# IN UTERO DIAGNOSIS OF SANDHOFF'S DISEASE

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SUMMARY: The first in utero diagnosis of Sandhoff's disease was made in an at-risk fetus by the demonstration of deficient β-N-acetyl-hexosaminidase A and B activities in amniotic fluid components the day of amniocentesis. These enzymatic deficiencies were determined by enzyme assay and electrophoresis using 4-methylumbelliferyl-β-N-acetyl-glucosaminide as substrate. The concentrations of the neutral glycosphingolipids were quantified in amniotic fluid; the level of the glycosphingolipid substrate, globoside, was markedly increased in amniotic fluid from the at-risk fetus compared to that of fetal controls. In addition, ultrastructural examination demonstrated pathologic glycosphingolipid accumulation in uncultured amniotic cells. These enzymatic, chemical and ultrastructural procedures provided the rapid and accurate in utero diagnosis of Sandhoff's disease within three days of amniocentesis. The in utero diagnosis was confirmed by the marked deficiencies of β-N-acetyl-hexosaminidase A and B in plasma and various tissues from the aborted fetus. These findings indicated that maternal hexosaminidases do not cross the fetal-placental barrier.

Sandhoff's disease, an inborn error of glycosphingolipid metabolism, results from the deficient activities of the enzymes, β-N-acetyl-hexosaminidase A and B (1-3) and is characterized by the neural and visceral accumulation of their substrates, $G_{M2}$ ganglioside, asialo-$G_{M2}$ ganglioside (NAcgal-gal-glc-ceramide), and globoside (NAcgal-gal-gal-glc-ceramide) (1-7). The progressive neurological deterioration and fatal course of this disorder are similar to Tay-Sachs disease, a related glycosphingolipidosis, which results from the deficient activity of hexosaminidase A only (8). Previously, hexosaminidase A and B activities were determined in normal amniotic fluid and uncultured amniotic fluid cells by enzymatic assay (9, 10) and electrophoresis (11, 12); these findings suggested that the rapid in utero diagnosis of this inherited enzymatic deficiency would be technically feasible the day of amniocentesis. In addition, amniotic fluid components were analyzed for chemical and ultrastructural evidence of pathologic substrate accumulation to further confirm the in utero diagnosis of this disorder.

MATERIALS AND METHODS: The heterozygous parents (7) of two previous siblings who expired from biochemically documented Sandhoff's disease (5-7) were referred because of their desire to have an unaffected child. Diagnostic transabdominal amniocentesis was performed at 13 weeks gestation and the amniotic fluid cells were separated from an aliquot of the amniotic fluid by centrifugation (100 x g). The amniotic fluid supernate and the centrifuged uncultured amniotic cells from the at-risk fetus and gestationally-matched fetal controls were immediately assayed for β-N-acetyl-hexosaminidase A and B activities as previously described (8, 13). Portions of the centrifuged amniotic cells were prepared for ultrastructural examination (14) and cultured (14) for subsequent enzymatic analyses.

Polyacrylamide gel electrophoresis was performed as previously described (15) using a discontinuous gel system (6% stacking and 8% plug and running gels) and 0.025M citrate phosphate buffer, pH 6.0. Samples of amniotic fluid and extracts (11) of uncultured and cultured amniotic cells (20 ul, 12 mg protein) were electrophoresed for 6 hours at 150 V, 100 mA, at 4°C. The bands of enzymatic activity were visualized using 4-methylumbelliferyl-β-N-acetyl-glucosaminide as substrate (11). The concentrations of the neutral glycosphingolipids were determined in both centrifuged and uncentrifuged amniotic fluid from the fetus at-risk and appropriate fetal controls by previously reported methods (16).

Following hysterotomy at 18 weeks of gestation, cerebral cortex and hepatic tissues as well as blood obtained by cardiac aspiration from the at-risk fetus and gestationally-matched fetal controls were assayed for hexosaminidase A and B activity. Protein concentration was determined on each tissue by the method of Lowry et al. (17).

RESULTS: Table 1 shows the level of total hexosaminidase activity and percent hexosaminidase B in the amniotic fluid supernate, uncultured and cultured amniotic fluid cells from the fetus at-risk and fetal controls; marked deficiencies of hexosaminidase A and B activities were demonstrated in each of

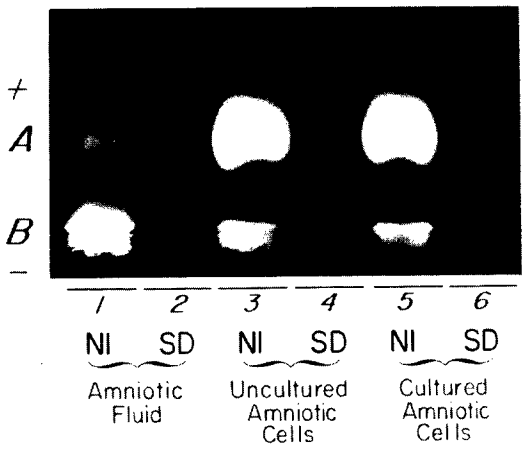


Fig. 1. Polyacrylamide gel electrophoresis of β-N-acetyl-hexosaminidase A and B. Amniotic fluid components in lanes 1, 3, and 5 from a normal fetus (Nl) and in lanes 2, 4, and 6 from the fetus diagnosed as having Sandhoff's disease (SD).

