Reactivity of amniotic fluid alpha-fetoprotein with concanavalin A in diagnosis of neural tube defects

CAROL J SMITH, PHILIP C KELLEHER, LUC BÉLANGER, LOUIS DALLAIRE

Summary and conclusions
Alpha-fetoprotein (AFP) concanavalin-A-affinity molecular variant patterns were determined in amniotic fluid samples from 10 pregnancies complicated by anencephaly (6), spina bifida (1), Turner’s syndrome (1), osteogenesis imperfecta congenita (1), and fetal death (1) and 20 normal pregnancies between 14-6 and 25-5 weeks of gestation. With the exception of one anencephalic pregnancy, the AFP concentrations in samples from women with a fetal abnormality were more than 5 SD above normal for gestational age. In every case, however, the proportion of total amniotic fluid AFP that was non-reactive with concanavalin A was significantly smaller in the presence of a fetal abnormality (mean 2%) than when the fetus was normal (mean 20%).

The results suggest that measuring the amount of concanavalin-A-non-reactive amniotic fluid AFP will be a valuable test for diagnosing fetal abnormality, especially when an increase in total amniotic fluid AFP concentration is equivocal or gestational age is uncertain.

Introduction
Increased alpha-fetoprotein (AFP) concentrations in amniotic fluid were first shown to be associated with anencephaly and spina bifida in 1972. Raised concentrations were then found in other fetal abnormalities. Amniotic fluid AFP concentrations, however, vary with the duration of pregnancy, so that the diagnosis of fetal malformations depends on accurate gestational aging.

AFP from man and rats consists of two molecular variants based on their reactivity with concanavalin A. Proportions of total AFP that do not react with concanavalin A differ in fetal serum and amniotic fluid: 2-6% of fetal serum AFP and 15-45% of amniotic fluid AFP does not react with concanavalin A. Increased amounts of AFP in amniotic fluid in the presence of neural tube defects are thought to result from transudation of fetal serum across exposed fetal membranes. We have therefore measured the proportions of concanavalin-A-non-reactive AFP in amniotic fluid samples from normal pregnancies and pregnancies in which there were fetal abnormalities as a test to detect leakage of fetal serum into the amniotic cavity.

Materials and methods
Amniotic fluid samples from 10 pregnancies complicated by anencephaly and other fetal abnormalities and 20 normal pregnancies between 14-6 and 25-5 weeks of gestation were obtained at St Justine’s Hospital, Montreal (LD), and stored at -60°C. The investigation was conducted as a retrospective, double-blind study. Samples were coded with use of a table of randomly assorted digits, and column chromatography (CJS and PCK) and AFP measurements (LB) were performed in random order.

Chromatography on concanavalin-A-agarose (Pharmacia Fine Chemicals) was carried out as described with the following modifications. Aliquots (0.1 and 0.2 ml) of each amniotic fluid sample were applied to columns of 5 ml volume or fresh concanavalin-A-agarose. Two fractions were collected with the equilibrating buffer, a 3 ml void fraction and the 5 ml containing the concanavalin-A-non-reactive proteins. An additional 5 ml fraction was eluted before adding glucose to the equilibrating buffer to a final concentration of 1 mol/l (18 g/l). Proteins reactive with concanavalin A were eluted in 15 ml. The capacity of the concanavalin-A-agarose matrix to bind human amniotic fluid proteins was at least eight times greater than the amount of protein applied to the columns. On rechromatography all of the concanavalin-A-non-reactive proteins in 15 ml aliquots of amniotic fluid eluted in the non-reactive fraction.

The AFP standard was prepared and the AFP measured in the amniotic fluid samples and four chromatographic fractions by radioimmunoassay. Neither chromatographic buffer affected the accuracy of the radioimmunoassay, as indicated by recovery experiments. The percentage of total amniotic fluid AFP that was bound by the concanavalin-A-agarose matrix was expressed as the percentage of total amniotic fluid AFP applied to the column that was not bound by the concanavalin-A-agarose matrix. Total protein concentrations were determined by the method of Lowry et al.

