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A Case of Prenatal Diagnosis of Hemophilia A

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Classic hemophilia, (hemophilia A), is an X-linked hereditary bleeding disorder affecting half of the male offspring of female carriers. Prenatal diagnosis offers an option, namely to restrict abortions to hemophilic fetuses only, and thus retain the chance of bearing normal sons. Recently, the authors have made a prenatal diagnosis of hemophilia A in an obligate carrier with a male fetus at 24 weeks of gestation by pure fetal sampling and accurate factor VIII coagulant assay, which was repeatedly less than 1% at 28 weeks of gestation.

Key Words: *Hemophilia A, Prenatal diagnosis, Obligate carrier*

INTRODUCTION

Classic hemophilia (hemophilia A) is an X-linked hereditary bleeding disorder affecting half of the male offspring of female carriers. Even today, the severe form of hemophilia takes a heavy toll of the physical, emotional and economic resources of the patient and his family.

Accordingly many carriers are usually adverse to bearing a hemophilic son and take steps to avoid pregnancy, or to terminate it if the fetus is male.

Prenatal diagnosis offers such parents an option, namely to restrict abortions to hemophilic fetuses only, and thus retain the chance of bearing normal sons. In order to implement this approach, there are two important factors; a reliable obstetric technique for sampling pure fetal blood, and performance of valid clotting factor VIII assay on the samples so obtained. Recently, the authors have made a prenatal diagnosis of hemophilia A in an obligate carrier, 24 weeks pregnant, with a male fetus. We report here what may be the first case of its kind in Korea.

MATERIALS AND METHODS

Fetal Blood Sampling

Fetal blood was obtained from the umbilical vein at the placental cord insertion at 24 weeks of gestation and from the heart because of failed cordocentesis at 28 weeks of gestation with use of a 20 gauge needle, 15cm long, under the guidance of ultrasound. Blood collection was immediately followed by anticoagulation with EDTA for hematocrit, red cell count, and red cell size distribution using Coulter counter (model S plus II), and with 3.2% sodium citrate for clotting assays with a ratio of 1:9. Evidence that the fetal blood sample was uncontaminated by maternal blood was obtained by Kleihauer-Betke fetal hemoglobin stain (>95% of fetal hemoglobin).

Coagulant Assays

After centrifugation of fetal citrated blood at 2000g for 15 min, one stage assays of factor VIII:C. Prothrombin time and activated partial thromboplastin time were measured using ACL coagulyzer (Instrumentation Laboratory). Coagulation factor V, IX, X activity were assayed using deficient substrate plasma (Stago) by one stage method. Von Willebrand antigen (VIIIR:Ag) was measured by enzyme immunoassay (Stago).

CASE SUMMARY

A pregnant, 26 year old was referred to Yong Dong Severance Hospital because of her family history; her father had died of a bleeding disorder and her sister had a hemophilic son. Four of her 7 paternal uncles had died at early ages due to undiagnosed bleeding disorders (Fig. 1).

Her obstetric history was shown as G3P1L1D0A1. On the basis of pedigree analysis combined with the likelihood ratio from laboratory tests and discriminative

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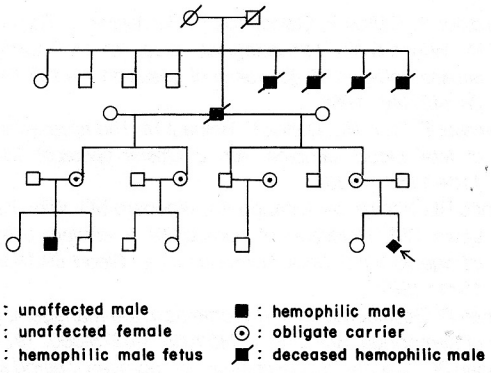

Fig. 1. Family Pedigree.

analysis, the client was regarded as an obligate carrier.

DNA based genetic prediction using BclI and XbaI polymorphism was done but the results were uninformative due to homozygosity of the mother.

Fetal sexing by chromosome study of amniotic fluid done at Koshin University hospital was reported to be male, which was confirmed by ultrasonogram.

At 24 weeks of gestation, cordocentesis was performed to collect fetal blood and the confident exclusion of maternal blood or amniotic fluid from the assay sample was made through the evaluation of RBC size, the Kleihauer-Betke acid elution test for HbF, and fetal Hct as shown in Table 1.

Factor VIIIC was measured in fetal samples as well as in samples from other family members including the father, mother, and 3 year old daughter as shown in Table 2.

