Quantitative and Qualitative Assay of Amniotic-Fluid Acetylcholinesterase in the Prenatal Diagnosis of Neural Tube Defects

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Summary. In 110 amniotic fluids the specific acetylcholinesterase was determined quantitatively and qualitatively. In the quantitative assay there were a considerable number of false positives and false negatives, although the mean value of the normal controls differed significantly from that of neural tube defect pregnancies. By the electrophoretic separation of acetylcholinesterase, however, all fluid samples with borderline alpha-fetoprotein levels or fetal blood contamination could be correctly classified. With the exception of one skin-covered spina bifida all neural tube defects in the second trimester could be identified by this method. The second fast-moving band characteristic of the specific acetylcholinesterase was also present in abdominal wall defects and intrauterine death.

Introduction

Since the original report of Brock et al. (1972) the determination of amniotic-fluid alphafetoprotein (AFP) has become a routine laboratory test for the prenatal diagnosis of neural tube defects (NTD). Elevated AFP levels, however, are not exclusively found in fetal NTD, but also in several other conditions, such as exomphalos, gastrointestinal atresias, congenital nephrosis, etc. (Seppälä 1977; Milunsky 1979) and recently in hemangiomas of the cord or the placental vessels (Bason et al. 1980; Schnittger et al. 1980).

Furthermore contamination with fetal blood may yield false-positive results. According to the second report of the U.K. Collaborative study on AFP (1979), the rate of false positives remains at 0.27% excluding the conditions mentioned above. Moreover the cut-off level is arbitrary, so that borderline values may render correct diagnosis difficult or impossible.

A new test substance for the prenatal detection of NTD has been proposed by Smith et al. (1979) and Chubb et al. (1979): a specific isoenzyme of acetylcholinesterase (ACHE) originating from the nervous system (Chubb et al. 1979) which migrates to the same position as the isoenzyme of AChE found in human cerebrospinal fluid (Smith et al. 1979).

The investigation presented here was designed to examine the validity of the quantitative AChE assay and of the electrophoretic separation of AChE with special consideration of cases where the AFP determination did not lead to clear results.

Materials and Methods

A total of 110 amniotic fluids from pregnancies with known fetal outcome were studied. All samples had been previously centrifuged to remove the cells. Of these, 85 fluids were tested retrospectively and had been stored frozen at -20°C for 1–60 months. At this temperature AChE is stable (Brock and Hayward 1980). The other 25 fluids were fresh. AFP was determined by rocket immunelectrophoresis (Laurell 1966). The specific AChE was estimated quantitatively by the photometric method of Ellman et al. (1961), as described by Smith et al. (1979).

The qualitative AChE test was performed on polyacrylamide gel electrophoresis. A slow migrating first band of unspecific cholinesterase; b the same sample with specific inhibitor; 2 → additional fast moving second band of specific acetylcholinesterase; c spina bifida; d the same sample with specific inhibitor; e anencephalus; f the same sample with specific inhibitor.
According to AFP concentration and fetal outcome, we divided the amniotic fluids into five groups prior to the examination of AChE:

1. Normal controls with an AFP level below 2 multiples of the median (MoM);
2. Borderline AFP values 2 MoM-3 MoM;
3. Elevated AFP concentrations due to fetal blood contamination;
4. NTD pregnancies;
5. Elevated AFP levels due to other fetal conditions.

**Normals:** For the controls, 70 clear samples of second-trimester amniotic fluids were used. Two pregnancies ended in a miscarriage and four were terminated (trisomy 18, trisomy 21, XXY and androgen medication); the other 64 went to term, and no abnormalities were found in the newborns. The quantitative assay yielded AChE concentrations between 0.90 and 9.00 mU/ml, the highest value originating from a pregnancy that resulted in a healthy newborn. The mean value was 2.88 mU/ml. In three cases with hydramnios, not included in the control group, we observed very low AChE levels (<1.2 mU/ml). Fetal outcome was normal.

The qualitative test for specific AChE was negative: none of the controls showed the second fast-moving band in the gel electrophoresis, but only the slow-moving first band.

