Inhibin A, activin A, placental growth factor and uterine artery Doppler pulsatility index in the prediction of pre-eclampsia

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KEYWORDS: activin A; inhibin A; PlGF; pre-eclampsia; screening; uterine artery Doppler

ABSTRACT

Objectives To evaluate whether the measurement of maternal serum inhibin A, activin A and placental growth factor (PlGF) at 12 + 0 to 16 + 0 weeks of gestation alone or in combination with second-trimester uterine artery Doppler pulsatility index (PI) is useful in predicting pre-eclampsia.

Methods This was a case–control study of pre-eclampsia. From pregnant women attending their first antenatal examination at 12–16 weeks we collected serum samples and stored them at −80 °C. All patients also underwent uterine artery Doppler examination to measure the PI at 22–24 weeks’ gestation. We retrieved for analysis frozen samples from women who then developed pre-eclampsia, as well as three control samples per woman, matched for gestational age and storage time. Inhibin A, activin A and PlGF were measured using an enzyme-linked immunosorbent assay (ELISA) by an examiner who was blinded to the pregnancy outcome.

Results There were 31 cases with pre-eclampsia and 93 controls. Second-trimester uterine artery PI and marker levels were expressed as multiples of the median (MoM). The uterine artery PI was increased in pregnancies with pre-eclampsia compared with controls (mean ± SD, 1.45 ± 0.31 MoM vs. 1.02 ± 0.23 MoM, P < 0.01), as were the level of inhibin A (mean ± SD, 1.57 ± 0.34 MoM vs. 1.06 ± 0.42 MoM, P < 0.001) and the level of activin A (mean ± SD, 1.68 ± 0.38 MoM vs. 1.06 ± 0.42 MoM, P < 0.001). The level of PlGF was decreased in pre-eclampsia compared with controls (mean ± SD, 0.69 ± 0.23 MoM vs. 1.00 ± 0.26 MoM, P < 0.001). Receiver–operating characteristics curves were analyzed for controls and cases and areas under the curve (AUC) were 0.796 (95% CI, 0.712–0.880, P < 0.001) for inhibin A, 0.823 (95% CI, 0.746–0.899, P < 0.001) for activin A, 0.831 (95% CI, 0.752–0.910, P < 0.001) for PlGF and 0.851 (95% CI, 0.783–0.920, P < 0.001) for uterine artery PI. The combination of activin A, inhibin A and PlGF using logistic regression analysis yielded an AUC of 0.907 (95% CI, 0.830–0.938, P < 0.001) with a sensitivity of 82% and a specificity of 80%. The combination of activin A, PlGF and PI gave an AUC of 0.925 (95% CI, 0.852–0.978, P < 0.001) with a sensitivity of 90% and a specificity of 80%. Combining all four markers gave an AUC of 0.941 (95% CI, 0.891–0.990, P < 0.001) with a sensitivity of 93% and a specificity of 80%.

Conclusion Early second-trimester serum inhibin A, activin A, PlGF and second-trimester uterine artery Doppler PI may add further information for the prediction of pre-eclampsia. The combination of the three serum markers and uterine artery Doppler PI has the highest prediction value for pre-eclampsia. Copyright © 2011 ISUOG. Published by John Wiley & Sons, Ltd.

INTRODUCTION

Pre-eclampsia is a pregnancy-associated disorder characterized by hypertension and proteinuria. It affects 5–8%1,2 of pregnant women and is associated with preterm labor and fetal growth restriction (FGR). Inadequate trophoblast invasion of the maternal spiral arteries during early gestation is thought to contribute to its etiology3,4. Campbell first introduced color Doppler ultrasound to investigate the uteroplacental circulation5. Uterine artery pulsatility index (PI) has been used as a marker of pre-eclampsia6 and FGR, in the presence of...
which it increases because of the elevation in uterine artery impedance\textsuperscript{7,8}.

Pre-eclampsia is also associated with a decreased level of serum placental growth factor (PlGF)\textsuperscript{9–13,19}, as well as increased levels of inhibin A and activin A\textsuperscript{14–16}.

The objective of this study was to evaluate whether the measurement of maternal serum inhibin A, activin A and PlGF at 12 + 0 to 16 + 0 weeks’ gestation or a combination of these biochemical markers with the second-trimester uterine artery PI are useful in predicting pre-eclampsia.

METHODS

This was a case–control study carried out between June 2007 and March 2008 in women presenting for routine antenatal care at 12 + 0 to 16 + 0 weeks. The couples were all Chinese. Blood samples were taken, centrifuged to extract the serum and stored at −80°C for subsequent analysis. Ethics committee approval was obtained from Shanghai First maternity and infant hospital and women gave their informed consent before samples were taken.