The fetus was considered to be affected because of a very prolonged activated partial thromboplastin time and the absense of factor VIIIC activity as shown in Table 2. The pregnancy was terminated at the parent's request after collecting blood from the heart and administering 4 ml of KCl (10mEq/l) at 28 weeks of gestation. Diagnosis was confirmed by concurring assays of both VIIIC and von Willebrand antigen as well as factor V, IX, and X as shown in Table 3.

Table 1. CBC findings in the mother and fetal cord blood at 24 weeks of gestation.

CBC	Mother	Cord Blood	Reference Range*
WBC ($X10^9$/l)	9.1	8.8	3.3-4.6
Differential (%)			
Segment form	55	24	3-12
Lymphocyte	44	69	76-88
Monocyte	0	2	0-3
Eosinophil	1	4	0-4
Basophil	0	1	0-2
Hb (g/dl)	10.3	14.7	11.6-13.2
Hct (%)	30.6	44.5	36.2-41.0
MCV (fl)	92.7	115.8	120-132
Platelet ($X10^9$/l)	213	264	216-301

* Forestier F et al (1986)

Table 2. Coagulation studies on fetal cord plasma at 24 weeks of gestation, and on plasmas from other family members and normal control.

	PT (sec)	APTT (sec)	Factor VIII:C(%)
Father	10.1	33.4	172.0
Mother	10.6	33.4	88.3
Daughter	10.4	37.0	54.5
Fetus	15.8	>120.0	<1.0
Control	12.0	31.2	95.0

Table 3. Coagulation factor assays of fetal blood at IUP 28 weeks.

Factors	Fetus	Reference Range at 27-31 wks (Ref)
VIII	<1.0	25-89 (Mibashan et al, 1980)
V	59.2	61-121 (Miller, 1989)
IX	5.5	6-14 (Forestier, 1988)
X	20.0	14-70 (Miller, 1989)
vWF:Ag	102.0	82-224 (Miller, 1989)

DISCUSSION

Hemophilia A results from a deficiency or abnormality of clotting factor VIII and is inherited as an X-linked trait (McKee, 1983). Severely affected patients suffer recurrent hemorrahges with their sequelae of arthropathy (Jones, 1977).

Treatment consists primarily of replacement therapy with factor VIII preparations (Firshein et al., 1979) but acquired resistance or complications associated with viral transmissions cause an emotional, financial and physical burden for both the patient and family (Shapiro, 1979; White et al., 1980).

As accurate prenatal sex determination becomes possible, many women who are known or potential carriers of hemophilia have chosen to terminate all pregnancies involving male fetuses to avoid the risk that a son will be born with hemophilia. At least half the male fetuses of these women are unaffected and accurate prenatal diagnosis of hemophilia would pro-

vide a better approach to genetic counselling (Firshein et al, 1979).

Prenatal diagnosis of hemophilia presents several difficulties (Mibashan et al, 1979). First of all, pure fetal blood is essential for the comprehensive assay of fetal factor VIII activity (Rodeck and Campbell, 1978). Maternal clotting factors would be misleading, and amniotic fluid dilutes the fetal sample as well as activating its coagulation (Bertina et al., 1981). Checks on fetal red cell count and hematocrit will give warning of dilution by amniotic fluid and assay of factor IX as well as factor VIII serves as a sensitive indicator of this possibility (Mibashan et al., 1979). Immunoradiometric assay of fetal factor VIIIC antigen can be performed on serum and so may dispense with anticoagulation (Peake and Bloom, 1978). Both factor VIII and IX are appreciably lower at mid-term than in the newborn, but are distinguishable from severe hemophilic levels with ease in the case of factor VIII, and by a much smaller margin in respect of factor IX (Mibashan et al., 1979). Normal plasma levels of fetal factors at mid-term was reported to be 44% with a range of 25-89% (Mibashan et al., 1980).

Nowadays an improved method for prenatal diagnosis by analysis of amplified DNA sequences, is widely applied (Kogan et al., 1987). The two most informative factor VIII intragenic polymorphisms, BclI and XbaI (Janco et al., 1987) were analyzed after polymerase chain reaction in this family, but the result was uninformative because approximately 30% of women will not be heterozygous for the BclI or XbaI polymorphisms (Wion et al, 1986; Janco et al, 1987). Other polymorphisms useful in the diagnosis of hemophilia carriers or prenatal cases are expected to be investigated currently or in the future.

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