7 Prenatal diagnosis of thalassaemia

Introduction:

At present a regular service for prenatal diagnosis of thalassaemia does not exist in any Muslim country. Consequently, the response of a Muslim community to the availability of prenatal diagnosis is largely unknown. A service for prenatal diagnosis was introduced for the first time in Pakistan in May 1994. This chapter describes the process of introducing the service and the response of the affected families to the availability of this facility in Pakistan. The study period extended from May 1994 to July 1996.

Methods:

Initial preparation:

The service for prenatal diagnosis of thalassaemia was introduced after initial preparation of training and establishment of DNA laboratory facilities, training of an obstetrician in fetal sampling, and consultation with the religious scholars on termination of pregnancy for a genetic disorder. After initial preparation the facility was advertised for the at risk couples.

Training and establishment of laboratory facilities:

I was trained at the Perinatal Centre, Department of Obstetrics and Gynaecology, University College Hospital, London. The training involved analysis of DNA samples from thalassaemia patients collected in Pakistan. At the end of six months training from July to December 1993 information on the pattern of mutations in the ethnic groups of Pakistan was also available.

The necessary hardware and consumables for PCR based analysis of thalassaemia mutations were procured from the UK. On my return back to Pakistan, in January 1994, I established a DNA laboratory at the Department of Haematology, Armed Forces Institute

of Pathology (AFIP) Rawalpindi. The laboratory was completely functional by March 1994.

Training of obstetrician:

In April 1994 I came to know that Dr. Yasmeen Raashid, a female consultant obstetrician at Lady Wellington Hospital Lahore, had training in Chorionic Villus Sampling (CVS). Dr. Raashid had worked under the supervision of Dr. Gerald Mason, Fetomaternal Consultant, Leeds General Infirmary. She could do CVS by the transabdominal placental aspiration technique. Due to the availability of the obstetrician it became possible to offer prenatal diagnostic service. At the same time an experienced female obstetrician (Dr. Nadra Sultana) from the Military Hospital, Rawalpindi was also sent for training in CVS to the Department of Obstetrics and Gynaecology, University College Hospital, London. She had training for three months under the supervision of Prof. C. H. Rodeck and on her return to Pakistan in August 1994 she started doing CVSs at AFIP. Dr. Sultana was trained in using CVS biopsy forceps by the transabdominal route.

Consultation with religious scholars:

During the initial preparation I had personal communications with two of the renowned religious scholars regarding Islam's view point on termination of pregnancy for a serious genetic abnormality. A personal meeting with Dr. Malik Ghulam Murtaza was arranged at Lahore. Dr. Murtaza was of the opinion that termination of pregnancy is permissible for thalassaemia if carried out before 120 days of gestation. The other scholar, Mohammad Taqi Othmani, was approached through a letter. The reply by Mr. Othmani (Appendix-A) stated that Islam permits termination of pregnancy for a genetic disorder provided (1) the diagnosis is made by an honest person (2) severe nature of the disorder is confirmed (3) termination of pregnancy is carried out before 120 days of gestation. He clearly prohibited termination after 120 days even if the fetus is affected by a serious genetic abnormality. He considered termination after 120 days like killing an affected child.

Advertisement:

After establishing the laboratory facilities, completing arrangements for CVS and obtaining the opinion of religious scholars on termination of pregnancy, the service was advertised. Most of the thalassaemics in Pakistan are treated at Fatimid Foundation

thalassaemia centres, in Karachi, Multan, Lahore and Peshawar. Another NGO has its treatment centre in Rawalpindi. This provides an easy way of communicating with the affected families. The administration and the medical staff of the two charity organizations were informed about the availability of prenatal diagnosis. They in turn started communicating the information to the affected families. Instruction booklets written in "Urdu" the local language, were also provided for distribution to the affected families. The service was also advertised in the newspapers. A few programmes about the significance of prenatal diagnosis were also broadcasted on Pakistan Television Network.

Booking a couple for prenatal diagnosis:

The couples from Lahore and its surrounding areas reported at the obstetrics out patient clinic of Lady Wellington Hospital, Lahore and those from Rawalpindi and its surroundings reported at the Department of Haematology, Armed Forces Institute of Pathology, Rawalpindi. At the time of booking, the complete procedure of prenatal diagnosis its associated risks and the chances of error in diagnosis were fully explained. The chances of having an affected or unaffected child and the possibility of termination of pregnancy was also discussed. The ethnic group, parental consanguinity and the source from where the couple came to know about prenatal diagnosis was noted. The gestation of the pregnancy and the position of placenta were ascertained by ultrasound examination and the date of CVS was booked. Information about the socio-economic status, educational status, number of children, psychosocial burden and financial burden of thalassaemia was also recorded on a pre-designed questionnaire (Appendix-B). This was done with a view of identifying the type of couples who requested prenatal diagnosis.

Blood samples (3-5 ml) from the parents and an affected child, if available, were collected in EDTA containers. The couples examined at Lahore were mostly sampled at the time of CVS and the samples were despatched to AFIP for further analysis. In 7/158 (4.4%) couples the husband's sample could not be obtained because of their non-availability (5) or lack of interest (2).

Chorionic Villus Sampling:

Dr. Yasmeen Raashid did sampling at Lady Wellington Hospital, Lahore by a free hand transabdominal ultrasound guided aspiration technique (Brambati et al, 1988). Dr. Nadra Sultana did CVSs at AFIP, Rawalpindi by transabdominal biopsy forceps (Rodeck and Nicolini 1989). Most of the CVSs done at Lahore were collected in culture medium (RPMI-1640) and were transported to AFIP in ice within 24 hours. The samples were usually transported by the father or a close relative of the couple. When a person was not available to carry the samples or when the CVS was done before a closed holiday, a delay of up to 36 hours was encountered. Due to a short supply of RPMI-1640, ten CVSs performed at Lahore were collected and transported in normal saline. The samples obtained at AFIP were collected in normal saline and were processed without delay.

