Comparison of Amniotic Fluid Alpha-Fetoprotein Reactivity to *Lens culinaris* Agglutinin and Concanavalin A in Crossed-Affinity Immunelectrophoresis: Ancillary Tests in the Prenatal Diagnosis of Severe Fetal Malformations

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Crossed-affinity immunelectrophoresis of alpha-fetoprotein in amniotic fluid from 135 normal and 39 abnormal pregnancies (mainly neural-tube defects) was performed with a lectin, agglutinin from *Lens culinaris*. Results were compared with findings reported for another lectin, concanavalin A. Three fractions of alpha-fetoprotein were obtained by reaction with *Lens culinaris* agglutinin. The most weakly reactive fraction correlated strongly with the concanavalin A nonreactive fraction. With both lectins, significantly lower concentrations of these fractions were found in all samples from abnormal pregnancies than in those from normal pregnancies. In eight cases with a fetal abnormality the total alpha-fetoprotein concentrations were below the lower limit for abnormal samples. All eight samples revealed fractions weakly reactive with *Lens culinaris* agglutinin below the lower 0.1% limit for normal samples; seven of these samples had fractions nonreactive with concanavalin A below this limit. Although a decrease in the two described fractions in normal pregnancies was found with increasing gestational age, we find either method to be valuable as an ancillary test in the prenatal diagnosis of fetal neural-tube defects and other malformations.

Measurement of alpha-fetoprotein (AFP) in amniotic fluid is currently accepted as the test of choice in the prenatal diagnosis of neural-tube defects (NTD), although it is agreed that additional tests are necessary to avoid misinterpretation of AFP values that are near the chosen cutoff level. Over the past two years several groups have described the potential value of AFP carbohydrate-microheterogeneity determinations for this purpose. AFP, a glycoprotein with a carbohydrate content of 4–5%, has been shown to consist of two fractions with respect to its affinity for the lectin concanavalin A (con A). The percentage of the fraction without affinity for con A (the "nonreactive" fraction) is significantly lower in amniotic fluid from pregnancies with an open NTD of the fetus than in fluid from normal pregnancies (1–7).

Other lectins have also been shown to reveal a carbohydrate-microheterogeneity of AFP. By use of *Lens culinaris* agglutinin (LCA), AFP can be separated into three distinct fractions (8, 9). However, to our knowledge the affinity of AFP for LCA has not been studied in the diagnosis of NTD. Our purpose here was to investigate the two reactive and the nonreactive fraction in this context, and to compare the results with our earlier findings for con A (6, 7). We gave special attention to samples from abnormal pregnancies in which the total AFP concentration was below the upper limit for normal samples, i.e., those giving a falsely negative result.

Materials and Methods

Amniotic fluid samples were obtained by amniocentesis in the second trimester of pregnancy. Samples were stored at –20 °C until analysis.

The total concentration of AFP was determined by rocket immunelectrophoresis (10). The mean and standard deviation for each week were generated from data from 1281 normal pregnancies.

The con A nonreactive fraction of AFP has been evaluated in 519 samples from normal pregnancies and the results are published in detail elsewhere (6, 7). LCA reactivity was determined for 135 of these samples. In the case of nine patients, two samples were obtained at different gestational ages, and both samples were included in the study of LCA reactivity. Results of con A crossed-affinity immunelectrophoresis on similar samples have been reported elsewhere (6). All of the above-mentioned samples were included, irrespective of the total AFP concentration.

In addition, 42 samples from pregnancies with an abnormal outcome were studied. These included 30 cases of fetal NTD, five cases of omphalocele/gastroschisis, three cases of intra-uterine fetal death, and four other cases (congenital nephrosis, sacral tumor, epidermolysis bullosa). The concentration of total AFP and the percentage of the con A nonreactive fraction was determined in all samples, but because of insufficient material in three cases only 39 samples were investigated by LCA. Of the 42 samples, 36 were obtained between 15 and 22 weeks of pregnancy, which is the appropriate period for prenatal diagnosis of NTD.

Crossed-affinity immunelectrophoresis was performed in a 1.5-mm-thick 10 g/L agarose gel at pH 8.6. Samples were diluted to a concentration of approximately 25 mg/L and 10 μL was applied. If the concentration was less than 25 mg/L, a correspondingly larger volume was applied.

The first-dimension electrophoresis was run at 5 V/cm in a gel containing, per liter, 3.81 × 10⁻⁵ mol of con A (Pharmacia Fine Chemicals, Uppsala, Sweden) or 0.64 × 10⁻⁹ mol of LCA (purified from lens seeds (11)) until a bromphenol blue-stained albumin marker had migrated 3.8 cm (con A) or 7 cm (LCA).

The second-dimension electrophoresis was run perpendicular to the first at 2 V/cm for at least 16 h into a gel containing 3.75 μL (0.56 μLCm⁻²) of anti-AFP antibody (DAKO Immunoglobulins Ltd., Copenhagen, Denmark) per milliliter. Between the first-dimension gel and the antibody-containing gel, a line of gel, 3 mm wide and containing AFP, was moulded before the second-dimension electrophoresis.

After the electrophoresis, the plate was dried and stained with Coomassie Brilliant Blue. The percentage distribution of fractions was determined by measuring the heights of the precipitates above the line precipitate.

Results

Within each week of the observed period the total concentration of AFP was found to follow a log normal distribution.

