Case Report

Prenatal Diagnosis in a Haemophilia A Family by Both Factor VIII Activity and Antigen Measurements

Shrimati Shetty, Kanjaksha Ghosh, Dipika Mohanty

Abstract

With the advent of molecular biology techniques prenatal diagnosis in haemophilia A is generally being performed by first trimester chorionic villus sampling followed by the DNA analysis using various polymorphic markers of factor VIII gene. Here we report antenatal diagnosis in a haemophilia A family performed in the second trimester by measuring both factor VIII : C and factor VIII : Ag in the fetal blood sample.

INTRODUCTION

Haemophilia A is a X linked recessive disorder producing absence or dysfunction of factor VIII : C molecule. The gene for factor VIII is very large containing 26 exons which spans 186 kb of genomic DNA. The mRNA transcribed from the gene is 9 kb and codes for a single chain containing 2332 amino acid residues.

The obvious drawbacks in the direct mutation detection in haemophilias are the large size of the gene, the complexity of mutations and the presence of de novo mutations in more than one-third of the cases. Therefore the indirect method of gene tracking using various polymorphic markers of factor VIII and IX genes is used to trace the mutant gene through families. However, there are certain limitations to this diagnostic strategy. The presence of homozygosity or noninformativeness of the polymorphic markers, the relatively limited number of polymorphic markers, the inaccuracy of the diagnosis in sporadic haemophilias and the requirement of the key family members including the affected individual make the gene tracking analysis not universally applicable to all haemophilia A families. Thus in antenatal diagnosis in haemophilia A families, the phenotypic evaluation of factor and antigen has still retained its significance. Further since the awareness about the carrier detection and prenatal diagnosis in the first trimester of pregnancy is still very low, some of the families are referred for a diagnosis in the first trimester of pregnancy is still very low, some of the families are referred for a diagnosis very late i.e. in the second trimester of pregnancy during which the chorionic villus sampling becomes ineffective and fetal blood sampling remains the only option.

We have been doing antenatal diagnosis in haemophilia families during the last few years by chorionic villus sampling followed by the indirect method of gene tracking using the various markers of factor VIII and IX genes. We report here the first antenatal diagnosis performed in the second trimester of pregnancy at our centre in a haemophilia a family by measuring both factor VIII : C and factor VIII : Ag.

CASE REPORT

A 28 years lady was referred to us from Islampur at 19.6 weeks of pregnancy for antenatal diagnosis of hemophilia A. She had one son with severe hemophilia A (VIII < 1%) and no other family members affected. The family was not aware of the antenatal diagnosis facility till them. As it was an advanced pregnancy, the female was sent for an USG to determine the sex of the fetus and it was found to be a male fetus. Accordingly the family was counselled about the probability of having a second child with hemophilia, the techniques involved in the diagnosis, the pitfalls and the rate of misdiagnosis by using the techniques. The family gave the consent for antenatal diagnosis. The family pedigree is shown in Fig. 1.

Fetal Blood Sampling

The fetoscopy was done in collaboration with USG department of Nowrosjee Wadia Maternity Hospital. A 26 gauge needle inserted into the umbilical blood vessel and 0.5 ml of fetal blood was aspirated for analysis. Possible contamination with maternal blood was excluded on the spot by a single, high MCV peak on a red cell size distribution plot using the channelizer.

Laboratory Investigations

Routine screening coagulation assays (PT, APTT, TT) were performed using commercial reagent (Dade Baxter, USA).
One stage factor assay was used for measuring factor VIII : C as described earlier. Von-Willebrand factor antigen was measured by Rocket immunoelectrophoresis by using an antibody raised in rabbit (Dako, Denmark). Factor VIII : Ag was measured by ELISA using commercial kit (Diagnostica Stago, France).

RESULTS

The results are shown in Table 1.

Both factor VIII : C and factor VIII : Ag levels were highly reduced i.e. less than 1% of the normal cord blood sample. The normal ranges for the various hemostatic parameters studied are given in Table 1. Thus a diagnosis of affected fetus was made.

The probability of carriership in the mother was calculated by taking a ratio of F VIII : C and VWF:Ag, which was 0.6. This value was much lower then a cut off value of 0.7 for the classification of the carriers and non-carriers.1

Following these investigations the family was informed about the reports and counselled accordingly. The mother underwent termination of pregnancy on the following day.

Subsequently, the DNA samples of the family members were subjected to gene tracking analysis using the polymorphic markers of the factor VIII gene ie IVS 18 Bcll, IVS 19 Hind 111 and DXS 52 St 14. However, the results were noncontributory due to homozygosity of these markers in the mother of the index case.

DISCUSSION

With the rapid advancement of molecular technique, phenotype evaluation is determined less and less significant in antenatal diagnosis of most of genetic disorders. However, in Hemophilias the genotyping technique have still not totally substituted phenotyping assays owing to the inherent drawback of the technique, the complexity of the gene and that of the underlying mutations.

