Prenatal diagnosis of homozygous familial hypercholesterolemia (FH) is of major importance because of the bad prognosis and the fact that this condition frequently causes death before the age of 20 years. Previously, this diagnosis was made by measuring low density lipoprotein (LDL) receptor activity on amniotic cells in culture. Although this procedure is reliable, it has major disadvantages since it takes a long time and can be done only in the few laboratories with experienced personnel. In this study, we describe a new faster procedure for prenatal diagnosis of homozygous FH based on a direct analysis in fetal serum of total cholesterol (TC), high density lipoprotein (HDL) cholesterol, triglycerides (TG), apolipoprotein (apo) B, and B-lipoproteins (B-LP). This procedure was made possible by a new method of fetal blood sampling during pregnancy that is simpler and safer than fetoscopy and by the knowledge of the normal fetal values of TC, TG, and apo B at various stages of gestation.

Methods

Family History

The parents were not related. The father, aged 30, was apparently in good health. Until his son's birth, he did not know of his hypercholesterolemia. Physical examination showed him to be entirely normal: neither lipid skin deposition nor cardiovascular complications were detected. Tests showed the following values: TC = 335 mg/dl, LDL cholesterol = 277 mg/dl, TG = 110 mg/dl, HDL cholesterol = 36 mg/dl, and apo B = 180 mg/dl. The child's mother was 29 years old and was a heterozygote for familial hypercholesterolemia, as was her mother. Xanthomas were present in both Achilles tendons. Tests showed the following values: TC = 330 mg/dl, LDL cholesterol = 278 mg/dl, TG = 80 mg/dl, HDL cholesterol = 36 mg/dl, apo B = 180 mg/dl.

Their first child was born in 1980 and was 20 months old when he was first examined. Since birth, homozygous FH had been suspected because of extensive cutaneous xanthomas and a TC level of around 1000 mg/dl. Fibroblast cultures tested in the Dallas laboratory of J.L. Goldstein and M.S. Brown showed a complete absence of LDL receptor activity, and allowed classification of this child as LDL-receptor negative.

Since the child did not respond to dietary or multiple drug therapy, a portacaval anastomosis was carried out when he was 28 months old. Despite surgical treatment and a fall in cholesterol values of about 40%, there was no regression of cutaneous lipid deposits after 2 years of survey.

Although there was no coronary heart disease at the age of 24 months, the clinical findings suggested a significantly impaired life expectancy. In September, 1983, the mother became pregnant for the second time and asked for prenatal counseling.

Amniocentesis

To diagnose homozygous FH, amniocentesis was performed at the 15th week of gestation by using the methodology cited by Brown and Goldstein. At the same time, two women who had had amniocentesis for other reasons were used as controls. Both women and their husbands had normal fasting blood TC and TG values. Cells from the amniotic fluids of the patient and the two controls were cultured for 1 month and sent to Dallas for analysis.
Table 1. Measurement of Low Density Lipoprotein Receptor Activity In Cultures of Amniotic Fibroblast Cells

<table>
<thead>
<tr>
<th>Subject</th>
<th>High affinity metabolism of $^{125}$I-LDL (ng/5 hr/mg protein)</th>
<th>$^{14}$O Olate incorporation into cholesteryl ($^{14}$C) olate (pmol/5 hr/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Binding</td>
<td>Internalization</td>
</tr>
<tr>
<td>Normal adult</td>
<td>287</td>
<td>1371</td>
</tr>
<tr>
<td>Receptor-negative FH</td>
<td>2.3</td>
<td>19</td>
</tr>
<tr>
<td>Control fetus 1</td>
<td>10.2</td>
<td>48</td>
</tr>
<tr>
<td>Control fetus 2</td>
<td>17.1</td>
<td>153</td>
</tr>
<tr>
<td>Study fetus</td>
<td>&lt;2</td>
<td>15</td>
</tr>
</tbody>
</table>

Fetal Blood Studies

Fetal blood was aspirated via the umbilical cord with a needle guided by ultrasound under local anesthesia at the 24th week of gestation. A 3 ml sample of fetal blood was easily obtained. To be sure that it was not contaminated by maternal blood or diluted by amniotic fluid, the sample was examined by using a Coulter Counter S plus II, which measures the mean corpuscular volume and the hematocrit. The sample was stained by the Kleihauer-Betke procedure. Enzymatic techniques were used to assay for TC, TG, LDL cholesterol (calculated according to the Friedewald-Fredrickson formula), and HDL cholesterol. Radial immunodiffusion determined apo B, and agarose gel electrophoresis measured lipoproteins.

For controls, we used samples from 48 normal fetuses whose blood had been sampled at the same stage of gestation for diagnosis of other diseases such as hemophilia, thalassemia, and rubella.

Confirmation of Diagnosis

A medical abortion was induced by using prostaglandins, and the same assays were carried out on fetal blood obtained by cardiac puncture. LDL receptor activity was tested on fibroblast cultures of a skin biopsy. Histologic sections of the heart, main vessels, and other organs were examined by routine microscopy.

