certain rare mutations seem to be fairly common in some Indian communities. In our series C15 (G→A) mutation is commonly found in communities of Maharashtra and Gujrat and therefore should be analysed as a priority, along with the five common mutations in diagnostic centres in these states. Although 55% of fetuses were at risk for homozygosity (Table 4), it was noted that they were mostly non-consanguineous, although endogamous. Compound heterozygosity of  $\beta$ -thalassaemia and HbE was seen in West Bengal and Bangladesh and HbS was seen in scheduled castes from central India where these haemoglobinopathies are prevalent (Sukumaran, 1974; Rao and Gorakshakar, 1990; De *et al.*, 1997). HbD-thalassaemia was seen only in Sindhi parents.

Although the Apo-B site is known to be highly polymorphic (Deka *et al.*, 1992; Saxena *et al.*, 1998), in Indian families tested by us, only 41% of the VNTR analysis were informative. Similarly, in 12 cases, the VNTR analysis in all four sites was not informative. This was mostly due to the fact that both parents had similar patterns of polymorphism at these sites, which may be due to endogamy in the Indian communities. All the non-informative cases mainly belonged to Sindhi, GB, GJ, MM, and MSC groups who had a high risk of homozygous mutations (Table 4). Therefore it is essential to search and include additional highly polymorphic microsatellite markers in these Indian communities for VNTR analysis.

For studies on twin fetuses, VNTR analysis of both chorionic villus samples was found to be useful when the two samples showed different alleles; it helped to confirm that the samples belonged to two different fetuses. Since in one of the twin pregnancies we inadvertently terminated the normal fetus because of the change in its position, we now ask the couple to wait until 16 weeks of gestation before testing, when the chance of fetuses changing their positions is minimum.

Thus, by adopting the strategies mentioned above we have been able to offer definitive diagnoses in 99.8% fetuses. As mentioned earlier, 27.4% of these fetuses were affected. In spite of social, cultural and family taboos regarding termination of pregnancy, 97.7% couples opted to terminate the pregnancy.

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