Mutation analysis of the parents:

Haematological diagnosis was available in only a few couples who requested prenatal diagnosis. Therefore prior to mutation analysis red cell indices were routinely done in all couples. Low MCV and MCH suggested thalassaemia trait whereas normal indices indicated either incorrect diagnosis or a silent β-thalassaemia mutation (Cap+1).

In the first round of mutation analysis the five most common mutations found in the ethnic group of the couple were tested. If the first round did not identify a mutation the second round of ARMS was done to screen for the uncommon mutations. The rare mutations were screened when the first two rounds failed to identify the mutation. In the last ten couples of the study, a multiplex ARMS PCR was also done. Three multiplex primer combinations were used to test each couple.

Where the father's DNA was not available, DNA from the affected child was used to characterize the father's mutation. The child's sample was tested in the usual way and when a mutation was found its normal allele was checked. The absence of a normal allele indicated that the father and the mother had the same mutation. If the affected child showed the normal allele the sample was further tested for other mutations.

Fetal diagnosis:

CVS is often contaminated with maternal decidua. Careful dissection under a microscope is essential to remove the maternal tissue (Cao and Rosatelli 1993). A stereo zoom microscope was used to clean the CVS. Branched and highly vascular villus structures were easily identified as compared to the more solid looking and much less vascular pieces of maternal decidua.

Mutation analysis:

Fetal diagnosis was accomplished by putting up ten ARMS reactions i.e. one for each parent, CVS in duplicate for the parent's mutation, one negative control for the parent's mutation, CVS in duplicate for the normal allele of the parent's mutation, negative and positive controls for the normal allele of the parent's mutations, and a reagent blank.

Linkage analysis:

When one or both mutations in the couple could not be identified by ARMS, diagnosis was done by linkage analysis. Six polymorphic sites closely linked to the β-globin gene were used as linkage markers.

Assessment of maternal contamination:

Maternal contamination was assessed in the first 10 CVSs by carrying out Apo-B VNTR analysis of the parents and the fetus (Rosatelli et al, 1992a). Although it would be preferable to do this on all samples to rule out maternal contamination as well as non-paternity, the cost must also be taken into account when applying this to a country such as Pakistan. Therefore in order to cut down the cost, this practice was discontinued and VNTR analysis was done only when difficulty was encountered in separating the maternal and the fetal tissues.

Follow-up:

The record of termination of pregnancy, complications of pregnancy, and postnatal out come following prenatal diagnosis was maintained in as many cases as was possible.

Response of the affected families to prenatal diagnosis:

At the end of the study, randomly selected 141 couples who visited Fatimid Thalassaemia Centre, Lahore for treatment of their children were interviewed to find out their response to the availability of prenatal diagnosis. The couples served as control for comparison with those who requested prenatal diagnosis. The response of the couples was recorded on a pre-designed questionnaire (Appendix-B) that included knowledge about the availability of prenatal diagnosis, any pregnancy after the availability of prenatal diagnosis, request for prenatal diagnosis in pregnancy (if any), request for prenatal diagnosis in future pregnancies and attitude towards termination of pregnancy.

Results:

Within 2-3 weeks from the announcement of the service the first couple came forward with a request for prenatal diagnosis. Several other couples who were avoiding a pregnancy came for counselling and information about the test. Thereafter the couples appeared at regular intervals and a steady increase in the number of requests for prenatal diagnosis was observed. During the two years of the study a total of 158 couples requested prenatal diagnosis. This included 118 (74.7%) Punjabi, 30 (19%) Pathan, 4 (2.5%) Baluchi, 4 (2.5%) Mohajir and 2 (1.3%) Sindhi couples. Fig: 7.1 shows the number of couples who requested prenatal diagnosis in each quarter of the year. The distribution of the couples according to the place of origin is presented in Fig: 7.2.

Almost all of the couples were informed about prenatal diagnosis by their doctors. Table: 7.1 gives a summary of the medium of information for the couples who requested prenatal diagnosis. A significant proportion (21%) was inspired by the experience of other couples who already had used prenatal diagnosis and many of whom had normal children due to the test. In 31% of the couples one member, usually the mother, had watched a programme on television. The information booklets had either not been delivered to all couples or many were not able to read because of illiteracy. Only two couples came with a request for prenatal diagnosis after having read the information booklet alone.

Table: 7.1. The medium of information for the couples who requested prenatal diagnosis.

Informed by:	**Number:**	**Percent:**
Treating doctor	153/158	96.8%
Television	45	28.5%
Other parents who had used prenatal diagnosis	33	20.9%
Information booklet	2	1.3%

