AMNIOTIC FLUID AND MATERNAL SERUM LEVELS OF CA125 IN PREGNANCIES AFFECTED BY DOWN SYNDROME: A RE-EVALUATION OF THE ROLE OF CA125 IN DOWN SYNDROME SCREENING

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SUMMARY

In a study of amniotic fluid from 91 Down syndrome cases and 240 controls, we have shown that the median value of CA125 in pregnancies affected by Down syndrome on the whole reflects those observed in the maternal serum from 106 affected cases and 238 controls. The median MOM for CA125 in amniotic fluid from Down syndrome pregnancies was 1.04 and in maternal serum 1.06, neither value being significantly different from that for the control population. We confirm our previous observation that CA125 is not a marker of Down syndrome and conclude that CA125 has no role to play in Down syndrome screening.

INTRODUCTION

Check et al. (1990a) first suggested the possibility that maternal serum CA125 levels might be predictive of an abnormal karyotype when they described elevated levels in three women who spontaneously aborted fetuses with chromosomal anomalies. Following this observation by Check et al., Spencer (1991) showed in a series of 25 second-trimester Down syndrome cases, that the maternal serum CA125 levels were not elevated above those of gestational and maternal age-matched controls. Furthermore, the study showed that maternal serum CA125 levels were increased in women in whom the pregnancy had ended in intrauterine death or in spontaneous abortion. Thus, Check et al.'s observation was most likely a result of the impending fetal death, rather than it being related to the chromosomal anomaly of the fetus. Since that time, further studies have been carried out by other groups which have been somewhat contradictory. Van Lith et al. (1991) initially showed low maternal serum CA125 levels in the early (9–10 weeks) gestation of nine pregnancies affected by Down syndrome, which when analysed as part of a larger study (Van Lith et al., 1993) of 29 Down syndrome cases between 9 and 18 weeks of gestation, showed no relation between maternal serum CA125 levels and Down syndrome, either in the first or in the second trimester. Norton and Golbus (1992), similarly analysing 26 aneuploid cases at 9–11 weeks' gestation, could also find no increase in maternal serum CA125 levels in such pregnancies. Van Blerk et al. (1992)
analysed maternal sera from ten cases of Down syndrome and gestational and maternal age-matched controls and found a lower [0·72 multiple of the median (MOM)] but not statistically different median level in Down syndrome cases compared with the controls. Van Blerk et al.‘s study also included the analysis of amniotic fluid CA 125 in nine pregnancies affected by Down syndrome and 109 control pregnancies. They observed three cases above the 95th centile and three cases either below or very close to the fifth centile, with the median being close to the 50th centile. They concluded that they could not demonstrate an association between Down syndrome and CA 125 levels in the second trimester. Hogdall et al. (1992), however, claimed that in studying 14 cases of Down syndrome and 61 controls in the first trimester, they found that maternal serum CA 125 was elevated and was a better marker thanAFP and hCG measured on the same samples. In the second trimester, they also claimed elevated levels of maternal serum CA 125 and suggested that improved detection could be obtained by using CA 125 in combination with AF P and hCG. Borri et al. (1993), in analysing CA 125 in amniotic fluid from 21 Down syndrome cases compared with 63 gestational age and maternal age-matched controls, found a significantly higher median of 1·41.

Considerable confusion therefore seems to exist in the literature as to the exact role, if any, of CA 125 in Down syndrome screening. In this study, we seek to re-examine the value of maternal serum CA 125 in a large series of second-trimester Down syndrome cases and to assess the levels of CA 125 in a similarly large series of amniotic fluid samples from affected cases, in an attempt to finally clarify the conflicting reports in the literature.

MATERIALS AND METHODS

Study population

The Paris set comprised amniotic fluid from 156 unaffected pregnancies spread across the 16th to 21st gestational week. The trisomy 21 samples included 77 samples within the gestational range 15–21 weeks. All samples had been stored as frozen aliquots at −20°C prior to being transported to the Romford testing centre on dry ice. The samples had been thawed once after arriving at the Romford centre for a previous study (Spencer et al., 1997). The majority of the affected cases had been offered amniocentesis on the basis of advanced maternal age (50/77). The range of sample storage to analysis time for the affected cases was 1–58 months (median = 20), and for the unaffected cases 1–10 months (median = 7).

The Glasgow set comprised amniotic fluid from 14 cases of trisomy 21 matched for gestational age, maternal age (± 1 year), and length of storage (± 3 months) with six amniotic fluid samples from unaffected pregnancies. Amniotic fluid samples had been stored as frozen aliquots at −20°C prior to being transported to the Romford testing centre on dry ice. The samples had been thawed once after arriving at the Romford centre for a previous study (Spencer et al., 1997). The majority of the affected cases had been offered amniocentesis on the basis of advanced maternal age (10/14). The range of maternal ages was from 20 to 40 years (median = 37) and gestational ages ranged from 16 to 22 weeks (median = 17). Gestational dating was by ultrasound in all cases. The range of sample storage to analysis time was 6–30 months (median = 12).

In total, there were 240 amniotic fluid samples from unaffected pregnancies and 91 samples from pregnancies with a trisomy 21 fetus.

