Prospective prenatal screening for fetal abnormalities using a quantitative immunoassay for acetylcholinesterase

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SUMMARY An immunoassay based on a monoclonal antibody specific for acetylcholinesterase (AChE) was used prospectively over a two year period to screen second trimester amniotic fluid for fetal abnormalities. All 26 cases of spina bifida, three of which had normal alphafetoprotein (AFP) concentrations and ultrasound scans, were detected by the immunoassay. Four clear fluids, with abnormal AFP concentrations, had normal AChE titres and yielded normal outcomes. Some difficulties were encountered with both the AChE immunoassay and polyacrylamide gel testing when amniotic fluids were contaminated with old blood. However, the quantitative nature of the AChE immunoassay and its independence of operator experience make it an ideal adjunct to AFP assay for routine screening of amniotic fluids.

Measurement of alphafetoprotein (AFP) in second trimester amniotic fluid samples is now a routine procedure for excluding fetal neural tube defects. The sensitivity for detection of anencephaly and open spina bifida is above 98%, while the false positive rate is below 0.5%. Differences are encountered in detecting some cases of spina bifida with small open lesions, which may also evade detailed ultrasound scanning. Occasional clear amniotic fluids may have raised AFP values in the absence of any fetal abnormality.

The introduction of the polyacrylamide gel electrophoretic test for cholinesterase isoenzymes has proved a useful adjunct to amniotic fluid AFP testing. A collaborative study showed that it could reduce the number of AFP based false positives without significant loss of sensitivity. However, gel electrophoretic analysis is a qualitative test and depends on the experience of operators in locating the characteristic fast migrating acetylcholinesterase (AChE) band which may signify a fetal neural tube defect. Densitometric scanning of stained gels is time consuming and is not widely used.

In an attempt to overcome these difficulties, we introduced a quantitative immunoassay for AChE, which used a monoclonal antibody which did not cross react with the more abundant non-specific cholinesterases found in amniotic fluid. We now report our experience in the prospective use of this assay in a routine prenatal diagnostic service laboratory.

Materials and methods

From mid-1984 to mid-1986, 1520 amniotic fluid samples were received for routine AFP testing. The outcome of pregnancy was traced on 1469 (97%), which are the subject of this study. On receipt of the fluids, cells were removed for karyotype analysis, and AFP and AChE measured immediately on the supernatants. AFP was measured by rocket immunoelectrophoresis using the standard system of gestation dependent multiples of the median (MoMs) to distinguish normal from raised values. AChE was assayed in a quantitative monoclonal antibody immunoenzymatic assay, with the cut off placed arbitrarily at 0-3 U/ml (figure). This corresponds to our earlier action line of 0-1 absorbance units. Where necessary polyacrylamide gel electrophoresis of cholinesterase isoenzymes was carried out as described.

Results

The outcomes of the 1469 pregnancies are summarised in the table. In 1401 cases, there was no visible abnormality in the child, although these did include nine neonatal deaths all with normal second trimester AChE and AFP values.

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TABLE Summary of outcomes of pregnancy with amniotic fluid AFP and AChE status.

<table>
<thead>
<tr>
<th>Outcome of pregnancy</th>
<th>Raised AChE Raised AFP</th>
<th>Raised AChE Normal AFP</th>
<th>Normal AChE Raised AFP</th>
<th>Normal AChE Normal AFP</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal outcome</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clear or slightly bloody fluid</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>1378</td>
<td>1382</td>
</tr>
<tr>
<td>Gross fresh blood in fluid</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Old blood in fluid</td>
<td>0</td>
<td>9</td>
<td>0</td>
<td>7</td>
<td>16</td>
</tr>
<tr>
<td>Anencephaly</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Open spina bifida</td>
<td>23</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>26</td>
</tr>
<tr>
<td>Closed neural tube defect</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Chromosome abnormality</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Intrauterine death</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>Spontaneous abortion</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Stillbirth, no apparent abnormality</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Miscellaneous abnormalities</td>
<td>1</td>
<td>4</td>
<td>0</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>32</td>
<td>18</td>
<td>4</td>
<td>1415</td>
<td>1469</td>
</tr>
</tbody>
</table>

FIGURE AChE concentrations in 1382 normal amniotic fluids (hatched lines) and 26 cases of open spina bifida, with abnormal (○) AFP and normal (■) AFP.

