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fetal blood sampling prenatal diagnosis

Prenatal Diagnosis of Alpha$_1$-Antitrypsin Deficiency by Analysis of Fetal Blood Obtained at Fetoscopy

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Summary

Two women had each borne a child who had alpha$_1$-antitrypsin (α_1AT) deficiency Pi ZZ and who developed liver cirrhosis. In subsequent pregnancies, the women requested prenatal diagnosis. Samples of blood from the two fetuses were obtained at fetoscopy. In a control group of five Pi MM fetuses aborted by hysterotomy, the mean α_1AT level was 0.73 g/liter. Of the two fetuses at risk, one had an α_1AT concentration calculated as 0.60 g/liter, *i.e.*, within the Pi MZ range. The electrofocusing pattern indicated a heterozygous Pi MZ phenotype which was confirmed at birth. The other fetus at risk had a markedly decreased concentration of α_1AT, 0.06 g/liter. Electrofocusing showed a homozygous Pi ZZ phenotype. Analysis of blood from the abortus confirmed these findings and thus the diagnosis of α_1AT deficiency.

Speculation

Most of the alpha$_1$-antitrypsin in amniotic fluid is derived from the mother. It therefore appears that the only possible way of making a prenatal diagnosis of alpha$_1$-antitrypsin deficiency is by examination of blood from the fetus. One fetus examined in the present study had an abnormal Z-pattern. This abnormality may indicate a disturbance of fetal liver function already *in utero*.

Most of the alpha$_1$-antitrypsin (α_1AT) in amniotic fluid is derived from the mother (1, 7) and is therefore inappropriate for prenatal diagnosis of α_1AT deficiency. The only possibility of making such a diagnosis seems to be the examination of blood from the fetus.

The development of techniques to obtain fetal blood *in utero* has made prenatal diagnosis possible in fetuses at risk for α_1AT deficiency. We here report the prenatal evaluation of two fetuses at risk for α_1AT deficiency Pi ZZ.

A preliminary report on case 1 has been published previously (5).

MATERIALS AND METHODS

CLINICAL MATERIAL

Case 1. A Pi MZ father and a Pi MZ mother had two healthy children and one Pi ZZ girl who had died of cirrhosis of the liver at the age of 3 years. In the 22nd wk of a subsequent pregnancy, a mixture of fetal blood and amniotic fluid was obtained at fetoscopy for prenatal diagnosis.

Case 2. A Pi MZ father and a Pi MZ mother had a Pi ZZ girl. The girl developed severe cirrhosis of the liver. At the age of 7 years, an unsuccessful attempt to construct a portacaval shunt was done. In the 20th wk of a subsequent pregnancy, pure fetal blood was sampled at fetoscopy.

The examination were done after informed consent from the women and the approval of the Ethical Committee at Lund, University Hospital.

Controls. Samples of maternal blood and amniotic fluid were collected from five women admitted for therapeutic abortion in the 16th to 20th wk of gestation. After the pregnancy had been terminated by hysterotomy, fetal blood was sampled by free flow from the pendant umbilical cord.

FETAL BLOOD SAMPLING

In case 1, ultrasound scanning showed that the placenta covered the entire anterior wall of the uterus. Insertion of the fetoscope (Dyonics "Needlescope," 170 mm) through the abdominal flank lateral to the placental margin failed to reach the amniotic cavity. Later, the uterus was found to have rotated slightly around its longitudinal axis, and the fetoscope was now successfully inserted. Placental vessels were punctured without any attempt to cannulate, and samples of the stream of blood that issued into the amniotic fluid were aspirated (2). In case 2, pure fetal blood was obtained by cannulation of a large vessel near the insertion of the umbilical cord into the placenta (18). In each woman, a sample of maternal blood was obtained from a cubital vein, and immediately before fetoscopy, a sample of amniotic fluid was obtained by fine-needle amniocentesis. The blood samples were immediately transferred to EDTA tubes.