Results

Some of the amniotic fluid flasks were contaminated with fungal growth, and thus full testing for receptor activity was impossible. Furthermore, the amniotic cells were epithelioid and not fibroblastic and grew more slowly than usual. The results of the assays performed on the amniotic fluid cells were available 2 months after amniocentesis (23rd week of gestation) and are given in Table 1. LDL receptor activity from the fetus under study was extremely low and comparable to results obtained from homozygotes who were receptor-negative for FH. Values from both control fetuses were lower than those previously tested in Dallas, and the possibility of heterozygous FH could not be excluded. For obvious reasons, the assay could not be repeated, and fetal blood sampling was proposed to the parents in order to confirm the results.

The values of serum lipid levels in the fetus under study and in 48 nonhyperlipidemic fetuses are shown in Table 2. TC was about eightfold higher in the index fetus as compared with the controls. Most of the TC was LDL cholesterol. TG were in the normal range, β-LP were higher, and apo B was more than sixfold normal value. The results were communicated to the parents who elected termination of the pregnancy. Lipid levels of the fetus were again assayed after abortion (Table 2). The LDL receptor activity on skin fibroblasts was 2% to 5% of the nor-

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Table 2. Lipid Levels and Agarose Gel Electrophoresis of the Affected Fetus Compared to Control Fetuses at the Same Stage of Gestation (18th to 24th Week)

<table>
<thead>
<tr>
<th>Assays</th>
<th>Fetal blood sample cord blood (mg/dl)</th>
<th>Control after abortion heart blood (mg/dl)</th>
<th>Normal fetuses (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol</td>
<td>543</td>
<td>496</td>
<td>66 ± 9</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>513</td>
<td>478</td>
<td>ND</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>20</td>
<td>16</td>
<td>ND</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>45</td>
<td>79</td>
<td>46 ± 9</td>
</tr>
<tr>
<td>Apolipoprotein B</td>
<td>275</td>
<td>273</td>
<td>43 ± 5</td>
</tr>
<tr>
<td>β-Lipoprotein</td>
<td>increased</td>
<td>increased</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND = not done.
Discussion

Study of LDL receptor activity in amniotic fluid obtained by amniocentesis is not possible before the 15th week of gestation, and the necessary cultures require a waiting period of at least 1 month, a period when the cells are exposed to the hazards of culturing and contamination. This long waiting period is a very difficult one for the mother for psychological reasons. In addition, the need to compare results to normal amniotic fluid cells requires simultaneous amniocentesis specimens from normolipidemic husbands.

Furthermore, the investigation of binding, internalization, and incorporation of 125I-labeled LDL in amniotic cells in culture requires a sophisticated technique that is available in only a few laboratories.

In the case reported here, fungal contamination of several culture flasks appeared after 1 month. Multiple handling during transportation might have been the cause of contamination. This made a definite diagnosis impossible. In any case, the LDL-receptor results left a margin of uncertainty between the diagnosis of heterozygous and homozygous FH.

The possibility of using fetal blood from the 18th week to the end of gestation opens new possibilities of testing for fetal pathology. Fetal blood sampling can be performed as an outpatient procedure under local anesthesia and can be repeated if necessary. In our series of 350 consecutive samplings during the second and third trimesters of pregnancy for various possible diseases, we have noticed no adverse effects on the fetuses. The rate of fetal loss has been extremely low, comparable to that of the normal obstetrical population (data not shown).

Little is known about lipid levels in utero in normal and pathological conditions. The relatively large amount of blood obtained by direct aspiration under ultrasound guidance allowed us to check for normal lipid values in a large number of fetuses at various stages of gestation. Normal TC was approximately 60 mg/dl at 24 weeks and 100 mg/dl at birth. In homozygous FH studied in childhood, TC ranges from 600 to 1200 mg/dl. Compared to normal, TC is increased fourfold in homozygotes and twofold in heterozygotes. The same ratio between normal and homozygous FH can be expected during fetal life. Indeed, Brown and co-workers found TC levels of 279 mg/dl in a 20-week-old homozygous fetus and 31 mg/dl in normal fetuses of the same age, a ratio of 9:1. According to our data, the ratio of TC for the index fetus compared to control fetuses was the same. The diagnosis of FH was confirmed after abortion by the absence of LDL receptor activity on fibroblasts. The normal findings on routine microcopy of fetal tissues is interesting; Buja and co-workers have already reported similar findings. Possibly the absence of fat deposits may be related to the intensive utilization of TC in tissue formation at this time of fetal development.

The improvement in rapidity of prenatal detection by this method was shown in a subsequent pregnancy in this couple in which cord blood analysis was done at the 18th week of pregnancy, allowing therapeutic abortion of another homozygous fetus.

Prenatal diagnosis of homozygous familial hypercholesterolemia is possible by direct analysis of fetal blood obtained by needle aspiration guided by ultrasound. The main advantages of this procedure as compared to the measurement of LDL receptor activity on cultured amniotic cells are its simplicity and speed. Results can be obtained on the same day, and fetal sampling can be easily repeated if necessary.

Acknowledgments

We thank Professor Taillemite for amniotic cultures and Joseph L. Goldstein and Michael S. Brown for assisting us with the assay and performing the 125I-labeled LDL metabolism tests.

References


Index Terms: homozygous familial hypercholesterolemia • prenatal diagnosis • fetal blood sampling • reference values
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