NORMAL OUTCOMES
In the 1382 cases where amniotic fluid was classified as clear or slightly bloody, there were no instances of raised AChE values. There were, however, four cases with unexplained raised AFP values (3-7, 4-0, 5-4, and 15-7 MoMs, respectively). These are classified as AFP false positives.

Of three cases with massive amounts of fresh blood in the fluid, two had both raised AChE and AFP values. Among the 16 cases where the amniotic fluid was black or dark brown owing to old blood, nine had AChE values in the abnormal range.

NEURAL TUBE DEFECTS
All six cases of anencephaly had raised AChE and AFP. All 26 cases of spina bifida had raised AChE, but of these three were AFP false negatives (figure). None of the four closed neural tube defects (two skin covered meningocele spina bifidas, one encephalolec, and one hydrocephalus) was detectable by either AChE or AFP.

OTHER ABNORMALITIES
Only one of seven intrauterine deaths and one of five spontaneous abortions had raised AChE. In each case the fluid was black or dark brown. Among the miscellaneous abnormalities there was one case with raised AFP and AChE; this was a haemangiomata of the placenta. The four with normal AFP but raised AChE resulted in a liveborn infant with multiple external dysmorphic features including cleft lip and palate, a 31 week neonatal death with unexplained oligohydramnios, a 25 week neonatal death with unexplained oligohydramnios, and a pregnancy that was terminated because the fetus had generalised hydrops.

Discussion
One of the major continuing problems in the routine use of amniotic AFP testing is that it fails occasionally to detect cases of spina bifida with small open lesions. Ultrasound scanning of each neural arch in both transverse and longitudinal directions may reveal these defects, but this is a time consuming process if there is no index of suspicion. Nicolaides and Campbell reported six cases of missed spina bifida in a total of 102 investigated over a five year period. Other operators may have difficulty in matching this experience.

There appears to be only one large reported series on the use of polyacylamide gel electrophoresis for routine screening of amniotic fluids for the characteristic AChE band. Aitken et al surveyed 3100 fluids and detected all 83 cases of open spina bifida by gel AChE analysis. However, there were seven false positives on AChE alone and another six where AChE failed to resolve positive AFP values in normal pregnancies. The Collaborative Acetylcholinesterase Study was restricted to amniotic fluids with positive AFP results, and thus did not address the question of detecting fetal abnormalities with normal AFP results.
In this report we show that the quantitative immunoassay was able to detect three cases of spina bifida which had normal AFP results and where ultrasound scanning gave no hint of abnormality. Retrospective gel electrophoretic analysis on these three samples also showed the AChE band quite clearly, but it is inconvenient to apply the cumbersome gel test to apparently normal samples.

We also found normal AChE values in four clear fluids which had unexplained raised amniotic AFP. These potential AFP false positives had unremarkable ultrasonar scans and went to term with the delivery of normal infants. In two amniotic fluid samples with gross amounts of fresh blood, both AFP and AChE were raised, and here the correct conclusion of a normal pregnancy rested on ultrasound scanning and the unsuitability of the fluid for biochemical analysis.

The most difficult amniotic fluid samples on which to perform AChE immunoassay are those which are black or brown in colour. Red cells have substantial amounts of AChE on the membrane surface, which is released after haemolysis and ageing. Much of this red cell derived AChE is particle bound and thus does not always penetrate a polyacrylamide gel and appear as a characteristic fast migrating band. Nevertheless, of the nine samples with old blood which gave false positive values in the quantitative AChE immunoassay, six also had positive polyacrylamide gel tests. This emphasises the importance of AFP assay for black or brown fluids.

We have described elsewhere the differential diagnosis of neural tube defects and ventral wall defects in amniotic fluid samples. However, the improvements in ultrasound equipment and operator skills suggest that this is a diminishing problem. In the two year period surveyed, all cases of exomphalos and gastrochisis were seen on ultrasound scan and there was no need for amniocentesis.

The conclusion of this study is that AFP and AChE testing should be run simultaneously on all amniotic fluid samples. We emphasise the merits of a cheap and rapid quantitative AChE immunoassay which in a modern laboratory should supersede the polyacrylamide gel test.

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References

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