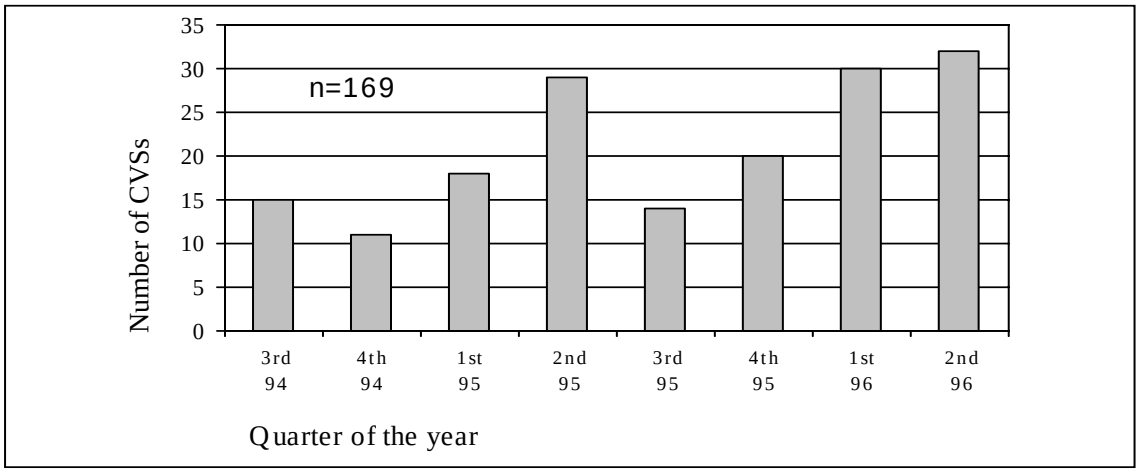

Fig: 7.1. The number of CVSs done for prenatal diagnosis of thalassaemia in the two study years.

Characteristics of the couples who requested prenatal diagnosis:

The characteristics of couples who requested prenatal diagnosis (cases) were compared with the randomly interviewed couples (controls) and the comparison was as follows:

Socio-economic status:

There were significantly more couples in the high socio-economic group (Table: 7.2) who requested prenatal diagnosis (p=0.0126).

Educational standard:

The educational standard of the couples who requested prenatal diagnosis was better as compared to the controls (Table: 7.3).

Fig: 7.2. Area wise distribution of 158 couples who requested prenatal diagnosis. Most of the couples came from areas in and around Lahore and Rawalpindi.

Number of children:

Only 2/158 (1.3%) couples who requested prenatal diagnosis did not have an affected child before the test. In one couple the father was a laboratory technician and therefore was well informed of thalassaemia. In the other couple the mother was a doctor who had an affected first cousin. Comparison between the two groups of couples (Fig: 7.3) showed a near normal distribution of the number of children in the controls. Whereas the distribution was markedly skewed towards the left in the couples who requested prenatal diagnosis. This was mainly due to the younger age group of the couples who requested prenatal diagnosis.

Effect of thalassaemia on family life:

The family life of 69% of the couples who requested prenatal diagnosis was affected a lot due to thalassaemia as compared to 83% in the controls (Table: 7.4).

Financial burden of thalassaemia:

There was a significant differences in the number of couples in the high income group between the cases and the controls (Table: 7.5). Financial burden of thalassaemia was also significantly different (Table: 7.6). Severe burden was felt by 58% of the couples who requested prenatal diagnosis as compared to 32% in the control group (p=<0.001).

Table: 7.2. Comparison of the socio-economic status of the group of couples who requested prenatal diagnosis and the controls.

Socio-economic status:	**Low:**	**Middle:**	**High:**
Cases (n=158)	99 (62.7%)	38 (24.1%)	21 (13.3%)
Controls (n=141)	110 (78.0%)	25 (17.7%)	6 (4.3%)
p:	0.224	0.279	0.0126

Table: 7.3. Comparison between the educational standard of the group of couples who requested prenatal diagnosis and the controls.

Education:	**None**	**Primary**	**Matric***	**Above matric**
Father's education:				
Cases (n=158)	12 (7.6%)	32 (20.3%)	43 (27.2%)	71 (44.9%)
Controls (n=141)	35 (24.8%)	29 (20.6%)	39 (27.7%)	38 (27.0%)
p:	0.0005	0.956	0.948	0.0267
Mother's education:				
Cases (n=158)	36 (22.8%)	54 (34.2%)	36 (22.8%)	32 (20.6%)
Controls (n=141)	61(43.3%)	45 (31.9%)	19 (13.5%)	16 (11.3%)
p:	0.0071	0.768	0.084	0.074

*matric is an educational standard that is equivalent to O-levels.

Table: 7.4. Comparison of the effect of thalassaemia on the family life of couples who requested prenatal diagnosis and the controls.

Effect on family life:	**A little:**	**Often:**	**A lot:**
Cases (n=158)	10 (6.3%)	39 (24.7%)	109 (69.0%)
Controls (n=141)	2 (1.4%)	22 (15.6%)	117 (82.9%)
p:	0.038	0.112	0.295

Table: 7.5. Comparison of the monthly income of the couples who requested prenatal diagnosis and the controls.

Monthly income (Rs):	**< 5,000:**	**5,000-10,000:**	**> 10,000:**
Cases (n=158)	99 (62.6%)	38 (24.1%)	21 (13.3%)
Controls (n=141)	108 (76.6%)	27 (19.1%)	6 (4.3%)
p:	0.267	0.410	0.0126

Table: 7.6. Comparison of the effect of thalassaemia on the financial situation of the couples who requested prenatal diagnosis and the controls.

