**ADAM12s in maternal serum as a potential marker of pre-eclampsia**

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**Objective** To examine whether maternal serum ADAM12s, a potential first- and second-trimester marker of fetal aneuploidy and fetal growth, had altered concentrations in the first or second trimester of pregnancies subsequently developing pre-eclampsia.

**Methods** ADAM12s was measured by a time-resolved fluoroimmunoassay developed by PerkinElmer Life Science. Maternal serum samples from women taking part in early first-trimester aneuploidy screening in whom the pregnancy resulted in pre-eclampsia (64) were identified from a cohort of 4390 singleton pregnancies in which uterine artery Doppler mean Pulsatility Index (PI) had been measured at 22–24 weeks. From amongst those cases delivering a normal term infant with birth weight greater than the 10th centile for gestational age 240 cases were selected as gestational age-matched controls. A second study group consisting of maternal serum taken at 22–24 weeks at the time of uterine artery Doppler in a group of 12 women developing pre-eclampsia were compared with 86 matched controls from a previously studied cohort of 24 cases and 144 controls. Serum ADAM12s concentrations were converted to multiple of the median (MoM) to take account of gestational age variation.

**Results** First-trimester maternal serum ADAM12s levels in women who developed pre-eclampsia were reduced with a median MoM of 0.71 which was further reduced in those delivering prior to 35 weeks (0.50). At the 5th centile of normal (0.48 MoM) ADAM12s identified 27% of cases with pre-eclampsia and 47% of those with early pre-eclampsia. Combining ADAM12s with PAPP-A from a previous study only resulted in a further 1% increase in detection of all women developing pre-eclampsia. However combining ADAM12s with mean PI increased the detection rate to 66%. In the second trimester at 22–24 weeks the maternal serum ADAM12s levels were increased in those women developing pre-eclampsia compared to controls (709 vs 486 ug/L, p = 0.045).

**Conclusion** ADAM12s in addition to being a potential marker of aneuploidy may also be a marker of pre-eclampsia. Further studies are required to see if this can improve on the clinical discrimination already provided by PAPP-A in the early first trimester. Copyright © 2008 John Wiley & Sons, Ltd.

**KEY WORDS:** screening; first trimester; aneuploidy; Doppler

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**INTRODUCTION**

Pre-eclampsia occurs in about 2% of pregnancies and is a major cause of maternal death worldwide. The underlying cause of pre-eclampsia is thought to be a circulatory maladaptation characterised by defective trophoblast invasion (Lyall, 2002). The consequent increased resistance in the uteroplacental circulation forms the basis of screening for the condition, by uterine artery Doppler measurement. Maternal serum samples from women taking part in early first-trimester aneuploidy screening in whom the pregnancy resulted in pre-eclampsia (64) were identified from a cohort of 4390 singleton pregnancies in which uterine artery Doppler mean Pulsatility Index (PI) had been measured at 22–24 weeks. From amongst those cases delivering a normal term infant with birth weight greater than the 10th centile for gestational age 240 cases were selected as gestational age-matched controls. A second study group consisting of maternal serum taken at 22–24 weeks at the time of uterine artery Doppler in a group of 12 women developing pre-eclampsia were compared with 86 matched controls from a previously studied cohort of 24 cases and 144 controls. Serum ADAM12s concentrations were converted to multiple of the median (MoM) to take account of gestational age variation.

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In the third trimester Gack et al. (2005) compared gene expression profiles from controls and pre-eclamptic tissue and found that ADAM12s represented the most upregulated transcript.

The aim of this present study was to evaluate the potential of ADAM12s in the first or second trimester to identify women who subsequently develop pre-eclampsia.