Sporadic cases of haemophilia comprise 30-50% of the total haemophilic population it is not possible to ascertain at which level of the pedigree, the mutations arose. One should also take into consideration the somatic and germline mosaicism. Involvement of most of the family members including the affected child would many a times makes it difficult to apply the linkage analysis. With the increase of HIV and other transfusion associated infection among haemophiliacs,2 more and more of such families are being encountered wherein all the affected members are diseased.

Further, the phenotype assessment by procoagulant activity alone might give erroneous results as the coagulant factors are known to be highly labile and any error in the sampling procedure might result in an error in the report. To overcome this, dual measurement of factor activity and antigen is advocated.3

The hemostatic parameters during intrauterine life varies at different gestation periods and has been reported earlier.4 Thus the factor levels should be calculated against these normal ranges and not to the adult values. One advantage of assessing antigen in fetal blood samples is that with minor contamination with amniotic fluid during the procedure, the variation in the antigen values may be low as compared to that of factor procoagulant activity, though correction factors may be applied. Difficulty may arise in cases where an affected member in the family is CRM+ with respect to VIII : C Ag; the fetal antigen levels may then be unreliable and diagnosis will depend on factor VIII : C assays. In severe haemophilia A cases such a situation is uncommon.5

In the absence of other family members or noninformativeness of the key female for all the available polymorphic markers, detection of intron 22 inversions should be the first line of investigation at least in case of severe haemophilia A cases. Though we have studied the prevalence of intron 22 inversions in our severe haemophilia A cases by Southern blotting technique,6 the routine application of this

<table>
<thead>
<tr>
<th>Subjects</th>
<th>PT (secs)</th>
<th>APTT (secs)</th>
<th>TT (secs)</th>
<th>F VIII : C (%)</th>
<th>VWF F : Ag (%)</th>
<th>F VIII : Ag (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A (Father)</td>
<td>13</td>
<td>35</td>
<td>18</td>
<td>94</td>
<td>108</td>
<td>87</td>
</tr>
<tr>
<td>1B (Mother)</td>
<td>14</td>
<td>36.5</td>
<td>19</td>
<td>62</td>
<td>104</td>
<td>55</td>
</tr>
<tr>
<td>1C (Mat. Uncle)</td>
<td>12</td>
<td>34</td>
<td>17</td>
<td>108</td>
<td>96</td>
<td>78</td>
</tr>
<tr>
<td>11A (Aff. Child)</td>
<td>14</td>
<td>102</td>
<td>18</td>
<td>&lt;1</td>
<td>94</td>
<td>2</td>
</tr>
<tr>
<td>11 B (fetus)</td>
<td>24</td>
<td>94</td>
<td>29.5</td>
<td>&lt;1</td>
<td>60</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Normal range (adult)</td>
<td>12-14</td>
<td>35-40</td>
<td>18-20</td>
<td>50-150</td>
<td>50-150</td>
<td>50-150</td>
</tr>
</tbody>
</table>

* (Fetus - 17-20 weeks)

*The normal range in the fetus is obtained by studying the hemostatic parameters in 23 fetuses (wherein there is no history of bleeding disorder in the family) in our laboratory.
technique in cases of antenatal diagnosis especially in families requiring a diagnosis in the second trimester of pregnancy becomes practically impossible due to the limitations of the laboratory in procuring the radiolabels as and when required. Nevertheless, second trimester diagnosis by measuring factor VIII antigen and factor VIII activity becomes the only option in cases of non-informativeness of the key female in the family.

REFERENCES


Book Review

Diabetic Foot: A Clinical Atlas
Sharad Pendsey

Dr Pendsey’s book, Diabetic Foot: A Clinical Atlas, is complete without being complicated. It quickly gets to the point of diagnosis and management. I am acquainted with multiple books on Diabetic Foot published over the last 30 years and I can truly say that this Atlas is outstanding.

Marvin E Levin
Professor of Medicine
University of Washington School of Medicine, USA

Dr. Pendsey, in this lucid and attractive text, clarifies the ways in which diabetic foot problems can be prevented and treated successfully. There is something here for everyone; no matter how experienced we think we are we can learn from Dr. Pendsey’s wisdom, gained from many years of practical experience managing diabetic feet. Dr. Pendsey’s superb volume will help all practitioners to improve outcome for the diabetic foot.

Michael E Edmonds and Alethea VM Foster
Diabetic Foot Clinic, King’s College Hospital, London, UK

I congratulate Dr. Sharad Pendsey on this excellent and well-illustrated review of the problems and the management of the diabetic foot. This book is highly readable, and does not scorn the occasional use of humour to make the reading of the book an enjoyable experience.

Paul Brand
Clinical Professor Emeritus, Dept. of Orthopaedic Surgery
University of Washington School of Medicine, USA

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