Effect on financial Situation:	**A little:**	**Often:**	**A lot:**
Cases (n=158)	19 (12.0%)	47 (29.7%)	92 (58.2%)
Controls (n=141)	48 (34.0%)	54 (38.3%)	39 (27.7%)
p:	<0.001	0.272	<0.001

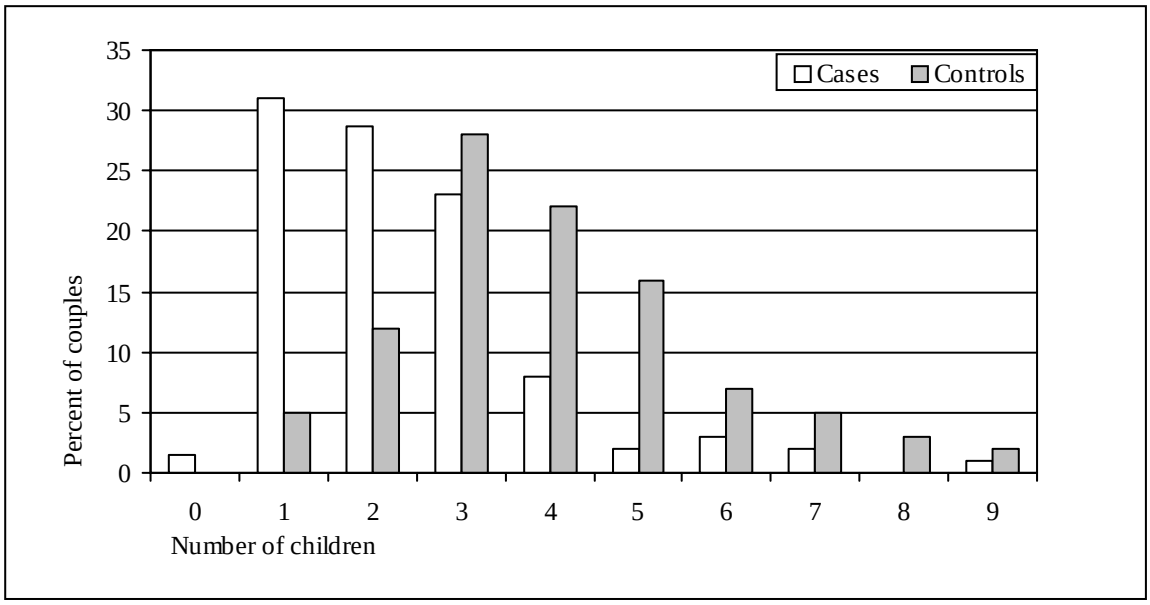

Fig: 7.3. Comparison of the number of children in the couples who requested prenatal diagnosis and the control group. The distribution in the control group is near normal whereas in the couples who requested prenatal diagnosis the distribution is markedly skewed towards the left. The data is presented as percent of the actual number of couples in each group.

Fetal diagnosis:

Chorionic Villus Sampling:

Out of 158 couples who requested prenatal diagnosis 9 (5.7%) used it twice and one couple used it three times. Seven couples who requested prenatal diagnosis more than once, had a previous diagnosis of an affected fetus. A total of 169 CVSs were done that included 148 for the couples who requested the test once, 18 for the couples who requested it twice and 3 for a couple who requested it thrice. There were 161 single pregnancies and four twin pregnancies. Of 169 CVSs 89 (53%) were done at Lahore and the remaining 80 (47%) were done at AFIP, Rawalpindi. Most of the CVSs were done between 10 and 16 weeks of gestation (Fig: 7.4). Only 11/169 (6.5%) were done after 16 weeks and two were done after 18 weeks. In 8 cases CVS was repeated because either the first attempt was not successful or the sample obtained was inadequate. Five of the later CVSs were done at AFIP by a CVS biopsy forceps, while the other three were done at Lahore where the placental aspiration technique was used.

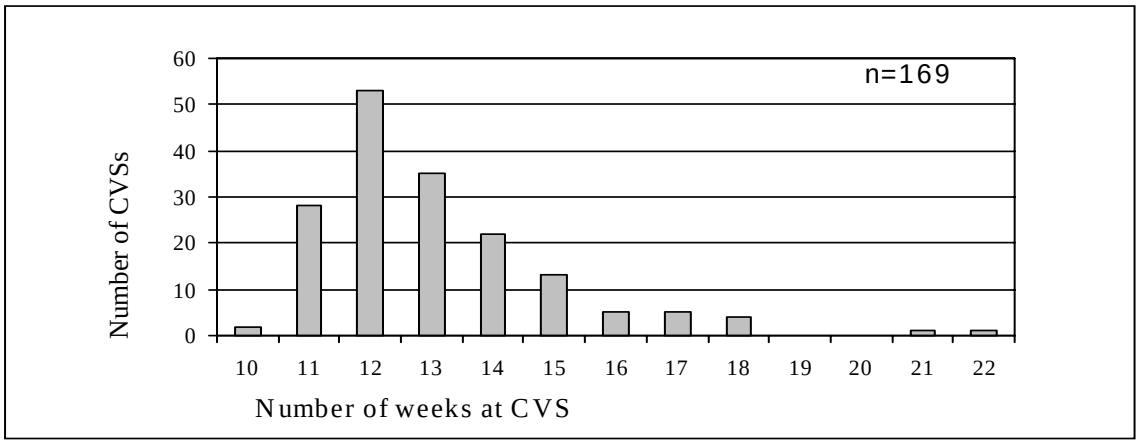

Fig: 7.4. The number of weeks of gestation in 169 CVSs carried out at Lahore and Rawalpindi.

Mutation analysis in the parents:

Measurement of red cell indices prior to mutation analysis was found to be useful. In one couple it indicated an incorrect diagnosis of thalassaemia. This was later confirmed by Hb-A_2 estimation. Bone marrow aspiration of the affected child showed the features of congenital dyserythropoietic anaemia. The couple was excluded from the study as CVS had not yet been carried out. In nine couples one of the parents had either completely

normal or borderline red cell indices. Five of these had Cap+1 mutation and the remaining four, who had borderline red cell indices had a severe β-thalassaemia mutation.

