PRENATAL DIAGNOSIS OF THALASSEMIA IN INDIA THE FIRST EXPERIENCE

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SUMMARY

Genetic counselling and second trimester prenatal diagnosis of thalassemia by globin synthesis on fetal blood obtained by fetoscopy was undertaken for the first time in India. 155 at-risk couples were counselled and 85% of them opted for this diagnostic facility over the last five years. All except one of the 131 couples who underwent testing had come for retrospective diagnosis. Twenty six homozygous fetuses were identified and these pregnancies were terminated. The fetal loss rate came down from 25% in the first year to 7.4% in the last year. There was one laboratory misdiagnosis. A follow up study for confirmation of diagnosis at birth or later was possible in 35 cases. Our preliminary experience has shown that such a programme is feasible and by and large acceptable. It is the first step towards initiation of community control of the disease.

INTRODUCTION

Beta-thalassemia or its interaction with structural hemoglobin variants like Hb S and Hb E is the commonest hereditary anemia in the Indian subcontinent. The incidence of B-thalassemia heterozygotes in certain communities like Gujratis, Punjabis and Sindhis is considerable, ranging from 5-15% (Mehta et al 1972; Sukumaran 1975; Bhatia et al 1976; ICMR Report 1989). Hb S occurs in a high frequency (10 to 40%) in tribal groups and Scheduled castes (Sukumaran 1975; Bhatia and Rao 1987). Hb E is found Primarily in Eastern India in a frequency ranging from 3 to 15% (Sukumaran 1975).

Based on these figures it is estimated that approximately 20 million people are heterozygous for any of these abnormal

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genes and more than 5000 children are born annually inheriting one of the major thalassemia syndromes. (Modell & Petrou 1983, Agarwal 1986).

Although rapid advances in clinical management will enable the patient to lead less stressful or near normal life, the financial burden involved is beyond the reach of the large majority of population (Agarwal 1986). This justifies the need for introduction of a prevention programme through prenatal diagnosis. The Indian Council of Medical Research established the first centre for second trimester prenatal diagnosis by globin biosynthesis in Bombay. This paper reviews our experience with the procedure over the last five years and its impact on introduction of a prevention programme.

MATERIAL AND METHODS

Genetic Counselling :

Both husband and wife were called for counselling. The entire procedure as well as the risks involved were explained and a written consent taken before undergoing the test. The carrier status in both partners was confirmed.

Fetal Blood Sampling :

This was done by fetoscopy under ultrasound guidance. A Storz operating fetoscope with an outer diameter of 2.7mm and a 27 guage blood sampling needle was used throughout the study. The procedure was carried out in the operating theatre with strict aseptic precautions under intravenous sedation by atropine (0.6 mg), pentazocine (30 mg) and diazapam (10 mg). 5.0 ml of 1.0% xylocaine was used for local infiltration anesthesia at the chosen site of entry. A small 3mm stab incision was made through the skin and under ultrasound guidance, the trocar and cannula was introduced in the amniotic cavity, taking care to avoid the placenta. A loop of umbilical cord was identified and the blood sampling needle advanced through the operating channel to puncture the vessels in the umbilical cord. Fetal blood was aspirated in a heparinized syringe. The volume of blood withdrawn ranged from 10 to 400 ml. After confirmation of an adequate fetal sample, the fetoscope was withdrawn and a sterile dressing done.

Assessment of the Sample withdrawn :

An MCV determination was done on an ERMA particle counter and a sample with an MCV of greater than 100 fl.was found to contain sufficient fetal cells to make a diagnosis. When a suitable sample was not obtained at the first attempt, the procedure was repeated after waiting for 1-2 weeks. The percentage of F cells in the sample was determined by fetal cell staining (Kleihauer et al 1957).

Laboratory diagnosis :

Globin biosynthesis was carried out by the .conventional carboxymethyl cellulose chromatography and assessment of B-chain radioactivity in relation to that of pre 4+4 and/or 2 chains (Alter, 1983). A maternal biosynthesis was done on an identical number of cells as the fetal sample for subtraction for maternal contamination in all cases where a 100% fetal sample was not obtained. Isoelectric focusing (IEF) was carried out in all cases where a 100% fetal sample was obtained (Basset et al 1978).

