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Digoxin-Like Substance in Amniotic Fluid – Fact or Artifact?

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Key Words

Digoxin

Digoxin-like immunoreactivity

Amniotic fluid

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Abstract

A digoxin-like immunoreactive substance (DLIS) has been reported in the amniotic fluid. Since radioimmunoassay kits are standardized using serum-based standards, we hypothesized that measurement of DLIS may be an artifact related to the low protein content of amniotic fluid. We analyzed 12 amniotic fluid samples before and after supplementation with lyophilized human serum. The means $\pm$ SDs for DLIS (nmol/l) at protein concentrations of 0, 32 and 63 g/l were 1.4 ± 0.16 , 0.6 ± 0.09 , and 0.4 ± 0.09 nmol/l, respectively. We, therefore, hypothesize that DLIS in amniotic fluid may in part be explained by a technical artifact.

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Introduction

A digoxin-like immunoreactive substance (DLIS) has been reported in the serum of neonates and in the serum and amniotic fluid of pregnant women [1–3]. The identity of DLIS is currently unknown, but its presence may complicate the interpretation of serum digoxin levels in pregnant women receiving digoxin therapy [2].

During the course of our studies on DLIS in various clinical conditions, we measured DLIS in amniotic fluid using a radioimmunoassay kit (Nuclear Medical Laboratories, Irving, Tex.). This product has been shown previously to give high values of DLIS in both amniotic fluid and neonatal sera [3]. Since radioimmunoassay kits are calibrated with serum-based standards (protein concentration 60–80 g/l), we hypothesized that the measurement of DLIS may be an artifact related to low protein concentrations in amniotic fluid.

Materials and Methods

Twelve amniotic fluid samples submitted to our laboratory for fetal lung maturity testing were used in the study. The mean gestational age was 37 weeks (range: 30–40 weeks). Testing for DLIS was carried out according to the manufacturer's instructions. To assess the matrix effect due to low levels of protein in

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amniotic fluid, we also measured digoxin in each fluid after supplementation with lyophilized normal human serum (Validate, General Diagnostics, Morris Plains, N.J.). The detection limit

(sensitivity) of the assay is 0.26 nmol/l and the interassay precision (coefficient of variation) is 7.3, 2.9 and 3.7 % at digoxin levels of 0.7, 2.9 and 4.7 nmol/l, respectively.

Results

The results are summarized in table I. Amniotic fluids assayed directly without protein supplementation contained apparent digoxin levels of 1.4 ± 0.16 nmol/l (mean $\pm$ SD). However, values of DLIS dropped to 0.6 ± 0.09 nmol/l at a protein concentration of 32 g/l, and to 0.4 ± 0.09 nmol/l at 63 g/l protein ($p < 0.01$). Also of note is the 'digoxin' value of 2.0 nmol/l seen with normal saline.

Table I. Concentration of DLIS in amniotic fluid with and without protein supplementation

Discussion

The digoxin kit supplied by Nuclear Medical Laboratories is calibrated with serum-based standards containing protein concentrations similar to human serum (60–80 g/l). The fact that protein is essential in the assay is illustrated by assaying normal saline which gives a 'digoxin' result of 2.0 nmol/l. This false result is eliminated by the addition of protein at a level of 63 g/l (table I). Likewise, most of the apparent DLIS estimated in amniotic fluid is eliminated by protein supplementation.

In summary, we have shown that DLIS in amniotic fluid can be reduced substantially by supplementing the fluid with human serum proteins. This technical artifact should be taken into consideration in the interpretation of results of DLIS in amniotic fluid.

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