Standard ARMS PCR:

The five common mutations accounted for 257/316 (81.3%) of the alleles (Table: 7.7). After the first round of PCR both members were assigned a mutation in 115/158 (72.8%) couples. In the second round of PCR another 54 (17%) of the alleles were identified and 153/158 (96.8%) of the couples were assigned mutations. After the final round of PCR 313/316 (99.1%) of the alleles were identified and 155/158 (98.1%) couples were completely assigned their mutations. In two of the remaining three couples only one parent and in the third couple both parent's mutations could not be characterized. In the first couple sequencing done at a later stage showed -88 (C-T) mutation and in the second couple sequencing of the entire β-gene failed to reveal a mutation. The sample from the third couple awaits sequencing.

Multiplex ARMS PCR:

The multiplex ARMS PCR proved to be technically feasible for mutation detection in all of the couples tested (Fig: 7.5). The results of multiplex and standard ARMS PCR were in complete agreement. However, the problem of false positive results with the primers described in Chapter: 3 became more pronounced because of the sensitivity of silver staining method. This was overcome by taking precautions already described and by loading only 1-2 μl of the amplified product on the gel. The method was quick and the addition of many different primers, that can be a potential source of error if addition of a primer is omitted were simplified. The polyacrylamide gels as compared to the agarose gels were more difficult to prepare and handle. The main advantage was the use of only six reactions per couple that could screen over 97% of the mutations. This saved a substantial amount of time and money.

Analysis of mutations in the fetal DNA:

While testing the fetal DNA parental samples were included to reconfirm the findings of initial screening. This was essential to prevent clerical mistakes. Negative and positive controls were also included to safeguard against technical errors. The presence of the

parental mutation and the absence of its normal allele in the test sample indicated a homozygous fetus (Fig: 7.6a). The presence of mutant and the normal alleles indicated a heterozygous fetus, a normal allele but no mutation indicated a normal fetus. In cases where both the parents had different mutations and both were also present in the fetal sample, a diagnosis of an affected fetus was made. Similarly, the presence of one of the mutations indicated a heterozygous fetus.

Linkage analysis:

In three couples where one or both of the mutations remained uncharacterized after screening with the ARMS had linkage analysis done. In two couples one mutation was known and the same mutation was also present in the fetal DNA. Linkage analysis in these couples was done as even partially informative markers were also helpful. In the third couple both mutations remained uncharacterized,, for diagnosis a polymorphism at 5'ψβ was informative and antenatal diagnosis was successfully accomplished (Fig: 7.6b).

Results of fetal diagnosis:

In the 169 fetuses (CVSs) diagnosed, 43 (25.4%) were affected (homozygous/compound heterozygous), 44 (26.0%) were normal, and 82 (48.5%) were heterozygous for thalassaemia.

Maternal contamination:

VNTR analysis in the first ten CVSs ruled out maternal contamination. Experience in the first ten CVSs showed that meticulous cleaning of the CVS is enough to safeguard against maternal contamination.

Time frame of prenatal diagnosis:

The usual time taken for a prenatal diagnosis varied between one day to one week. In couples where mutation analysis had previously been done, the CVS results were usually available the following day. The results were considerably delayed where CVS was done on the same day as blood was collected. Linkage based diagnosis took up to one week.

Table: 7.7. Characterization of β-thalassaemia mutations by ARMS in 158 couples who requested prenatal diagnosis.

ARMS PCR:		**Results:**	
Round:	**Mutations screened:**	**No of alleles detected:**	**No of couples resolved:**
1st	Fr 8-9 (+G) IVSI-5 (G-C) Fr 41-42 (-TTCT) IVSI-1 (G-T) Del 619 bp	257/316 (81.3%)	115/158 (72.8%)
2nd	Cd 5 (-CT) Cd 30 (G-C) Cd 30 (G-A) Cd 15 (G-A) Fr 16 (-C) Cap +1 (A-C) IVSII-1 (G-A)	54/316 (17.1%)	153/158 (96.8%)
3rd	Hb-E IVSI minus 25 (-25bp) Fr 47-48 (+ATCT) IVSI-1 (G-A) Cd 39 (C-T)	2 (0.6%)	155/158 (98.1%)
-	Uncharacterized mutations:	3 (0.9%)	-
Total:		313/316 (99.1%)	155/158 (98.1%)

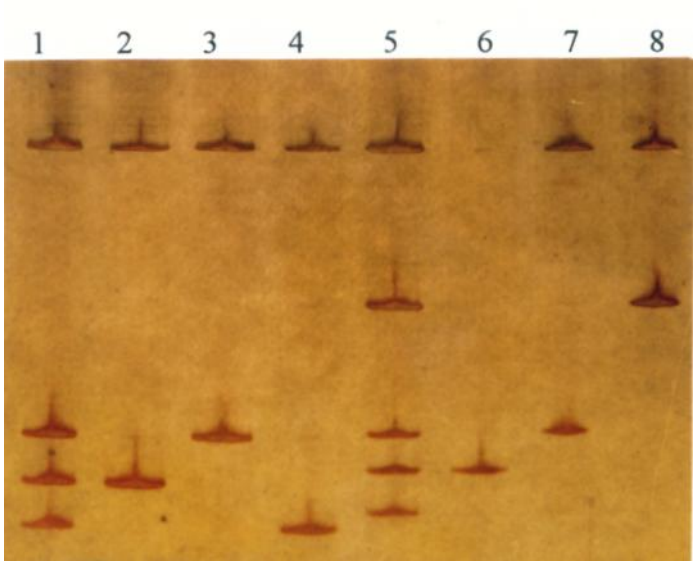

Fig: 7.5. Silver stained polyacrylamide gel electrophoresis of multiplex ARMS PCR for β-thalassaemia mutations. Lane-1 and 5 show allelic ladders for AD-2 and AD-1 respectively. Lane 2, 3, and 4 show AD-2 multiplex reactions positive for Fr 16, Cd 30, and Cd 5 respectively. Lanes 6, 7, and 8 show AD-1 multiplex reactions positive for del 619, IVSI-5, and Fr 41-42 respectively. Control bands of 861 bp are visible in all lanes except lane 6 where it indicates homozygous del 619.