The Oldchurch sample set consisted of maternal serum from 106 cases of Down syndrome collected during routine screening for neural tube defects and Down syndrome from hospitals within our catchment area during the period 1989–1996. Affected cases were confirmed either by second-trimester amniocentesis and karyotyping or by karyotyping after the birth of an affected infant. Samples had been stored as aliquots at −20°C prior to analysis for CA 125 and were not part of a previous CA 125 study (Spencer, 1991). In order to compare CA 125 in normal pregnancies, 238 samples from unaffected pregnancies collected between 14 and 20 weeks of gestation were retrieved from our frozen sample bank from samples collected over the same time period. All serum samples had previously been analysed prospectively for AFP and free beta hCG as part of our routine screening programme (Spencer and Carpenter, 1993). Amniotic fluid samples had been analysed in a previous study (Spencer et al., 1997) for AFP, total hCG, free beta hCG, and unconjugated oestriol (U E 3).

Analytical methods

Amniotic fluid CA 125 was measured in duplicate using the ELISA-CA 125 (CIS U.K. Ltd, High Wycombe, U.K.) immunoradiometric assay.
Samples were diluted 1 in 50 in assay diluent before analysis. The samples were analysed over five assays and the within-assay precision was better than 3 per cent at 5 U/ml, 1·5 per cent at 20 U/ml, and 5 per cent at 200 U/ml. Between-assay precision was better than 4 per cent at 5 U/ml, 5 per cent at 20 U/ml, and 6 per cent at 200 U/ml.

Maternal serum CA125 was measured using the Diagnostic Products Corporation Immulite automated chemiluminescent immunoassay system and the OM-MA method (Euro/DPC, Llanberis, U.K.). The samples were all analysed within the same day and the within-day precision of control samples run across the working day was 6·5 per cent at 10 U/ml, 5·5 per cent at 25 U/ml, and 8·7 per cent at 72 U/ml.

Statistical analysis

Results for each analyte were expressed in multiples of the median (MOM) for unaffected pregnancies of the same gestational age, derived from the regressed weighted log10 medians for each analyte when appropriate and from the overall control population median when no gestational analyte variation was observed. Statistical analysis of the data was performed using A stute, a statistical software add-in for Microsoft Excel 5 (DDU Software, University of Leeds, U.K.).

RESULTS

Table I shows the observed and regressed median data for amniotic fluid CA125 and the observed medians for maternal serum CA125 across the second-trimester period. For the amniotic fluid, the median values showed a statistically significant increase (P<0.01, Mann–Whitney U-test) across the second-trimester period, whilst for maternal serum there was no significant change (P>0.05) across this period. The overall control population median in maternal serum was 9·35 U/ml, being some 300- to 600-fold higher in amniotic fluid.

In the trisomy 21 pregnancies, the median maternal serum CA125 was 1·06; this was not significantly different from the 0·99 observed in the control group (P=0.3936, Mann–Whitney U-test). The tenth to 90th centile for the trisomy 21 pregnancies was 0·56–2·73 (log10SD was 0·3222), and for the controls 0·48–1·84 (log10SD was 0·1555). An examination of the effect of fetal sex on the median MOM in amniotic fluid for the affected pregnancies showed a female:male ratio of 1:15:1:01 and for comparison, a similar analysis for the control population showed a ratio of 1:00:0:98. A Mann–Whitney U-test showed a P value of 0·3824 and 0·6106, respectively, indicating no statistically significant difference between the presence of a male or female fetus.

<table>
<thead>
<tr>
<th>Gestation (weeks)</th>
<th>Amniotic fluid</th>
<th>Maternal serum</th>
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<tbody>
<tr>
<td></td>
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<td>Observed</td>
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<td>15</td>
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Kolmogorov–Smirnov tests of goodness-of-fit to a Gaussian distribution was demonstrated for both amniotic fluid and serum CA125 \( (P < 0.01) \). Maternal serum CA125 was not correlated with maternal age \( (r = 0.0608) \) or with maternal weight \( (r = 0.0005) \).

Women in the control group were asked to self-indicate whether they consider themselves to be smokers or non-smokers. Previous studies have shown that self-indication of smoking status is a very reliable method of smoking assignment (Spencer, 1993). When the control population was analysed by smoking status, the maternal serum CA125 levels were found to be significantly lower \( (P = 0.0397, \text{Mann–Whitney } U\text{-test}) \) in the smoking group, with a median of 0.79 compared with 1.02 in the non-smoking group.

**DISCUSSION**

CA125 levels in amniotic fluid of normal pregnancies are approximately 300–600 times higher than in maternal serum at the same gestation. Unlike in maternal serum where levels are constant across the second trimester, as shown previously...
Clear that maternal serum CA125 levels were not elevated in the Down syndrome group. Clearly the data of Hognall et al. (1992) are at odds with our larger series, as is the study of Borri et al. (1993) with respect to amniotic fluid. Our new large study of CA125 in amniotic fluid and second-trimester maternal serum reconfirms our original view that CA125 is not a marker of Down syndrome and that the initial observation of Check et al. (1990a) was confounded by fetal death or impending fetal death, which has been shown to cause an increase in the level of maternal serum CA 125 (Check et al., 1990b; Spencer, 1991; Ocer et al., 1992).

REFERENCES