IDENTIFICATION OF FETAL BLOOD

The proportion of fetal and maternal red blood cells was estimated by the Kleihauer technique (8). In addition, the relation between HbA and HbF was assessed by electrofocusing at pH 3 to 10 (4).

EVALUATION OF THE DILUTION FACTOR

The ratio of fetal blood to amniotic fluid in the fetoscopic samples was calculated from the hematocrit, assuming that the mean hematocrit in pure fetal blood at midpregnancy is 37% (3, 15). In case 1, the concentration of antichymotrypsin in fetal blood and amniotic fluid was also used in the calculation.

PROTEIN QUANTITATION

The concentration of α_1AT was estimated by electroimmunoassay (11).

ELECTROFOCUSING TECHNIQUE

The fetoscopic samples were reduced with cysteine to a final concentration of 30 μmoles/ml to achieve a clear microheterogeneous pattern and less background staining. The freshly prepared cysteine solution used consisted of 0.3 mM cysteine-HCl, 2 M

glycine, and 0.01 M EDTA with pH adjusted to 7.4 with NaOH. A volume of 10 μl of the cysteine solution was mixed with 90 μl of plasma or serum and stored overnight at 4°C.

Ready-prepared, thin-layer polyacrylamide gel plates with Ampholine at pH 4 to 5 (LKB, Bromma, Sweden) were used with LKB 2117 Multiphore apparatus cooled at 15°C by an external cryostat (16). Electrode strips were moistened with 1.0 M glycine at the cathodal end 1.0 M phosphoric acid at the anodal end. The gel was prerun at 1 hr with an LKB 2103 power supply at 500 V, 50 mA, and 20 W maximum. Samples of 15 μl cysteine-reduced plasma were applied to filter papers (10 x 15 mm) on the gel. They were placed along the cathodal edge of the gel about 10 mm from the cathodal strip with about 1 mm between the papers to avoid mixing of the samples. For the analysis, the power supply was set at 1400 V, 50 mA, 20 W for 1 hr, and thereafter increased to 30 W for another 2 hr. Total effective running time, including prerun, was 4 hr.

After completion of electrofocusing, the gel was placed in fixative consisting of trichloroacetic acid (10%) and sulfosalicylic acid (5% w/v) for 30 to 60 min. Staining was performed in 0.4% (w/v) of Coomassie R 250 and the destaining in a solution of methanol, acetic acid, and distilled water (35:10:55, v/v) for 45 min. The microheterogeneous pattern of α_1AT was clearly visible after another 60 min in the destaining solution.

IMMUNOFIXATION TECHNIQUE

Reduced samples were diluted with 0.05 M glycine to a final α_1AT concentration of approximately 0.08 g/liter assuming that the mean α_1AT concentration in a normal population is 1.35 g/liter (6).

Fifteen μl of the dilution was subjected to electrofocusing as earlier described. Immunofixation was carried out on the surface of the electrofocusing gel after completion of the run using the "immunoprint technique" (17). A cellulose acetate membrane (Sepraphore III, Gelman) was soaked in α_1AT antiserum (IgG-fraction, DAKO, Denmark) diluted with a 0.1 M phosphate buffer pH 7.4 (1:4). After completion of the run, the antibody-impregnated cellulose acetate membrane was placed over the expected α_1AT area on the polyacrylamide gel for 1 hr. The membrane was soaked in phosphate buffer overnight, stained for 5 min in 0.2% Coomassie R 250, and destained in the methanol, acetic acid, and water mixture.

RESULTS

CONTROLS

Samples obtained at five hysterotomies (therapeutic abortions of fetuses with Pi MM patterns) in the 16th to 20th wk of gestation showed that the mean concentration of α_1AT in amniotic fluid was 0.16 g/liter (range, 0.14 to 0.24); in fetal plasma, 0.73 g/liter (range, 0.70 to 0.80); and in maternal plasma, 1.84 g/liter (range, 1.60 to 2.10), assuming 1.35 g/liter as the mean concentration in adult plasma (6).