**METHODS**

Maternal serum samples from women taking part in early first-trimester aneuploidy screening (Spencer et al., 2003) in whom the pregnancy resulted in pre-eclampsia (64) were identified from a previously published cohort of 4390 singleton pregnancies in which uterine artery Doppler mean PI had also been measured at 22–24 weeks (Spencer et al., 2005). Women had given written informed consent for surplus blood taken for routine diagnostic procedure to be used for regulated research approved by our Institution Review Board. Pre-eclampsia was defined according to the guidelines of the International Society for the Study of Hypertension in Pregnancy (Brown et al., 2001). The pre-eclamptic group were further classified as early onset (n = 34) or late onset (n = 30) based on whether the case required delivery before 35 weeks (early onset). From amongst those cases delivering a normal term infant with birth weight greater than the 10th centile for gestational age, 240 cases were selected as gestational age-matched controls. PAPP-A and free β-hCG levels were available from routine first-trimester testing using the Kryptor analyser (Brahms AG, Berlin, Germany). Table 1 summarises the maternal characteristics of the two groups. All samples had been stored as aliquots and had not previously been thawed.

A second study group consisting of maternal serum samples taken at 22–24 weeks at the time of uterine artery Doppler in a group of 24 women developing pre-eclampsia were compared with 144 matched controls. This cohort has previously been described (Spencer et al., 2006). Of this cohort, 12 cases of pre-eclampsia and 86 controls had sufficient volume of serum for analysis of ADAM12s.

Table 1—Maternal characteristics and marker levels in the control group and the pre-eclamptic group in the first trimester

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Pre-eclampsia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>240</td>
<td>64</td>
</tr>
<tr>
<td>Mean maternal age</td>
<td>30</td>
<td>29</td>
</tr>
<tr>
<td>(years)</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>Caucasian</td>
<td>95%</td>
<td>86%</td>
</tr>
<tr>
<td>Cigarette smoker</td>
<td>15%</td>
<td>16%</td>
</tr>
<tr>
<td>Nulliparous</td>
<td>40%</td>
<td>50%</td>
</tr>
<tr>
<td>Median free β-hCG</td>
<td>1.00</td>
<td>0.92</td>
</tr>
<tr>
<td>MoM at 11–14 weeks</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>Median PAPP-A MoM at</td>
<td>1.00</td>
<td>0.84</td>
</tr>
<tr>
<td>11–14 weeks</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>Uterine artery mean PI at 22–24 weeks</td>
<td>1.02</td>
<td>1.56</td>
</tr>
</tbody>
</table>

Maternal serum ADAM12s was measured by a research time-resolved fluorimmunoassay developed along similar lines to that described by Laigaard et al. (2005b) with the exception that the final biotin-streptavidin-europium detection step was replaced by a direct europium labelling of the 8F8 second monoclonal (PerkinElmer Life Science, Turku, Finland). The assay was performed in a manual form as described by Cowans and Spencer (2007) with the analysis done in duplicate with the assayist blinded to pregnancy outcome. The final fluorescence was read with a Victor 1420 fluorimeter (PerkinElmer Life Science, Turku, Finland). A standard curve was created from dilutions of a third-trimester serum pool calibrated against recombinant ADAM12s and the results of the unknowns interpolated from the standard curve. Samples with a coefficient of variation in the duplicates worse than 10% (amounting to 35 samples—less than 10% of the total samples analysed) were scheduled for repeat analysis.

ADAM12s concentrations were expressed as MoM in normal pregnancies by performing a weighted linear regression of median ADAM12s concentration against gestational age in days to derive gestational day–specific medians. The ADAM12s concentrations in the cases were similarly converted to MoM using the derived normal medians at each gestation. The control group and the pre-eclamptic group were compared using non-parametric Mann–Whitney tests of the median MoMs with a probability of <0.05 denoting significance.

To assess the potential value of combining ADAM12s with other biomarkers such as PAPP-A and mean Pulsatility Index (PI) we used the distribution of these markers in pre-eclamptic cases and controls from a previous study (Spencer et al., 2005) and the distribution of ADAM12s in cases and controls from this study. We used standard statistical modelling techniques to compare detection rates using either marker alone or in combination and assessed the performance using Receiver Operator Characteristic (ROC) curves.

