Comparison of Amniotic Fluid Alpha-Fetoprotein and Gel-Acetylcholinesterase Tests With Fetal Blood Contamination

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Abstract: *The addition of 5% fetal serum, equivalent to 1.5 ml fetal blood in 20 ml of amniotic fluid, can result in a false positive amniotic fluid AFP test. The qualitative AChE test requires the addition of more than 3 ml of fetal blood to be abnormal. We recommend a repeat amniocentesis whenever both tests are positive despite the presence of fetal blood, but this may be avoided when the presence of fetal blood appears to explain a slightly elevated AFP (≥+3 SD<+5 SD) and the AChE and sonogram are normal. (Thai J Obstet Gynaecol 1989; 1 :1-5)*

Key words: alpha-fetoprotein, acetylcholinesterase,fetal blood contamination

Neural tube defects (NTDs) include anencephaly, spina bifida and encephalocele, most of which can be diagnosed in the second trimester by two amniotic fluid (AF) tests, alpha-fetoprotein (AFP) assay and acetylcholinesterase (AChE) analysis. AChE can be measured quantitatively or analysed qualitatively by gel-electrophoresis[1-4].The latter is frequently used in combination with AFP assay[5], and a laboratory engaged in the diagnosis of NTDs should probably perform both tests[6].

Because the concentration of AFP in fetal serum is between 100 and 200 times higher than in AF, false positive AFP tests can result from fetal blood (FB) contamination. The AChE test appears to be less influenced by FB contamination [1,4,7,-11], although some reported false positive results have been attributed to this. Maternal blood contamination does not affect either the AFP or AChE test[10].

The present study was designed to compare the amount of FB contamination required to produce an elevated AF-AFP and a positive AChE gel - electrophoresis test.

Materials and Methods

Fetal blood was collected from the umbilical cord of a normal 24 weeks infant delivered prematurely because of an incompetent cervix. Clear AF was obtained from a 17 weeks gestation with a normal AFP level and AChE test. 1%, 5%, 10%, 25% and 50% dilutions of cord serum were made in AF.

AChE electrophoresis was carried out by disc technique as previously described[10]. Two tests were run on each sample; the first without and the second with the specific AChE inhibitor BW284C51. A positive result was defined as a clear band in front of the non-specific cholinesterase which disappears with addition of the specific inhibitor. Positive and negative controls were included.

AF-AFP was measured by radioimmunoassay using a double antibody technique[10].

Results

AF-AFP levels, the equivalent concentrations of FB and the effects of different amounts of FB contamination on the qualitative AChE test are shown in Table 1 and in Fig. 1. By the definition used, the AChE test was not positive until the concentration of FB exceeded 10%. However, a faint but specific band was seen at concentrations of 5% and 10%.

The 17 weeks gestation AF sample used as diluent contained 1.03 mg% AFP and was negative for AChE. AFP measured 9.84 mg% in the cord serum at 24 weeks gestation and the AChE was clearly positive (Fig. 1).

Table 1. AFP levels and AChE results at different concentrations of FB contamination

Fetal serum in %	Equivalent [1] FB in %	FB in 20 ml of AF in ml	AFP in mg% and (SD)	AChE result
1	1.5	0.3 [2]	1.09(<2SD)	0
5	7.5	1.5	1.91(+2-3SD)	±
10	15.0	3.0	3.29(>+5SD)	±
25	37.5	7.5	6.84(>+5SD)	±
50	75.0	15.0	8.64(>+5SD)	±