Results
The table gives the amniotic fluid AFP concentrations and AFP concanavalin-A-affinity molecular variant patterns in the samples from the two groups. Total protein concentrations were above normal in five of the abnormal pregnancies. Amniotic fluid AFP concentrations greatly exceeded the mean values expected for gestational age in all cases. Without exception, however, the percentage of total amniotic fluid AFP that did not react with concanavalin A was lower in the presence of a fetal abnormality (mean 2-4%) than when the fetus was normal.

Amniotic fluid AFP concentrations and AFP concanavalin-A-affinity molecular variant patterns in presence of fetal abnormalities

| Case No | Fetal abnormality | Gestational age (weeks) | Total protein concentration (g/l) | AFP concentration (mg/l) | % Concanavalin-A-non-reactive AFP
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Spina bifida</td>
<td>14.6</td>
<td>3.2</td>
<td>65</td>
<td>52</td>
</tr>
<tr>
<td>2</td>
<td>Anencephaly</td>
<td>16.0</td>
<td>9.6</td>
<td>480</td>
<td>32</td>
</tr>
<tr>
<td>3</td>
<td>Fetal death</td>
<td>17.5</td>
<td>11.9</td>
<td>300</td>
<td>19</td>
</tr>
<tr>
<td>4</td>
<td>Anencephaly</td>
<td>18.0</td>
<td>5.7</td>
<td>220</td>
<td>19</td>
</tr>
<tr>
<td>5</td>
<td>Turner’s syndrome with hydrops</td>
<td>18.5</td>
<td>10.0</td>
<td>1200</td>
<td>20</td>
</tr>
<tr>
<td>6</td>
<td>Anencephaly</td>
<td>20.0</td>
<td>9.1</td>
<td>180</td>
<td>15</td>
</tr>
<tr>
<td>7</td>
<td>Anencephaly</td>
<td>22.0</td>
<td>6.6</td>
<td>61</td>
<td>35</td>
</tr>
<tr>
<td>8</td>
<td>Anencephaly</td>
<td>23.0</td>
<td>3.3</td>
<td>12</td>
<td>22</td>
</tr>
<tr>
<td>9</td>
<td>Anencephaly</td>
<td>24.0</td>
<td>6.5</td>
<td>62</td>
<td>25</td>
</tr>
<tr>
<td>10</td>
<td>Osteogenesis imperfecta congenita</td>
<td>25.5</td>
<td>14.8</td>
<td>400</td>
<td>14</td>
</tr>
</tbody>
</table>

Normal fetuses (n = 20) (Mean ± SD) 161 ± 0.7 32 ± 1.3 12.5 ± 4.3 20.1 ± 6.6

*Percentage of total amniotic fluid AFP not bound by concanavalin-A-agarose matrix. (ng concanavalin-A-non-reactive AFP/ng AFP applied to column in 0.2 ml aliquot of amniotic fluid) / 100.
normal (mean 20.1%). The difference in mean percentages between the two groups was highly significant (P < 0.001). The values listed in the table are based on the results of column chromatography of the 0.2 ml aliquot of amniotic fluid, but differences of the same level of significance were obtained with values based on the 0.1 ml aliquot. The AFP concanavalin-A-affinity molecular variant patterns did not correlate with concentrations of either the total amniotic fluid protein or total amniotic fluid AFP. More important, the percentage of concanavalin-A-non-reactive AFP was not related to gestational age within the range of ages tested.

Discussion
Since the AFP concentration varies with the duration of pregnancy its use as a diagnostic index of neural tube defects may yield false-positive results. An observed value may be taken as normal if gestational age has been overestimated and abnormal if age has been underestimated. This difficulty is reflected in amniotic fluid AFP concentrations that are not normally distributed.11 Multiple monoamniotic pregnancy may also be associated with raised amniotic fluid AFP concentrations when the fetuses are normal.11 Some laboratories11 have therefore extended the normal range of AFP values to include 5 SD above the mean.