DIAGNOSTIC CASES

On examination by the Kleihauer technique, all the fetoscopic samples were found to contain >95% fetal red blood cells. Electrofocusing run on hemolysates from isolated red cells showed that the proportions between the two HbF and the minor HbA fractions were typical in all the samples, indicating fetal origin.

CASE 1

In all samples obtained from case 1, the fetal blood was diluted with amniotic fluid. In the sample with the highest hematocrit, that is the least diluted sample, it was 15%. In this sample, the concentration of α_1AT was 0.38 g/liter; in pure amniotic fluid, 0.24 g/liter. Calculated from the hematocrit, the ratio of fetal blood to amniotic fluid was about 2:3 in the fetoscopic sample. The antichymotrypsin concentration in amniotic fluid is higher than in fetal blood. The mixture will thus have a lower concentration than pure amniotic fluid depending on the dilution with fetal blood. The ratio of the mean α_1AT to antichymotrypsin concentration in the controls was 12:1. When the concentrations in amniotic fluid, 0.110 g/liter, and in the mixture, 0.076 g/liter, were compared with the corresponding α_1-AT values, a similar ratio of approximately 2:3 was obtained. From this ratio, the fetal plasma concentration of α_1AT was calculated as 0.6 g/liter. This value is consistent with a fetal contribution of α_1AT, at least from a Pi MZ subject. Assuming the same relation between the α_1-AT concentration and the Pi-type in a fetus as in a newborn, a Pi ZZ fetus would have about 10% of the normal fetal plasma α_1AT concentration, that is, about 0.1 g/liter, and a Pi MZ fetus would have 70%, that is, about 0.5 g/liter (12). The electrofocusing of this sample showed that the density of the major Pi M bands was greater than that in pure amniotic fluid, suggesting a fetal contribution of M protein. The identity of the α_1AT fractions was further demonstrated by immunofixation (Fig. 1). Thus, the α_1AT concentration and the electrofocusing pattern showed that the fetus had at least one PiM gene and, accordingly, did not have α_1AT deficiency.

The pregnancy was uncomplicated and a healthy girl was born in the 41st wk of gestation. Analysis of plasma from the newborn infant showed a heterozygous Pi MZ pattern, and the concentration of α_1AT was 0.95 g/liter.

CASE 2

The sample obtained in case 2 was collected by cannulation of a large vessel near the insertion of the cord. The hematocrit was 39%. The α_1AT concentration in the fetal plasma was 0.06 g/liter, in amniotic fluid, the α_1AT concentration was 0.15 g/liter. The fetal plasma had a Pi ZZ pattern both at electrofocusing and immunofixation (Fig. 2). However, the pattern was not normal; thus a doubling of the major bands was observed even after reduction. The Pi MZ pattern of amniotic fluid was identical with that of case 1. The findings indicated an α_1AT-deficient fetus. The pregnancy was terminated, and examination of blood from the abortus confirmed the diagnosis of severe α_1AT deficiency.

The examination of the liver from the abortus showed sings of cholestasis. No inclusion bodies were visible in the hepatocytes.

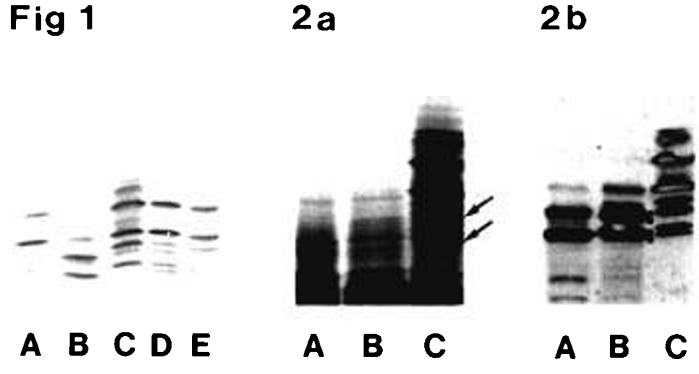

Fig. 1. The immunofixation pattern after electrofocusing at pH 4–5 of plasma samples from case 1 and from two normal controls. *A*, plasma from a MM control; *B*, plasma from a ZZ control; *C*, amniotic fluid from the MZ mother; *D*, plasma from the MZ mother; *E*, mixture of fetal blood and amniotic fluid from the fetus. The Z-band contribution in the amniotic fluid is relatively weak (*C*). The Z-band in the mixture of fetal blood and amniotic fluid (*E*) is very faint, compared with that of pure amniotic fluid (*C*), as less protein material was applied.