Follow-up of diagnosis :

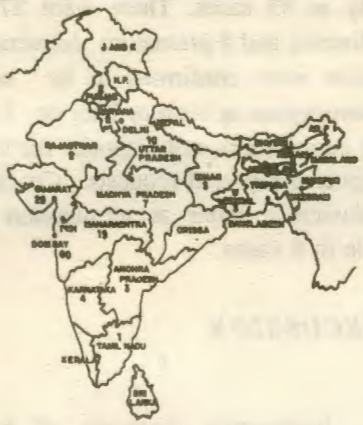
Fetuses diagnosed as normal or

heterozygous were followed up at birth or at 6 months of age for confirmation of diagnosis. When the fetus was diagnosed as homozygous, the parents were advised to terminate the pregnancy. Termination was done by extra amniotic Emcredyl (1% Ethacridine Lactate-150 ml) instillation with pitocin.

RESULTS

A total of 155 couples referred from various regions in India were counselled. 38% of the couples were residing in Bombay while the remaining had come from various other parts of the country (Fig.1). While 57%

FIG-1 STATEWISE DISTRIBUTION OF 155 COUPLES REFERRED FOR PRENATAL DIAGNOSIS



FIGURES INDICATE NUMBER OF COUPLES

of the cases belonged to the Gujrati, Punjabi or Sindhi communities where the incidence of B-thalassemia is high, there were several cases from other caste groups like Marathas, Brahmins, Schedule Caste, Muslim, Bengalees, Artisans & Christians. 24 couples (15.4%) refused to undergo prenatal diagnosis and majority of them decided to continue with the pregnancy. While all these couples had at least one previous child with B-thalassemia major, 8 of them (53%) did not have any normal child. Of the 131 couples who underwent testing, only one couple had come for prospective fetal diagnosis before having an affected child. In 94% of the couples tested, both partners were heterozygous for classical high Hb A2 B-thalassemia, in 2 couples one partner had silent Hb A2 B-thalassemia, in 2 others one o73 partner had Hb D-thalassemia, in one couple one partner had B-thalassemia and one couple each was at risk for Hb E thalassemia and Hb S thalassemia.

The age of the mothers ranged from 18 to 37 years. Details of fetal blood sampling are shown in fig.2. In majority of the cases fetoscopy was done between 18 to 20 weeks of gestation. A pure fetal sample was withdrawn in 69% of cases while a maternal contamination of more than 50% was present only in 4 cases. A fetal sample for diagnosis could not be obtained in 13% of cases and this was more so when the placenta was anterior (Fig.2)

FIG-2 NUMBER OF ATTEMPTS AT FETAL BLOOD SAMPLING BY FETOSCOPY AND QUALITY OF SAMPLE WITHDRAWN

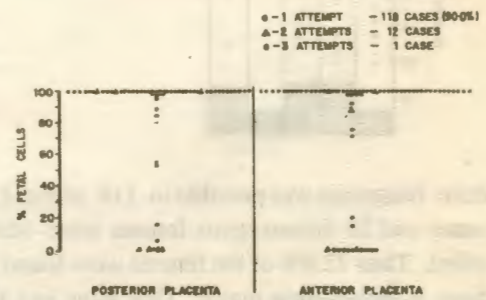
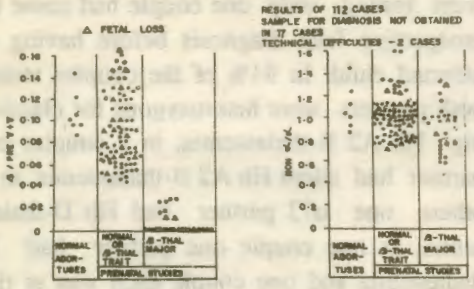


Fig.3 gives the B/pre 4+4 ratios and non 2/2 ratios in normals and heterozygotes ranged from 0.7 to 1.6 and in the homozygotes from 0.57 to 1.3.

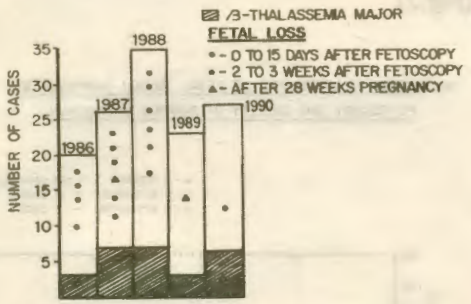
FIG-3. PRENATAL β / PRE γ+γ AND NON α/α RATIOS IN NORMAL AND THALASSEMIC FETUSES



Isoelectric focusing gave concurrent results to column chromatography.