Our table shows that leakage of fetal serum into the amniotic fluid was reflected by a change in the AFP concanavalin-A-affinity molecular variant pattern. In normal amniotic fluid samples 12-37% of the AFP did not react with concanavalin A. Leakage of fetal serum, containing about 2%, concanavalin-A-non-reactive AFP, reduced the proportion of concanavalin-A-non-reactive AFP in the amniotic fluid to 1.2-5.2%. This observation is particularly important because the AFP concanavalin-A-affinity molecular variant pattern was independent of gestational age.

Case 8 shows the value of determining the AFP concanavalin-A-affinity molecular variant patterns when the increase in amniotic fluid AFP concentration is equivocal. The fetus was anencephalic but the amniotic fluid AFP concentration was 12.0 mg/l at 23 weeks of gestation. This was less than 5 SD above the mean expected for gestational age, yet the percentage of concanavalin-A-non-reactive AFP was clearly lower than normal. On the other hand, in two of the normal pregnancies at 16 weeks' gestation the amniotic fluid AFP concentrations were 19.0 and 24.0 mg/l—that is, between 2 and 3 SD above the mean expected for gestational age. The percentages of concanavalin-A-non-reactive AFP, however, were 14.9 and 17.9 respectively, which were comparable with the mean of 20.5 in the remaining 18 normal pregnancies. Thus in normal multiple monoamniotic pregnancies the percentage of concanavalin-A-non-reactive AFP would probably be normal even if the amniotic fluid AFP concentration and maternal serum AFP concentration were raised. 

The abnormal pregnancies we studied comprised three cases of fetal malformation, one fetal death, and six cases of anencephaly. All were associated with defects in the integument or lymphatic (Turner's syndrome) or capillary (ostegenesis imperfecta congenita) development, and the decreased percentage of concanavalin-A-non-reactive AFP observed was consistent with leakage of fetal serum into the amniotic fluid. A raised amniotic fluid AFP concentration in ostegenesis imperfecta congenita has not apparently been reported, but is not unexpected in view of the thin skin and capillary fragility in such cases.13

For use in the clinical laboratory the determination of amniotic fluid AFP concanavalin-A-affinity molecular variant patterns may be simplified into two main steps. (1) Apply 0.2 ml amniotic fluid to a 5 ml bed volume column of concanavalin-A-agarose, discard the first 2.5 ml eluted from the column with the equilibrating buffer (void fraction), and collect the next 7.5 ml (concanavalin-A-non-reactive AFP) eluted with the equilibrating buffer. (2) Measure the AFP in the 7.5 ml column eluate and amniotic fluid sample and calculate the percentage of total amniotic fluid AFP not bound by the concanavalin-A-agarose matrix:

$$\text{ng concanavalin-A-non-reactive AFP/ ml} \times 7.5 \times 100$$

$$\text{ng amniotic fluid AFP/mL} \times 0.2$$

We intend to see whether the abnormal AFP concanavalin-A-affinity molecular variant patterns observed in amniotic fluid in cases of neural tube defects and certain other fetal malformations are reflected in maternal serum AFP.

We are grateful for the excellent technical help of Miss Diane Hamel and Miss Sharon O'Brien. The work was supported by USPHS research grant CA-15222 from the National Cancer Institute, National Institutes of Health (CJS and PKC), Medical Research Council of Canada grant MA-6478 (LB), and le Réseau de Médecine Génétique du Québec (LD). LB is a scholar of the Medical Research Council of Canada.

Requests for reprints should be addressed to: Dr Carol J Smith, Department of Medicine, University of Vermont, Given Building, Burlington, Vermont 05405, USA.

References

(Accepted 8 February 1979)