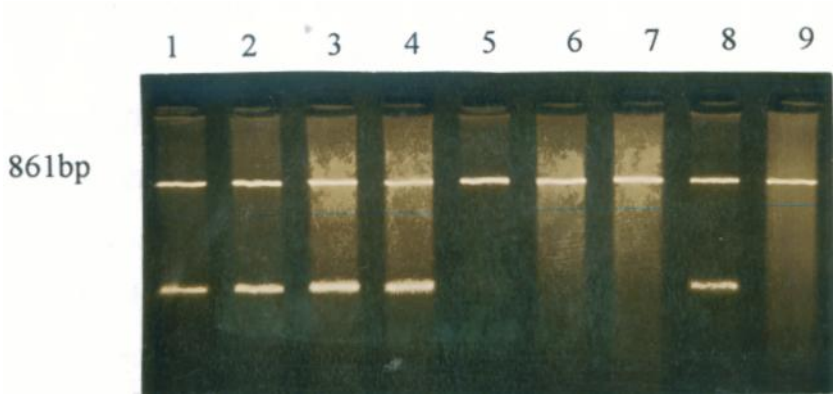

Fig: 7.6a. Ethidium bromide stained agarose gel electrophoresis of a standard ARMS PCR for prenatal diagnosis of thalassaemia. All lanes show 861bp control bands. Lanes 1-4 show 285 bp bands of IVSI-5. Lane 1 and 2 contain parent's samples while lane 3-4 contain fetal DNA in duplicate. Lane 5 is a negative control for IVSI-5. Lanes 6-7 show absence of bands for the normal allele of IVSI-5 in the fetal DNA. Lane 8 and 9 are positive and negative controls for the normal allele of IVSI-5. The results indicate a homozygous fetus.

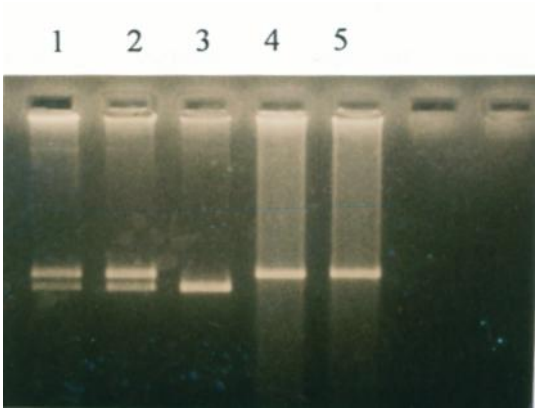

Fig: 7.6b. Prenatal diagnosis by linkage analysis at the 5'ψβ polymorphism. Figure shows the Hinc-II digest of the PCR amplified fragments after electrophoresis on agarose gel. Lane 1: father (-/+), Lane 2: mother (-/+), lane 3: affected child (+/+) and lane 4 & 5: CVS (-/-). The findings suggest that thalassaemia mutation is linked to "+" site and the CVS is "Normal".

Follow-up:

Complete follow-up was possible in 117/158 couples the rest were lost to follow-up.

Complications of CVS:

In 78 pregnancies that were not terminated and were available for follow-up, 7 (8.9%) spontaneously terminated within 2 weeks following a CVS. The complications occurred in 3/37 (8.1%) and 4/41 (9.7%) at the centres in Lahore and Rawalpindi respectively. The miscarriages at Lahore occurred during the first 20 CVSs whereas at Rawalpindi these were evenly spread over the entire study period.

Termination of pregnancy:

Out of 43 women diagnosed with an affected fetus, 42 were available for follow-up and all but three (93%) terminated their pregnancy. In 32/39 (82%) cases termination was done before 15 weeks of gestation (Fig: 7.7). In three cases termination was done at or after 17 weeks (17, 18 and 21 weeks). Three couples did not terminate pregnancy due to religious reasons. In all three the CVS was carried out between 13 and 14 weeks of gestation and the results were conveyed within one week of the test. These couples felt that the pregnancy was far too advanced to terminate. In a twin pregnancy one fetus was affected and the other was normal, as selective termination could not be carried out, the parents requested termination of pregnancy. It was not possible to access most of the terminated fetuses, but in two fetuses referred for DNA analysis the diagnosis of homozygous thalassaemia was reconfirmed.

Post natal outcome:

Sixty two children born after prenatal diagnosis of “normal” or “trait” were available for follow-up. Twenty five children were brought to AFIP for reconfirmation of prenatal diagnosis. In the remaining children reconfirmation of diagnosis was not possible as the couples had come from far off places. Prenatal diagnosis was reconfirmed in 25 children by Hb-A_2 or DNA analysis. In 3/37 couples that were contacted by letter informed that the child was tested at a local laboratory and the prenatal diagnosis was correct. The remaining 34 children were found to be apparently healthy and none required blood transfusions. The laboratory reconfirmation of diagnosis in these children was not done.

Since the introduction of prenatal diagnosis no misdiagnosis has been reported. None of the children born after CVS had any apparent congenital malformation. Three couples with a fetal diagnosis of thalassaemia major refused termination of pregnancy. One of the couples was lost to follow up. The other two children have transfusion dependent thalassaemia major.