TABLE 1. TOTAL HEXOSAMINIDASE ACTIVITY* AND PERCENT HEXOSAMINIDASE B IN AMNIOTIC FLUID COMPONENTS

| Source | Amniotic Fluid | | Uncultured Amniotic Cells | | Cultured Amniotic Cells | |
|---|---|---|---|---|---|---|
| | Total Hex | % Hex B | Total Hex | % Hex B | Total Hex | % Hex B |
| Fetus at-risk | 12.5 | 80 | 3.8 | 60 | 86 | 16 |
| Fetal Control Mean | 842 | 76 | 231 | 67 | 3665 | 34 |
| and Range (n=4) | 580-1020 | 70-89 | 110-498 | 64-81 | 1110-5960 | 26-41 |

*Total Hexosaminidase activity (Total Hex) expressed as nmoles substrate hydrolyzed/hr/ml fluid or mg protein cells; hexosaminidase B (thermostable hexosaminidase, 50°C.) expressed as percent of total hexosaminidase activity (% Hex B). Standard reaction conditions were used (8, 11).

these amniotic fluid components. Figure 1 shows the polyacrylamide gel electrophoresis of the amniotic fluid supernate, uncultured and cultured amniotic fluid cells from the fetus at-risk and a 14 week gestation fetal control; the hexosaminidase A and B components were easily identified in these sources from the fetal control, but absent in those from the at-risk fetus. These deficiencies were confirmed by enzymatic assay and electrophoresis in the amniotic fluid supernate and in uncultured amniotic cells the day of amniocentesis, and subsequently, in the cultured amniotic cells which were harvested four weeks later.

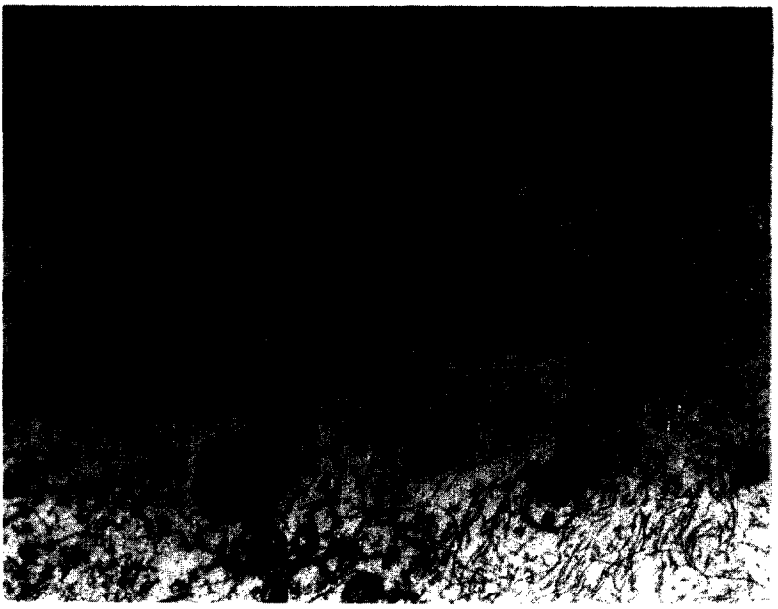

Fig. 2. Electron photomicrograph of a portion of an uncultured amniotic fluid cell from the fetus diagnosed as having Sandhoff's disease. Multivesicular bodies are accumulated in lysosomes (X 22,500). Amniotic cells were fixed successively in 0.1% and 1% glutaraldehyde, then in equal volumes of 1% osmic acid and 0.5% glutaraldehyde for 1.5 hr. at 4°C. Following routine electron microscopy procedures for dehydration, embedding and staining, thin sections were examined in a Phillips 200 electron microscope.

TABLE 2. CONCENTRATIONS OF NEUTRAL GLYCOSPHINGOLIPIDS IN AMNIOTIC FLUID

| Glycosphingolipid | Uncentrifuged Fluid | | Centrifuged Fluid | |
|---|---|---|---|---|
| | Fetus at-risk | Control Mean* | Fetus at-risk | Control Mean* |
| | nmoles/ml | | | |
| Glucosyl Ceramide | 9.4 | 4.5 | 8.6 | 3.9 |
| Lactosyl Ceramide | 4.9 | 4.3 | 2.0 | 2.7 |
| Trihexosyl Ceramide (gal-gal-glc-ceramide) | 0.6 | 0.6 | 0.5 | 0.4 |
| Globoside | 12.6 | 0.4 | 7.5 | 0.4 |

*Control Fluids obtained at 16-22 weeks gestation, n=3.

