Prenatal Diagnosis of Haemophilia-A: A basis for the Pakistani Families

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Abstract

Objective: To determine the feasibility of a PCR based strategy for prenatal diagnosis of Haemophilia-A in Pakistani Families.
Design: Prospective.
Setting: Department of Haematology Armed Forces Institute of Pathology, Rawalpindi.
Subjects: Five families with at least one child affected with Haemophilia-A. Each family comprised of father, mother, affected child and fetus when present.
Main outcome measures: Short Tandem Repeat (STR) analysis in the Intron 22 of factor VIII gene.
Results: PCR based analysis of the STR in intron 22 of factor VIII gene showed that the marker was informative in 4/5 study families and could be used in these families for the prenatal diagnosis of Haemophilia-A. In two families prenatal diagnosis was carried out by Chorionic Villus Sampling at 10-13 weeks gestation and the results in both the cases showed a carrier female fetus.
Conclusion: Linkage based prenatal diagnosis of Haemophilia-A by an intragenic STR marker is feasible in most of the Pakistani families. The long term response of the Haemophiliac families to the availability of prenatal diagnosis remains to be seen. The STR marker can also be used for carrier detection of female subjects in the affected families (JPMA 49:230, 1999).

Introduction

Haernophilia-A is the commonest inherited bleeding disorder which is caused by the absence or defective funtioning of plasma coagulation factor VIII. It affects about I in 5000 males without ethnic or geographic limitations. The disease is caused by a defect in the factor VIII which is an essential co-factor in the activation of factor X by factor Ixa. The dramatic haemorrhages and the devastating role of therapeutic concentrates in transmission of the acquired immune deficiency syndrome have made Haemophilia-A the subject of great medical interest. Classically, the disease is inherited in an X-linked recessive manner. However, one third of the patients do not have any family history and they represent spontaneously developing mutations[1].
Haemophilia-A can be effectively managed by adequate replacement therapy but the morbidity and mortality are high when treatment facilities are not available. Lack of organization of the health services and the meagre health facilities in Pakistan pose a challenge for the management of Haemophilia. In this situation prevention of the birth of new haemophiliacs by prenatal diagnosis and selective termination of the affected pregnancies can play an important role. This prospective study was carried out to investigate the practical easibility of linkage based prenatal diagnosis of Haemophilia-A in Pakistani families.

Material and Methods

Five families with at least one child suffering from Haemophilia-A were studied. Each family comprised of father, mother and an affected child. Haematological diagnosis of Haemophilia-A was

made by the standard methods[2]. The study families were counselled according to the internationally accepted guidelines[3] and prenatal diagnosis was offered. The families were further investigated by DNA analysis.

Gene analysis

DNA extraction: From each individual 3.0 ml of blood was collected in EDTA. The red cells were lysed by a buffered solution of Triton X-100 followed by lysis of the nucleated cells by 2% solution of SDS in Tris buffer (pH 8.3). Further DNA extraction was carried out by the phenol chloroform method[4].

PCR Amplification for the Short Tandem Repeats (STRs): A dinucleotide repeat STR sequence in the Intron 22 of factor VIII gene was amplified by Polymerase Chain Reaction (PCR) using a set of primers: 5'-TTC TAA GAA TOT AGT GTG TGT G-3' and 5'-TAA TGC CCA CAT TAT AGA-3' (Lalloz et al, 1994). The PCR was carried out in 100 ml reaction mixture containing 300 ng of each primer (Pharmacia, UK), 2.5 units of AmpliTaq (Perkin Elmer, UK), 200 mmol of each dNTP (Advanced Biotechnologies, UK) and 300-500 ng of genomic DNA. The PCR buffer contained 1.5 mmol MgCI2, KCL and Tris-EDTA (pH 8.3). The thermal cycling included 40 cycles each comprising of 35 sec denaturation at 94°C, annealing at 56°C for 1 min 30 sec and extension at 72°C for 1 mon. Polyacrylamide gel electrophoresis ot the amplified products: PCR amplified products were run on a 6% denaturing polyacrylarnide gels at 10 Watts constant power on an instrument form Bio-Rad, UK. The gel measured 20 X 16 X 0.4 cm. After electrophoresis the gels were stained in silver nitrate.