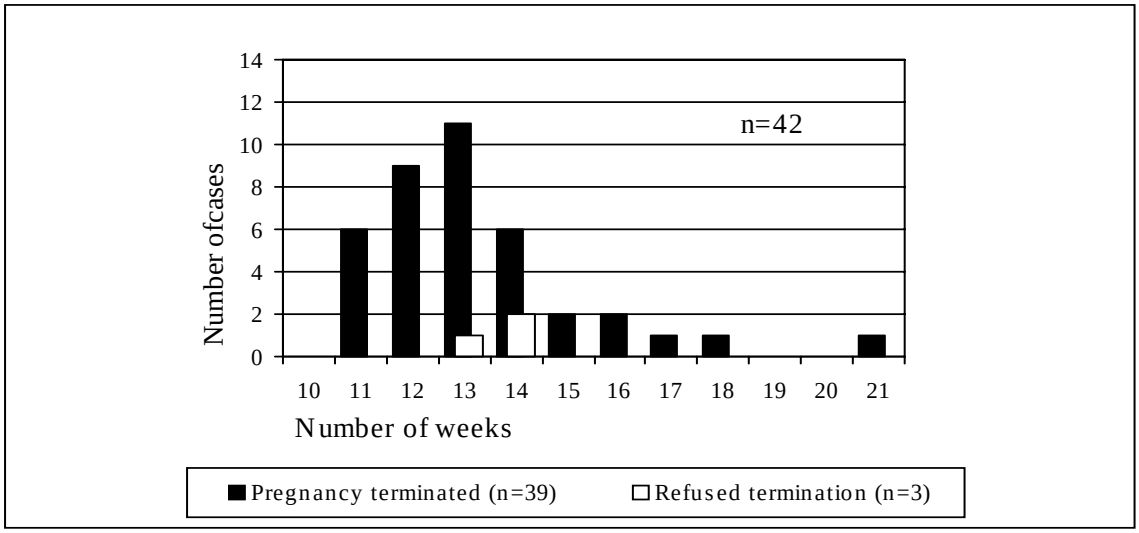

Fig: 7.7. The response of the couples to termination of pregnancy after fetal diagnosis of thalassaemia major.

Response of the families to prenatal diagnosis:

Knowledge about the availability of prenatal diagnosis:

Out of 141 randomly interviewed couples, 101 (72%) knew the test was available in Pakistan. Three couples had no interest in prenatal diagnosis because they had already completed their families. The remaining 38 (27%) were completely ignorant about the test facility.

Attitude towards prenatal diagnosis:

During the period when prenatal diagnosis was available 62 couples had a pregnancy. Two women (3.1%) aborted spontaneously prior to prenatal diagnosis. Twenty five of the remaining 60 couples (41.6%) were either not aware of the test or came to know about it very late and therefore did not request prenatal diagnosis. There were 3/60 (5%) couples who were not aware of their risk because they did not have an affected child. In 32 couples who had a pregnancy and were aware of the test facility, 16 (50%) requested it and another

two were waiting to use the test (Table: 7.8). Two couples preferred to terminate the pregnancy rather than use prenatal diagnosis. Twelve out of 32 (37.5%) couples knew about prenatal diagnosis at the time of pregnancy but did not use it due to reasons given in Table: 7.9. Six couples had no clear explanation for refusing prenatal diagnosis, two couples avoided the test due to cost and in another two the mothers were afraid of the test. There was a serious disagreement between the parents in two couples i.e. one favoured the test and the other did not.

Response of the couples to the possibility of prenatal diagnosis in a future pregnancy showed that 29 (20.6%) had completed their families. In the remaining 112 couples, 102 (91%) were in favour of prenatal diagnosis, and six (5.4%) were not sure. Only 4/112 couples (3.6%) were not in favour of prenatal diagnosis. Of the 102 couples who favoured prenatal diagnosis, 75 (73.5%) would request it unconditionally, but 27 (26.5%) would request it if it were free of cost. Most of the later 27 couples (93%) were from the low income group.

Attitude towards termination of pregnancy:

When questioned, 124/141 (87.4%) couples felt they would terminate an affected fetus. Only 2% had a negative attitude towards termination of pregnancy (Fig: 7.8).

Table: 7.8. Response to prenatal diagnosis of 32 couples who had a pregnancy and were also aware of the test facility.

Response:	**Number (%):**
Requested prenatal diagnosis	16/32 (50%)
Preparing to request prenatal diagnosis	2/32 (6.3%)
Did not request prenatal diagnosis	12/32 (37.5%)
Terminated pregnancy prior to prenatal diagnosis	2/32 (6.3%)
Total	32 (100%)

Table: 7.9. Reasons for not requesting prenatal diagnosis in 12 couples who had a pregnancy and also knew that the test was available.

Reasons:	**Number (%):**
Unclear reasons	6/12 (50%)
Disagreement between the parents	2/12
Fear of undergoing the test	2/12
High cost of the test	2/12
Total	12 (100%)