Fig. 2. *a*, the electrofocusing pattern of samples from case 2 after direct staining. *A*, pure fetal blood obtained from the abortus; *B*, pure fetal blood obtained at fetoscopy. The fetal blood obtained at fetoscopy (*B*) had an atypical Z-pattern with double major bands. *C*, plasma from the MZ mother. *Arrows*, normal Z_1 and Z_2 fractions. *b*, the corresponding immunofixation pattern indicating that the abnormal fractions (*dots*) represent α_1AT.

DISCUSSION

Recently, Roth *et al.* (19) used only amniotic fluid proteins to determine the phenotype of a fetus at risk for α_1AT deficiency. However, it has been shown that amniotic fluid proteins are mainly of maternal origin (1, 7). Our results clearly show that the fetal α_1AT phenotype is fully expressed in fetal plasma in mid-pregnancy and that the amniotic fluid protein cannot be used for prenatal diagnosis of α_1AT deficiency.

We have thus shown that the phenotype and the concentration of α_1AT can be determined in a fetoscopic sample of fetal blood even when diluted with amniotic fluid. However, pure fetal blood is preferable, as shown in case 2. Immunofixation of good quality is a prerequisite because the actual area on the electrofocusing pattern can sometimes be covered by other smearing proteins, which render interpretation more difficult. Also heparin, when used as an anticoagulant instead of EDTA, can have the same effect on the pattern. Why the Z-allele contribution was weaker in the MZ amniotic fluid is unknown.

In case 2, the Z-pattern was abnormal (Fig. 2), suggesting a disturbed α_1AT-production by the liver. This disturbance may be due to the liver disease confirmed by the cholestasis observed at autopsy. However, in another study we found no abnormalities in the Pi patterns of 60 Pi ZZ infants including seven with neonatal cholestasis studied at about 3 months of age (Jeppsson and Sveger, unpublished results). Inasmuch as to our knowledge case 2 is the first fetus with α_1-AT deficiency and cholestasis studied *in utero*, the significance of the abnormal PiZ pattern is unknown.

The liver and the yolk sac produce α_1AT, but the contribution from the yolk sac is no longer detectable after the 11th wk of gestation (1). At about 10 wk, the α_1AT concentration is 70% of the adult level, which is reached at 26 wk (1). At term, the mean serum level is 150% in the fetus compared with 200% in the mother (10). A maternofetal transfer of α_1AT has, to our knowledge, not been measured directly. However, judging from comparison of genetic types of transferrin (13), Gc-globulin (7), orosomucoid (7), and albumin in amniotic fluid and maternal serum, most amniotic fluid proteins are of maternal origin. Our findings showed the same for α_1AT. With the methods used, however, it was not possible to detect a 5 to 10% contribution from the fetus to the amniotic fluid proteins.

In Scandinavia, 1 out of 1500 individuals has α_1AT deficiency Pi ZZ (9). In a prospective study of 120 Swedish Pi ZZ infants, 11% developed neonatal cholestasis, and another 7% had other clinical symptoms of liver disease one out of every two infants with neonatal cholestasis was small for gestational age, which may indicate that the infant had the disease already *in utero* (20). It has been estimated that the risk for two Pi MZ parents to have a child with α_1AT deficiency Pi ZZ and congenital liver disease progressing to cirrhosis is 1 to 2% (14, 20).

Thus, prenatal examination for α_1AT deficiency may be considered in fetuses of women who have already borne a Pi ZZ child with sever liver disease and who request prenatal diagnosis in a subsequent pregnancy.

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