[1] Packed cell volume 33%
Mean FB count 2.8 x 10^9 rbc/ml

[2] 20 ml of AF contaminated with 1% FS contains 0.3 ml of FB or 8.4 x 10^8 rbc

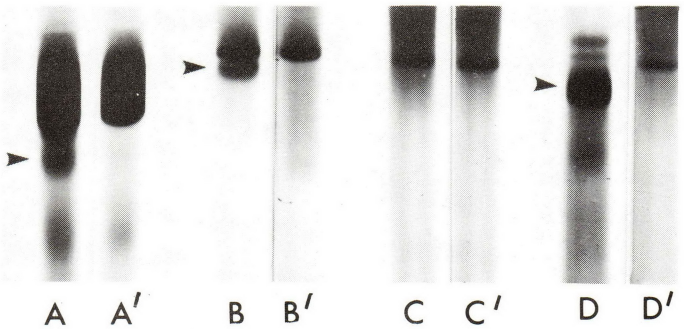

Fig 1 (a) AChE gel electrophoresis. AA'= cord blood, BB' = AF from open NTD, CC' = AF from normal 17 weeks pregnancy, DD' = AChE positive control.
In each pair the left hand tube contains no inhibitor and the right hand tube contains the specific inhibitor. ▲ denotes specific AChE band.

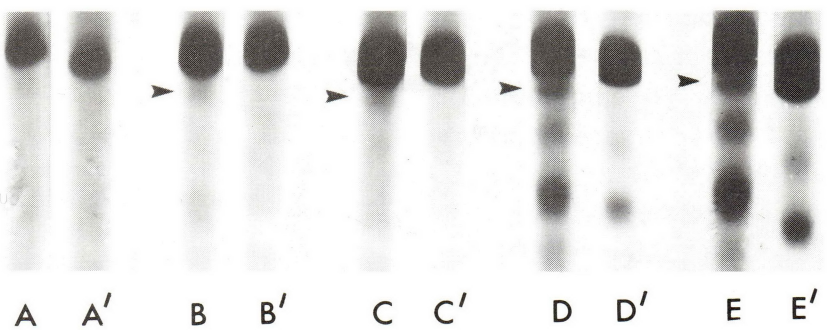

Fig. 1 (b) AChE gel electrophoresis. AA' = AF with addition of 1% cord serum, BB' = AF with addition of 5% cord serum, CC' = AF with addition of 10% cord serum, DD' = AF with addition of 25% cord serum, EE' = AF with addition of 50% cord serum. B and C show a faint specific band.

Discussion

Most fetal abnormalities which affect AF-AFP levels produce elevations which measure more than 5 SD above the mean. However, levels of between +3 and +5 SD are suggestive of some abnormality. In a recent analysis of 34,000 routine second trimester AF-AFP assays, the risk of a fetal abnormality was 22.6% when levels measured between +3 SD and +5 SD and 86% for those above +5 SD. Contamination with FB accounted for 36% of 72 false positives in the former group and 68% of 19 false positives in the latter group. A second test, less sensitive to FB contamination, could help identify these false positives, and in some instances might avoid a repeat amniocentesis. A qualitative AChE test using gel electrophoresis appears to fulfill this role and in our experience is much less affected by FB contamination than AFP. However, false positives have occurred with this test, usually with heavy FB contamination. In the series

reported above, we had 8 false positives with AChE, 7 apparently due to FB contamination.

In this study, an unequivocally positive AChE test occurred only with the addition of more than 10% FB. This was equivalent to more than 3 ml of FB in 20 ml of AF, an unlikely event since this amount to about 25% of the total fetal blood volume at 17 weeks gestation. At this level of contamination, the AF-AFP measured more than +5 SD above the mean. Additional dark bands in front of the specific AChE were also very suggestive of blood contamination (Fig. 1). The very faint band noted on the AChE gel with 5% and 10% FB contamination usually did not interfere with interpretation of results. All open NTDs produced a clear band which disappeared with addition of the specific inhibitor. A difference in the response of AFP and AChE to FB contamination would be even more apparent earlier in gestation because AF-AFP levels are about 20 times higher at 17 weeks compared with 24 weeks. Although AChE levels in AF at 17 weeks are not recorded, studies in other species, as well as higher levels in premature human gestations, show some decrease with gestational age, but this decline appears to be much less than for AFP.

From this study we conclude that repeat sonography and amiocentesis is indicated whenever the AF-AFP measures ≤+ 3 SD and the AChE test is positive, regardless of FB contamination. However, if the AF-AFP measures between +3 and + 5 SD above the mean, and the AChE is negative, a repeat amniocentesis may be avoided if FB is present, providing careful sonography excludes the presence of NTDs or other defects.

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