**Borderline AFP:** Five cases with borderline AFP values and known fetal outcome were examined. The amniotic fluid was taken between the 17th and 18th week of pregnancy. AChE values ranged from 2.82-6.06 mU/ml in the quantitative test. None of the fluids had a fast-moving second band in gel electrophoresis (Table 1). All pregnancies continued normally. One infant (case 2) had a severe ABO erythroblastosis and one (case 3) was delivered premature, but had no abnormalities. Normal babies were born in cases 1 and 4. In case 5 the slow migrating band was absent (Fig. 2d), and the female infant was still-born with bilateral cystic kidney dysplasia combined with atresia of the anus, urethra, and vagina (Schmidt et al. 1981).

**Fetal blood contamination:** There were four samples of amniotic fluid with borderline or slightly elevated AFP values (up to 3.5 MoM). In our opinion this was due to fetal blood contamination since fetal erythrocytes were shown to be present by the Kleihauer test. In the twin pregnancy (case 8) the elevation might have been caused by the small exomphalos of one of the twins (there were two amniotic sacs and only one was punctured). The other twin was normal. None of these fluids had a fast second band in gel electrophoresis (Fig. 2e), and the values of AChE by the quantitative assay did not differ significantly from those of the controls. They ranged from 1.56 to 7.50 mU/ml (Table 2). Case 6 with the relatively high AChE activity of 7.50 mU/ml ended in an abortion. No abnormalities were found in the newborns in cases 7 and 9.

**Neural tube defects:** Thirteen amniotic fluids of anencephalic fetuses were examined (Table 3). In ten cases, tested in the second trimester (nos. 10-19) the AChE concentration was 3.19-19.30 mU/ml with a mean of 8.10 mU/ml. They all showed a distinct second fast-moving band in gel electrophoresis (Fig. 2c). Three fluid samples (cases 20-22) were obtained in late third-trimester pregnancies. These samples had low AChE activity (0.70-2.22 mU/ml) and only one of them displayed a clear second band in gel electrophoresis, while it was absent in the other two (Fig. 3b).

The other open forms of NTD included five cases of spina bifida (nos. 23-27), three as part of Edward's syndrome and four cases of encephalocele (nos. 29-32) one as part of Meckel's syndrome. All of them revealed two bands in gel electrophoresis (Fig. 3a). In case 28 we did not see the second fast-moving band in the qualitative AChE test. As AFP was also normal, the pregnancy went to term: an infant with a skin-covered meningomyelocele combined with an extensive hydrocephalus was delivered by cesarian section and died shortly after birth. The case with Meckel's syndrome (no. 30) had a high AChE activity of 24.76 mU/ml; the others (nos. 23-29 and 31-32) ranged between 2.82-7.20 mU/ml (Table 3). The mean value was 7.97 mU/ml.

**Other elevated AFP levels:** In seven instances of 1800 amniotic fluids routinely examined, we found clearly elevated AFP values (up to 75 MoM) not due to NTD or fetal blood contamination (Table 4). All except one had a correspondingly high AChE level (>6.0 mU/ml). Four showed the specific

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**Table 1. AChE in amniotic fluids with borderline AFP values (2-3 MoM)**

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Week of pregnancy</th>
<th>AFP (µg/ml)</th>
<th>AChE (quantitative) mU/ml</th>
<th>AChE (qualitative)</th>
<th>Fetal outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>17</td>
<td>32.8</td>
<td>3.85</td>
<td>Negative</td>
<td>No abnormalities</td>
</tr>
<tr>
<td>2</td>
<td>17</td>
<td>34.3</td>
<td>4.07</td>
<td>Negative</td>
<td>ABO-Erythroblastosis</td>
</tr>
<tr>
<td>3</td>
<td>16</td>
<td>38.5</td>
<td>2.82</td>
<td>Negative</td>
<td>Premature infant, no malformations</td>
</tr>
<tr>
<td>4</td>
<td>17</td>
<td>32.8</td>
<td>3.85</td>
<td>Negative</td>
<td>No abnormalities</td>
</tr>
<tr>
<td>5</td>
<td>17</td>
<td>35.0</td>
<td>6.06</td>
<td>Negative (atypical)</td>
<td>Potter's syndrome, multiple malformations</td>
</tr>
</tbody>
</table>