**RESULTS**

ADAM12s showed an increase in concentration across the gestational window 78–97 days. The relationship could be described by the regression equation $y = 13.785x - 738.71$ with a correlation coefficient of 0.319 ($p < 0.0001$) (Figure 1). The overall median MoM in the first-trimester pre-eclamptic group was significantly reduced to 0.712 MoM (95% Confidence Interval 0.573–0.815; $p < 0.0001$) whilst in the early pre-eclamptic group this was even further reduced to 0.498 (95% Confidence Interval 0.388–0.597; $p < 0.0001$). Figure 2 shows the box and whisker plots of ADAM12s MoM in controls, all pre-eclamptic cases and those delivering early. $Log_{10}$ ADAM12s MoM fitted a Gaussian distribution as assessed by the Shapiro-Wilk test with a probability of $p < 0.05$. The $Log_{10}$ standard deviation in the control group was 0.1768 and in the pre-eclamptic group 0.2417. The 5th centile of normal was 0.4819 MoM and at this cut-off 16 of 34 (47%) of early
cases would have been identified compared with 17/64 (26.6%) of all pre-eclamptic cases. ADAM12s was significantly correlated with PAPP-A MoM in the controls ($r = 0.364$) and also amongst the pre-eclamptic cases ($r = 0.382$) but was not correlated with free $\beta$-hCG in controls ($r = 0.055$) or cases ($r = 0.109$).

When we investigated the potential value of ADAM12s compared with other potential biomarkers either alone or in combination using ROC curves we found that the area under the curve increased from 0.584 with PAPP-A to 0.694 with ADAM12s and 0.714 with both combined. At a 5% false-positive rate the addition of PAPP-A to ADAM12s increased detection by only 1%. However when ADAM12s was combined with mean PI, the area under the curve increased to 0.881. At a 5% false-positive rate the detection rate for this combination was 66%. The respective ROC curves are shown in Figure 3.

In the second-trimester pregnancies at 22–24 weeks there was no noticeable variation in ADAM12s concentration across this short gestational window. The median ADAM12s in controls was 486 ug/L with 5th and 95th centiles of 241 and 1137 ug/L, respectively. In the cases with pre-eclampsia the median ADAM12s was 709 ug/L and this was significantly higher by Mann–Whitney test ($p = 0.045$); expressed as MoM this was equivalent to a median MoM of 1.49. Figure 4 shows the box and whisker plots of ADAM12s in controls and pre-eclamptic cases.

**DISCUSSION**

The results of this study have confirmed that in the first trimester, levels of ADAM12s are reduced in pregnancies that will subsequently go on to develop pre-eclampsia. In those cases that developed severe pre-eclampsia necessitating early delivery (prior to 35 weeks), the levels of ADAM12s are even lower. In this study the size of the reduction was more than seen in the study by Laigaard et al. (2005b) and also more than seen in the same sample series for the marker PAPP-A (Spencer et al., 2005). Combining ADAM12s with PAPP-A did not improve detection significantly although combining ADAM12s with mean PI from uterine artery Doppler measurements made at 22–24 weeks resulted in an increased detection to 66% which was 4% higher than when PAPP-A was used (Spencer et al., 2005).
We have also confirmed that ADAM12s levels increase during the first trimester mirroring those in our previous publication with this same assay (Cowans and Spencer, 2007) and contrary to those observed in a previous study with a different assay (Laigaard et al., 2006b). We also found a significantly narrower standard deviation of log_{10} MoM ADAM12s in the control group which was half that of a previous study (0.3734, Laigaard et al., 2006b) and which was in agreement with that seen in a previous study (0.1735, Cowans and Spencer, 2007) using the new ADAM12s method. Whether this tightening of standard deviation is related to method improvement or whether it reflects an issue of instability of ADAM12s in previous studies (Cowans and Spencer, 2007) remains to be ascertained. The dotted-line connects the nearest observations within 1.5 IQRs (inter-quartile ranges) of the lower and upper quartiles. The crosses (+) indicate possible outliers.

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REFERENCES