Chorionic Villus Sampling (CVS)

Fetal sampling was done by a transabdominal free hand ultrasound guided aspiration technique[6]. The aspirated placental tissue was collected in RPMI-1640 and was transported to the lab within 24 hours of collection. In the lab the CVS was meticulously cleaned under a stereo dissecting microscope and any maternal tissue contaminating the fetal villi was removed. The fetal tissue was transferred to a 2% buffered solution of SDS along with 20mg of proteinase-K (Sigma Chemicals, USA). The subsequent DNA extraction was done by the phenol-chloroform method.

Results

The results of the five study families are summarized in Table 1.

Table. Summary of the results of counselling and DNA analysis in haemophiliac families.

Family	Ethnic origin	Family history of haemophilia	STR analysis	Prenatal diagnosis
No: 1	Punjabi	None	Informative	Carrier female fetus
No: 2	Punjabi	None	Informative	Willing to use in future
No: 3	Punjabi	None	Informative	Carrier female fetus
No: 4	Punjabi	None	Non-Informative	Interested but STR pattern was non-informative
No: 5	Pathan	Present	Informative	Non interested

Family 1

This was a Punjabi family who had an affected child. There was no other history of haemophilia in the family. The mother was 10 weeks pregnant. The STR analysis .at intron 22 showed the mother was heterozygous and the father had a single band. The affected child had inherited the mutant chromosome from the mother (Figure 1).

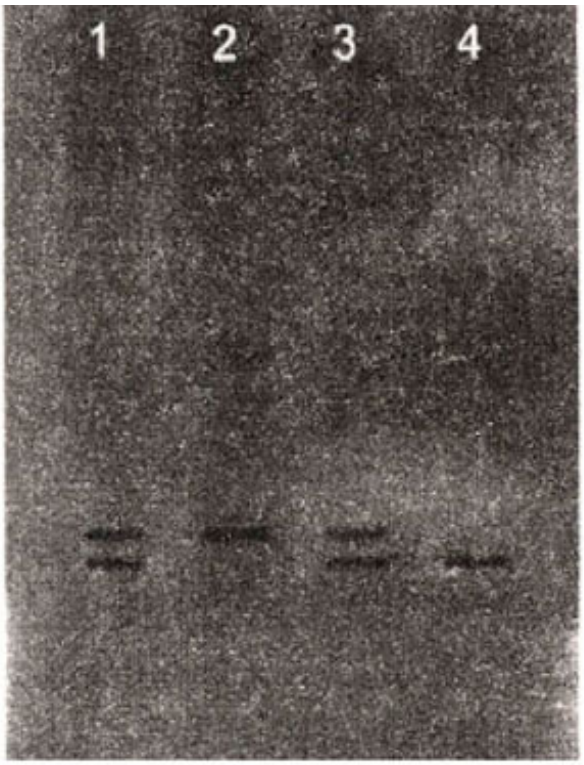

Figure: Polyacrylamide gel electrophoresis after PCR amplification of the STR in intron 22 of factor VIII gene in the Family No: 1. Mother (lane 1) shows two bands each representing an X-chromosome and the father (lane 2) shows single band representing one X-chromosome. The affected child (lane 4) also has one band that has been inherited from the mother (representative of the mutant X-chromosome). The fetus (lane 3) has two bands that are consistent with the diagnosis of a carrier female fetus.

The results indicated that the family was informative and prenatal diagnosis could be accomplished. Chorionic Villus Sampling was done at 12 weeks of gestation. The fetal DNA revealed heterozygous pattern suggestive of a carrier female fetus.

Family 2

This was also a Punjabi family with one affected child. There was no previous family history of haemophilia. The STR allele pattern at intron 22 was informative. The couple was informed and was advised to report at 10-12 weeks of gestation in any future pregnancy. The couple were willing to use prenatal diagnosis in future pregnancies.

Family 3

This was a Punjabi family with one affected child. There was no previous family history of haemophilia. The SIR pattern was informative. Mother was pregnant and the couple was willing to use prenatal diagnosis. CVS, done at 13 weeks of gestation, showed result suggestive of a carrier female fetus.