The overall yearwise results are shown in Fig.4. During 1989, the procedure was discontinued for 5 months to fulfil a new Government regulation for procurement of a licence before undertaking any interventional procedure. Diagnosis was possible in 114 of the 131 cases and 26 homozygous fetuses were identified. Thus 22.8% of the fetuses were found to have B-thalassemia major. One fetus had Hb D trait and one had Hb D-thalassemia. There was one laboratory misdiagnosis. The fetal sample had 80% maternal contamination. After subtraction for maternal contamination, the B/pre 4+4 ratio of the fetus was 0.056 indicative of a heterozygous condition. However a few months after birth, the child presented with anemia and was found to have B-thalassemia major. There was a decrease in the rate of fetal loss from 25% in the first year to 7.4% in 1990. Majority of the fetal losses (17/21) were within a week of the procedure and were related to the duration of fetoscopy. When the duration of the procedure exceeded 20 minutes, there was a much higher risk of fetal loss. The two fetuses were lost after 28 weeks pregnancy and may not be related to the procedure. In 2 cases a diagnosis could not be given due to technical difficulties in the laboratory.

FIG-4 YEARWISE ANALYSIS OF RESULTS



Majority of the women with a favourable diagnosis must have delivered already. A follow up of prenatal diagnosis was possible only in 35 cases. There were 27 full term deliveries and 8 premature deliveries and the babies were confirmed to be normal or heterozygous at birth or later on. The remaining couples did not respond for a follow up inspite of repeated requests. Confirmation of thalassemia major at termination was possible in 8 cases.

DISCUSSION

Intrauterine diagnosis of hemoglobin disorders was first attempted in 1974 (Kan et al 1975) and has now been performed in more than 10,000 fetuses at about 30 centres worldwide. Fetal blood sampling in the second trimester of pregnancy and diagnosis by globin synthesis was the initial method. However, due to its limitations of a greater risk of fetal loss and a later therapeutic abortion if indicated, majority of the centres have gradually replaced this procedure with DNA analysis on chronic villi samples in the fir

trimester (Alter 1988).

India is a vast country with a diverse population and many religions and cultures. B-thalassemia is prevalent in many caste groups. Fetal diagnosis of hemoglobinopathies was started in the country with the objective of providing a diagnostic facility at a nominal cost for Indian patients who until then had to get the diagnosis done in Western countries incurring an expenditure affordable by only a few families.

The present analysis covers a period of 5 years from inception of this centre in 1986 to 1990. As is apparent from the map,62% of families have travelled long distances and from various corners of the country against many odds to avail of this facility. This shows the overall acceptibility of the programme and intensifies the need for having regional centres to undertake such investigations.

15% of the couples refused to undergo the test after genetic counselling. While every effort was made to convince them, we had to understand and respect the social, psychological, religious and cultural norms of the people.

Some of the couples were not prepared to undertake the risk of fetal loss involved and some did not wish to terminate the pregnancy if indicated.

An older obstetric procedure like fetoscopy was used due to a relatively poor quality ultrasound machine that was available to us. The longer duration of fetoscopy was partly attributed to the absence of a Coulter channalyser in the vicinity of the operating room. The obstetrician had to wait till the sample was taken to the laboratory for an MCV determination and the result brought back before withdrawing the scope. Inspite of this, the percentage of fetal losses which was considerable at the beginning, steadily decreased over the years. In a large prevention programme in Greece, the rate of fetal loss was 28.6% in the first year but came down to 1.9% after 6 years (Loukopoulos et al 1985).

We used a B/preu+u ratio of 0.03 to differentiate between homozygous and heterozygous fetuses. This was quite distinct from the lower limit (0.04) found in heterozygotes. Using this cut off point, 22.8% of fetuses were diagnosed as homozygous which is slightly lower than the expected 25%. Isoelectric focusing was found to be reliable and rapid and an excellent back up for diagnosis in 100% fetal samples.

Prenatal diagnosis although offered retrospectively so far in all except one case has on the whole been found to be feasible and acceptable as seen in our preliminary endeavour. With our recent updating of the ultrasound equipment and acquisition of a Coulter channalyser, fetoscopy will now be replaced by cordocentesis which should further bring down the obstetric complications. At the same time our efforts are directed at establishing the DNA technology for first trimester diagnosis on chorionic villus biopsy. The transition from retrospective to prospective fetal diagnosis will require education of the population and large scale screening programmes to identify heterozygotes prospectively before reproduction. We have already initiated such screening in high risk communities (Gorakshakar et al 1990). This will help to initiate a community control programme which has been shown to be successful in several countries (W.H.O. 1983).

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