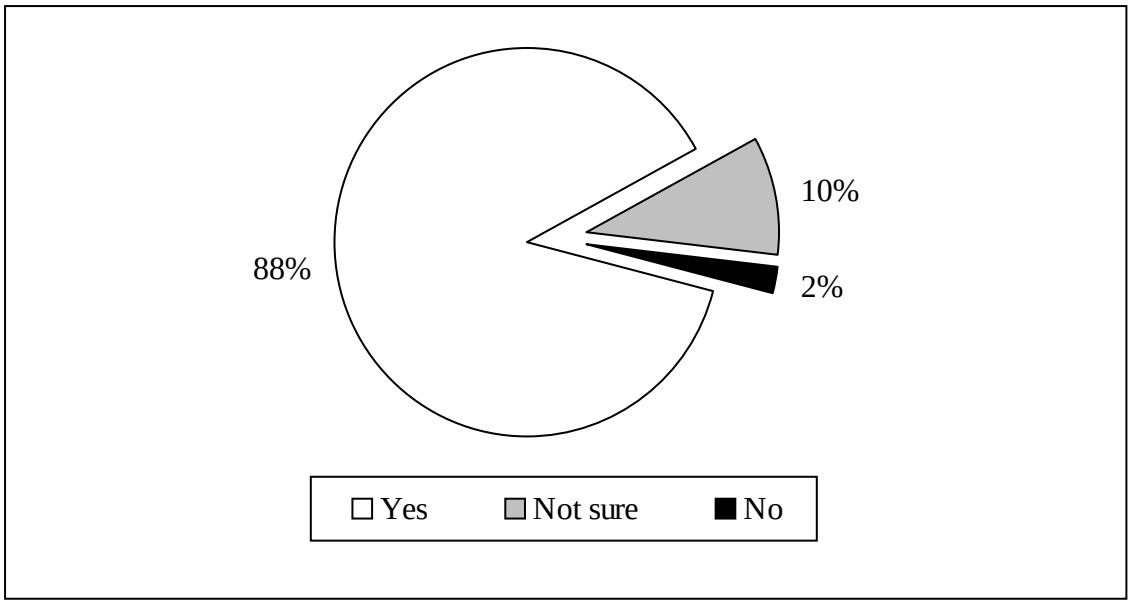

Fig: 7.8. Attitude of the randomly interviewed thalassaemic couples towards termination of pregnancy.

Discussion:

Technical feasibility:

The first step in fetal diagnosis is the analysis of parent's mutations. In this study direct mutation analysis was possible in over 98% of the couples. This makes it the method of choice for fetal diagnosis in most cases. The procedure is quick and reliable and is also cost effective. The multiplex ARMS can further reduce the time required in mutation analysis from two days to just one day. Similarly, the cost of mutation analysis per person can also be reduced considerably (Rs. 500 ($12.5) instead of Rs. 800 ($20); Chapter: 8).

It is important to ascertain the correct diagnosis of thalassaemia before mutation analysis is done. Thalassaemia is the commonest cause of transfusion dependent anaemia in Pakistan. Experience at AFIP shows that congenital dyserythropoietic anaemia (CDA), an autosomal recessive disorder, is the next most common cause of transfusion dependency (unpublished observation). Both the disorders have similar clinical picture. Therefore it is likely that a small proportion of patients having transfusions in Pakistan may actually be suffering from CDA. It may be useful to screen the couples who request prenatal diagnosis by red cell indices as it can help in differentiating between thalassaemia and other causes of transfusion dependency.

Chorionic villus sampling is the best approach for fetal sampling because it can be done in the first trimester of pregnancy and the associated risk of miscarriage is also less than 1% (Centre for Disease Control and Prevention, Atlanta 1995). The results of this study indicate that over 90% of Pakistani couples would request early prenatal diagnosis. Therefore CVS is the best option for fetal sampling. Procedure related complications in this study were high (9%). However in the early part of a new programme high miscarriage rates are not unusual (Old et al, 1986). The use of rigid biopsy forceps is more traumatic and may be associated with more frequent complications than aspiration needles (Golbus and Appelman 1990). The rate of complications is also related to the operator skill. CVS should be done at a centre where good ultrasound support is available. The samples can be easily transported to a central DNA laboratory either in RPMI-1640 or even normal saline.

CVS is often contaminated by maternal decidua and its meticulous cleaning is essential (Cao and Rosatelli 1993). VNTR analysis can rule out maternal contamination (Rosatelli et al, 1992a). This also safeguards against errors due to non-paternity. In this study financial constraints precluded VNTR analysis in most of the cases and meticulous cleaning of the CVS had to be accepted as adequate in the local circumstances.

In this study fetal diagnosis was possible by direct mutation analysis in over 98% of the couples. In the remaining couples it was possible to use RFLP as backup support. Inclusion of appropriate controls and reagent blanks (Kwok and Higuchi 1989) increased

the confidence in accuracy of diagnosis. The follow-up in 62 children born after a prenatal diagnosis indicates that the method is accurate and reliable for large-scale application.

Response of the couples to prenatal diagnosis:

Almost all of the couples in this study were retrospectively identified and they had at least one affected child. Their response to the availability of the test and to termination of pregnancy was very good. The couples who requested prenatal diagnosis mostly had small families, better education and higher socio-economic status. The response of the randomly interviewed at risk couples showed that the majority (72%) knew about the test and over half of the couples also had a positive attitude towards it. Careless attitude and the cost of the test were seen as the main reasons for avoiding the test. Sustained efforts at counselling the at risk couples can substantially improve the attitude of the couples. Subsidizing the cost of the test can also improve the acceptance rate for prenatal diagnosis especially amongst the low socio-economic group of couples.

Prenatal diagnosis and termination of pregnancy:

The community would accept the concept of prenatal diagnosis if it is compatible with the religious and cultural beliefs. Two renowned religious scholars in Pakistan, when asked to give opinion on permissibility of prenatal diagnosis and termination of pregnancy for thalassaemia gave a verdict in its favour provided the termination is carried out before 120 days (17 weeks) of gestation. The couples who were reluctant to use prenatal diagnosis were greatly relieved to learn that terminating a pregnancy is permissible under special circumstances. Most of the couples who requested prenatal diagnosis in this study had little hesitation in terminating an affected pregnancy. Only 3/42 (7%) couples refused termination and all three did so on religious grounds. A high rate of acceptance of termination may be because only a selected group of couples, who were prepared to accept termination of pregnancy, requested the test. However most of the prospectively interviewed couples (87% in this study) would terminate an affected fetus.