Family 4

This was also a Punjabi family with an affected haemophiliac child. There was no previous family history of haemophilia. Mother was 8 weeks pregnant and the couple had requested prenatal diagnosis. However, DNA analysis of the couple and the affected child showed a pattern that was non-

informative, mother being homozygous for the polymorphism and therefore prenatal diagnosis could not be done.

Family 5

This was a Pathan family with an affected haemophiliac child and a positive family history of haemophilia in the maternal side of the family. STR analysis showed a pattern that was informative for carrying out prenatal diagnosis. The couple were counselled but they did not report back for further follow-up.

Discussion

The factor VIII gene comprises of 186 kb of DNA and is located at the distal end of the long arm of X-chromosome. Due to the large size of the gene the number of mutations causing Haemophilia-A is also very large[7]. Therefore the prenatal diagnosis of haemophilia by direct mutation analysis is not feasible in most cases. An alternative method for its prenatal diagnosis is by linkage analysis in which several restriction sites linkedto the factor VIII gene can be used[8,9]. More recently, Short Tahdem Repeats (SIRs) linked to the factor VIII gene have been identified that can be used for the linkage based diagnosis of Haemophilia-A 5,.[10,11]

A di-nucleotide repeat SIR sequence in the intron 22 of factor VIII gene has been used for the prenatal diagnosis of haemophilia[5]. Since the SIR is located on the Xchromosome a male would have only one allele (one Xchromosome) that appears as a single band on the electrophoresis gel. A female heterozygous for the SIR allele would have two bands (one allele on each of the two X-chromosomes). However, a female who is homozygous at this locus would also show a single band. In a family comprising of father, mother nad an affected child it is possible to ascertain, by linkage analysis, that which maternal X-chromosome is carrying the mutant factor VIII gene. If the mother is homozygous for the STR allele, a distinction exists between the mutant and the normal factor VIII gene (chromosome) and therefore the linkage based diagnosis is not possible. The proportion of the families in a population in whom an SIR can be informative depends on its overall level of heterozygosity.

The present study, which is also the first of its kind in Pakistan, has provided some information on the level of heterozygosity for an STR in the intron 22 of the factor VIII gene and the feasibility of its usefulness as a linkage marker for prenatal diagnosis of haernophilia in Pakistani families. The results indicate that PCR based approach for SIR analysis at this locus is a quick and reliable method that can be useful in a Pakistani setting. Although the number of families studied is small but the results indicate that the SIR analysis can be useful in most families (4/5 in the present study). A major drawback of a linkage based approach is an assumption that the mother is an obligate carrier of haemophilia. In 4/5 study families there is no previous family history of haemophilia which indicates that the affected children may represent de-novo mutations. If this is the case then the actual risk of recurrence in the subsequent pregnancies would be much lower than anticipated. Another potential source of rror in a linkage based diagnosis of a genetic disease is that in about I % of the cases the marker and the mutant gene could dissociate due to a meiotic crossing over[12]. However, the risk of such an eventuality would be very small with an intragenic marker like the SIR in the intron 22 of the factor VIII gene.

Although the advances in the treatment of haemophilia have greatly improved its prognosis in the West but the treatment facilities in Pakistan are far below the required standard. Prevention of the births of new cases can be a cost effective method of reducing the burden of the disease on the affected families (Hoyer 1994). Another useful application of the linkage based diagnosis of haemophilia-A is the detection of female carriers[10].

The results of this study need to be substantiated by large prospective studies on the acceptability of the prenatal diagnosis of haemophilia in our target population. The results of a similar prenatal diagnostic

service for thalassaemia, a very common inherited disorder in Pakistan, that was introduced in 1994[13] are very encouraging[14]. A relatively poor response by the haemophiliac families to a prenatal diagnostic service available since the last 3 years could be due to lack of awareness amongst the families as well as the medical community about the availability of the facility. In addition it may also be due to rarity of hemophilia per se (1:10,000 live male births) as compared to p-thalessemia (3-5% of population).

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