Screening for Tay-Sachs Disease *in Utero* Using Amniotic Fluid[1] (35479)

JOAN FRIEDLAND, GUTA PERLE, ABRAHAM SAIFER, LARRY SCHNECK, AND BRUNO W. VOLK

Isaac Albert Research Institute and the Birth Defects Center of the Kingsbrook Jewish Medical Center, Brooklyn, New York 11203

The most reliable method for prenatal diagnosis of a number of genetic disorders where the specific enzyme defect is known has been the analysis of the cultured amniotic cells for the missing enzyme which in the case of Tay-Sachs disease (TSD) is hexosaminidase A (1). The cultured cells often require 3 or more weeks for adequate growth and occasionally the cells are not successfully cultured. Uncultured cells have also been used but there is a question of their reliability. In the case of a bloody tap the uncultured cells may be contaminated with a sufficient quantity of white blood cells to cloud the results of the analysis even when only an insignificant quantity of serum will contaminate the amniotic fluid itself. In addition, the quantity of uncultured cells available may be so small as to make the analysis of the uncultured cells difficult. Therefore the acrylamide gel electrophoretic method for analysis of hexosaminidase A has been applied to amniotic fluid as a possible method of detecting TSD *in utero*.

Material and Methods. The acrylamide gel electrophoresis method described by Friedland *et al.* (2) has been used with the following modification: To each gel 0.15 ml of a mixture (2 vol amniotic fluid and 1 vol 40% sucrose) was applied. The electrophoresis was carried out for 5 min at 1 mA/tube and then for 1 hr and 35 min at 3.5 mA/tube. Originally the gels were incubated with the substrate according to Friedland *et al.* (2) but recently the gels were incubated for 1 hr at 37° completely submerged in the same buffered substrate. The gels were scanned using a modified Turner TLC scanning fluorimeter. For comparison, the quantitative technique for analysis of hexosaminidase A with heat fractionation was used on the fluid (3).

Results. To date 38 control fluids and 5 fluids from mothers who have had previous children with TSD have been analyzed. In all of the control amniotic fluids, there was identifiable hexosaminidase A activity by acrylamide gel electrophoresis (Table I). In 4 of the 5 amniotic fluids from mothers of Tay-Sachs children, hexosaminidase A was present at greater than 15% of the total hexosaminidase activity on acrylamide gels. In these 4 cases, hexosaminidase A activity has been found in the uncultured cells and in the cultured cells of the 3 cases where they were available. In 3 different amniotic fluid samples from the fifth case no hexosaminidase A was detected in the amniotic fluid by the acrylamide gel method. Because of the failure of the cultured cells to grow in this case, only uncultured cells were available. These uncultured cells showed only trace quantities of hexosaminidase A, presumably the result of contaminating white blood cells. In this previously reported case (4), the diagnosis of fetal TSD was verified by enzymatic analysis on the aborted fetus. This mother is again pregnant and is represented by one of the other four cases.

The heat fractionation results on the case where TSD was detected showed between 8 and 10% hexosaminidase A. As shown in Table I, the lower limit of the controls was 5% so that this method which may have an error as large as 10% would not have been successful in distinguishing between the Tay-Sachs fetus and some of the normal controls.

Discussion. The exact limits of detection of hexosaminidase A by the acrylamide gel are

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TABLE I. Hexosaminidase in Amniotic Fluid.

Sample	Total hexosaminidase (n*M*/hr/ml; mean)	% Hexosaminidase A acrylamide gel	% Hexosaminidase A heat fractionation
Normal pregnant women (38)	658	Mean 21.9 (4.2–35.6)	Mean 20.9 (5.7–32)
Tay-Sachs mothers predicted normal (4)	722	Mean 22.2 (17.3–27.6)	Mean 22.9 (19.7–27)
Tay-Sachs mothers predicted Tay-Sachs (1)	529	None detected	(8.1–10.2)

unknown. It is possible that a small amount of this activity would not be detected by this method. Since the mechanism of the synthesis of amniotic fluid is not entirely understood, it is also possible that either the chorion or the maternal serum may contribute a small amount of hexosaminidase A to the amniotic fluid. The former source would be small since the predominant form of hexosaminidase in the chorion as determined in this laboratory is hexosaminidase B. During pregnancy the serum of a Tay-Sachs carrier also contains mainly hexosaminidase B with hexosaminidase A being only 15 to 33% of the total at the time amniocentesis is performed. These contributions, if they exist, might produce an overlap between normal amniotic fluids with a low level of hexosaminidase A and Tay-Sachs amniotic fluid. So far sufficient data are unavailable to rule this out, so the results of amniotic fluid analysis should be confirmed, if possible, on uncultured and cultured cells. However, to date, amniotic fluid has provided us with large quantitites of rapidly analytical material from which we have obtained preliminary diagnosis which so far have proved to be as accurate as the diagnosis obtained on the cultured and uncultured cells.

Finally, it must be emphasized that these tests are useful in diagnosing the classical form of TSD only. The occurrence of hexosaminidase A in the fetus does not rule out the diagnosis of G_{M2}-gangliosidosis since at least two cases of late infantile or juvenile G_{M2}-gangliosidosis have been reported where hexosaminidase A has been shown to be only partially deficient (5, 6). In addition, a form of the disease where there is a total absence of hexosaminidase activity is reported to occur in a non-Jewish case of G_{M2}-gangliosidosis (7).

Summary. The amniotic fluid from 38 control and 5 heterozygote TSD mothers were analyzed by acrylamide gel electrophoresis and heat fractionation for the percent of hexosaminidase A activity. This study indicates that amniotic fluid, when analyzed by acrylamide gel electrophoresis, can be used for the prenatal diagnosis of TSD.

ADDENDUM

Since this paper was submitted for publication, the amniotic fluids of 35 additional normal (non-Tay-Sachs) pregnant women, 3 pregnant Tay-Sachs mothers (predicted normal) and 2 pregnant Tay-Sachs mothers (predicted Tay-Sachs) were analyzed for total hexosaminidase and hexosaminidase A content with both the heat fractionation and acrylamide gel procedures. The results obtained extend and confirm the findings reported in this paper.

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