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Received June 16, 1982; accepted Sept. 20, 1982.
The mean, 95th percentile, and three, four, and five standard deviations above the mean (SDAM) were calculated accordingly (Figure 1). Of the 36 samples obtained between 15 and 22 weeks of gestation, eight were found to have AFP concentrations between three and five SDAM.

Crossed-affinity immunoelectrophoresis of AFP with LCA in the first-dimension gel revealed three fractions of AFP: a nonreactive, a weakly reactive, and a strongly reactive fraction. With con A, two fractions were seen, a strongly reactive and a nonreactive fraction (Figure 2). It has been shown previously (9) that the con A nonreactive and the LCA weakly reactive fraction measured on the same sample are of approximately the same magnitude. There was considerable overlap among normal and abnormal samples for the LCA nonreactive and for the LCA strongly reactive fractions (not shown). Thus these fractions are not considered any further.

The normal range for the LCA weakly reactive fraction of AFP, calculated from 135 samples form normal pregnancies and the values found in the samples from abnormal pregnancies, is shown in Figure 3. All eight samples with a total AFP concentration below five SDAM were found to have a LCA weakly reactive fraction that was below the 0.1% lower limit.

Determination of the normal range for con A non-reactive AFP is described in detail elsewhere (6). Values for the con A nonreactive AFP in the abnormal samples in relation to the normal range are illustrated in Figure 4. All eight samples with a total AFP concentration below five SDAM had a con A nonreactive fraction less than the calculated 1% lower limit. In seven cases the values were below with 0.1% lower limit.

The con A nonreactive and the LCA weakly reactive fractions correlated strongly, with nearly a 1:1 relationship between the two \( r = 0.95, y = 1.44 + 1.00x \) (Figure 5).

A 2.2% per week decrease in the con A nonreactive fraction of AFP in serial samples has been reported elsewhere (6). Similarly, in nine patients from whom two samples were obtained at different gestational ages, the LCA weakly reactive fraction was observed to decrease by an average of 2.3% per week between 15 and 19 weeks of gestation.

**Discussion**

In the present study we have chosen five SDAM as the upper limit for normal concentrations of AFP in amniotic fluid. This cutoff level is recommended in some studies on amniotic fluid AFP in the prenatal diagnosis of NTD (12, 13). The concept of multiples of medians as cutoff limits has been recommended by others (14). However, no matter how the upper limit for normal values is determined, concentrations of AFP found in samples from normal pregnancies and those found for pregnancies with a malformation of the fetus overlap (15). With a chosen upper limit of five SDAM, a relatively large proportion of our samples from abnormal pregnancies fell between three and five SDAM, i.e., below the upper limit for normal samples.

By applying the lectin affinity immunoelectrophoresis to these samples, a definite diagnosis of a fetal anomaly could be made in almost all cases. With the use of con A, values below the strict lower limit of 0.1% were found in seven of eight cases, and even in the remaining sample, the value was below the 1% lower limit, strongly suggesting a fetal anomaly and indicating the necessity of further assessment.

With LCA, the weakly reactive fraction is the one of clinical interest. In all eight cases, values for it were below the lower 0.1% limit.

It is not possible, from the available data, to decide which method is to be preferred. There appears to be a slightly better discrimination between normal and abnormal samples with the use of LCA, in particular if only the eight samples with equivocal results on the total AFP concentration are considered. However, the LCA crossed-affinity immuno-electrophoresis is technically more difficult to perform, and great care has to be taken to secure a separation of AFP into three distinct precipitates (16). Still, the reproducibility (CV) of both methods is the same, approximately 6% (see ref. 16). The difference in the number of samples used for determining the normal ranges for the two lectins may also have influenced the results to some extent.

There was a very strong positive correlation between results by the two methods. Although the specificity of these two lectins is considered to be the same with respect to affinity to monosaccharides (α-D-mannose and glucose), they
demonstrably possess the ability to recognize fine differences in more complex carbohydrate structures (17). It is therefore tempting to speculate that the LCA weakly reactive fraction and the con A strongly reactive fraction in fact may be "complementary," given the very strong negative correlation between them. For clinical purposes, however, the LCA weakly reactive and the con A nonreactive fractions of AFP are the fractions of interest.

In agreement with others (4), we found (6) a decrease in the con A nonreactive fraction of AFP with advancing gestational age; in this study we also found a similar decrease in the LCA weakly reactive fraction of AFP. The implication of these findings is that, with advancing gestational age, interpretation of a low con A nonreactive/LCA weakly reactive fraction becomes increasingly difficult. Still, misinterpretation of results is rather unlikely. If an apparently low fraction is found in a normal pregnancy with an underestimated gestational age, the total AFP concentration is likely to be low and thus not lead to any suspicion of an anomaly. Likewise, if an apparently high total-AFP concentration is found in a normal pregnancy with an overestimated gestational age, the probable finding of a high fraction will exclude the presence of a fetal malformation.

It is generally agreed today that any laboratory performing analyses of amniotic fluid AFP in the prenatal diagnosis of NTD should have access to additional tests, because the overlap in values from normal and abnormal pregnancies inevitably leads to equivocal interpretation. We have found the determination of heterogeneous fractions of AFP useful in this context. With the increasing demand for more selective diagnoses, the role of lectin crossed-affinity immunoelectrophoresis of AFP in the prenatal diagnosis of NTD remains to be seen because, like determination of total AFP, is unable to discriminate between the different types of malformations.

This study was supported by Den Lægevidenskabelige Forskningsfond for Sønderjylland, Ringkøbing og Ribe Amt. The expert technical assistance of Mrs. Jette Rasmussen is gratefully acknowledged.

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