Table 2 shows the concentrations of neutral glycosphingolipids in both centrifuged and uncentrifuged amniotic fluid from the at-risk fetus and fetal controls; the levels of globoside were 30-fold greater in the uncentrifuged fluid and 18-fold greater in the centrifuged amniotic fluid compared to the levels in control fluids, respectively. The increased concentrations of globoside could be detected visually on thin-layer chromatography plates.

TABLE 3. TOTAL HEXOSAMINIDASE ACTIVITY* AND PERCENT HEXOSAMINIDASE B IN FETAL PLASMA, CEREBRAL CORTEX AND LIVER

| Source | Plasma | | Cerebral Cortex | | Liver | |
|---|---|---|---|---|---|---|
| | Total Hex | % Hex B | Total Hex | % Hex B | Total Hex | % Hex B |
| Sandhoff Fetus 1 (18 wk gestation) | 11 | 54 | 110 | 31 | 32 | 63 |
| Control 1 (20 wk gestation) | 1190 | 28 | 368 | 29 | 1365 | 38 |
| Control 2 (21 wk gestation) | 1870 | 33 | 432 | 26 | 2159 | 36 |

*Total Hexosaminidase activity (Total Hex) expressed as nmoles substrate hydrolyzed/hr/ml plasma or per mg protein tissue; hexosaminidase B (thermostable hexosaminidase, 50°C.) expressed as percent of total hexosaminidase activity (% Hex B). Standard reaction conditions were used (8, 11).

Additional diagnostic evidence was obtained by ultrastructural examination of the uncultured amniotic fluid cells. Figure 2 is a photomicrograph showing abnormal lysosomes containing multi-vescular bodies similar to those found in the tissues of patients with Sandhoff's disease (6, 7). The chemical and ultrastructural demonstrations of substrate accumulation required three days of laboratory analysis.

On the basis of these data, the pregnancy was terminated by hysterotomy at 18 weeks of gestation. Table 3 shows that cerebral cortex, hepatic tissue and plasma from the at-risk fetus were markedly deficient in hexosaminidase A and B activities compared to those in fetal controls, confirming the *in utero* diagnosis.

DISCUSSION: The analysis of amniotic fluid components obtained by amniocentesis has made the prenatal diagnosis of an increasing number of inborn errors of metabolism a reality. The *in utero* diagnosis of Sandhoff's disease was made the day of amniocentesis by the enzymatic and electrophoretic demonstration of deficient hexosaminidase A and B activities in the amniotic fluid supernate and uncultured amniotic fluid cells. The ultrastructural demonstration of lysosomal glycosphingolipid accumulation in uncultured amniotic cells provided further diagnostic confirmation within three days of the amniocentesis; these pathologic morphologic findings are analogous to those allowing the prenatal diagnosis of Type II Glycogenosis (18).

Previously in patients with Sandhoff's disease, the demonstration of accumulated globoside in the urinary sediment provided the biochemical diagnosis of this disorder (6, 7, 11). Since amniotic fluid is derived in part from fetal urine, it was of interest to determine if globoside had accumulated in the amniotic fluid of the at-risk fetus. Since most workers centrifuge the amniotic fluid, utilizing the amniotic cells for culture and analyses, the concentrations of the neutral glycosphingolipids were determined in both centrifuged and uncentrifuged amniotic fluid from the at-risk fetus and fetal controls; the increased levels of globoside in both sources provided additional diagnostic information.

The in utero diagnosis of Sandhoff's disease was confirmed enzymatically by the demonstration of deficient hexosaminidase activities in the plasma and tissues obtained from the aborted fetus. The deficient hexosaminidase activities in the amniotic fluid components, plasma, and various tissues from the affected fetus indicates that the maternal hexosaminidases do not cross the fetal-placental barrier. Therefore, in Sandhoff's disease, the fetal accumulation of the glycosphingolipid substrates occur early in utero. Further enzymatic, chemical, and morphologic studies of this fetus will be reported elsewhere (14).

Thus, the in utero diagnosis of Sandhoff's disease, for the first time, has been accomplished by rapid and reliable methods. The use of enzymatic, chemical and ultrastructural techniques permits the rapid diagnosis of an affected fetus, reduces the interval between amniocentesis and diagnosis, and eliminates the need for the sophisticated techniques as well as the time and expense required for the establishment of tissue culture lines. Furthermore, these combined techniques provide sufficient cross-check of methodology so artifacts can be avoided. Thus, the genotype of an at-risk fetus can be accurately defined in utero, allowing at-risk heterozygotes for Sandhoff's disease the ability to have only phenotypically normal offspring.

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