In cases in which no major fetal defect was detected, women were invited to undergo an additional screening study for pre-eclampsia and undergo Doppler measurement of the uterine artery PI at 22–24 weeks, performed by experienced sonographers who had obtained The Fetal Medicine Foundation’s certificate of competence in placental and fetal Doppler. Uterine artery PI was calculated as the mean PI from three similar consecutive waveforms\textsuperscript{14,17,18,20}. All examinations were carried out transabdominally using a C3–5 mHz curvilinear transducer (Envisor 2540A, Philips Medical Systems, ShenYang, China).

Pre-eclampsia was defined as systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg on at least two occasions 4 h apart, developing after 20 weeks of gestation in previously normotensive women with proteinuria of 300 mg or more in 24 h, or two readings of at least +1 on dipstick analysis of midstream catheter urine specimens if 24-h urine collection was not available\textsuperscript{2}.

Blood samples were retrieved from those women who developed pre-eclampsia, and three gestational age-matched normal pregnancies were used as controls for each case. The samples were tested for inhibin A, activin A and PlGF levels using solid-phase sandwich enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Minneapolis, MN, USA) by technicians who were blinded to the clinical outcome. According to the manufacturer, fresh quality-control samples were used at concentrations of 50 and 1000 pg/mL for PlGF, 50 and 1000 pg/mL for inhibin A and 50 and 2500 pg/mL for activin A. The interassay coefficients of variation for the respective low and high concentrations were 5.64% and 7.96% for PlGF, 6.89% and 11.56% for inhibin A and 4.27% and 9% for activin A.

Statistical analysis

The inhibin A, activin A and PlGF results were expressed as multiples of the median (MoM)\textsuperscript{14,20,21}. Basic demographic characteristics including weight and height as well as serum inhibin A, activin A and PlGF results in pregnancies with pre-eclampsia and normal pregnancies were compared by unpaired t-test. The sensitivity and specificity for different cut-offs of each variable in detecting pre-eclampsia were calculated and depicted as receiver–operating characteristics (ROC) curves. Multiple logistic regression analysis was used to model the combination of inhibin A, activin A, PlGF and uterine artery PI. The statistical software package SPSS 17.0 (SPSS Inc, Chicago, IL, USA) was used for all data analysis.

RESULTS

We recruited 650 pregnant women to the study. Of these, 37 (5.7%) did not deliver at our hospital and were lost to follow-up, leaving a study cohort of 613 pregnancies. Thirty-one women developed pre-eclampsia, giving an incidence of 5.1%, consistent with the literature\textsuperscript{1}. There were 93 gestational age-matched normal pregnancies as controls.

At the time of blood sampling there was no statistically significant difference in maternal age, body mass index and gestational age, between pregnancies that developed pre-eclampsia and normal controls (Table 1). However, gestational age at delivery and birth weight were lower in pregnancies that developed pre-eclampsia compared with controls.

In pregnancies that developed pre-eclampsia compared with controls, the uterine artery PI was increased (mean ± SD, 1.45 ± 0.31 MoM vs. 1.02 ± 0.25 MoM, P < 0.001; Figure 1a), as was the level of inhibin A (mean ± SD, 1.57 ± 0.34 MoM vs. 1.08 ± 0.43, P < 0.001; Figure 2a) and the level of activin A (mean ± SD, 1.68 ± 0.38 MoM vs. 1.06 ± 0.42 MoM, P < 0.001; Figure 3a). In contrast, the level of PlGF was decreased in pregnancies that developed pre-eclampsia compared with controls (mean ± SD, 0.69 ± 0.23 MoM vs. 1.00 ± 0.26 MoM, P < 0.001; Figure 4a). Figures 1b, 2b, 3b and 4b show the ROC curves for each marker.

The ROC curves for combinations of markers in the prediction of pre-eclampsia are shown in Figure 5 and areas under the curves (AUC) are detailed in Table 2. Combining activin A, inhibin A and uterine artery PI using logistic regression analysis yielded an AUC of 0.907 (95% CI, 0.830–0.938; P < 0.001) with a sensitivity of 87% at a specificity of 80%. A combination of activin A, PlGF and uterine artery PI gave an AUC of 0.925 (95% CI, 0.852–0.978; P < 0.001) with a sensitivity of 90% at a specificity of 80%. The combination of all four markers gave an AUC of 0.941 (95% CI, 0.891–0.990; P < 0.001), with a sensitivity of 93% at a specificity of 80%.

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Prediction of pre-eclampsia

Table 1 Demographic characteristics in the pre-eclampsia study group and normal controls

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Cases</th>
<th>Controls</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>31</td>
<td>93</td>
<td>—</td>
</tr>
<tr>
<td>Primiparous</td>
<td>28</td>
<td>89</td>
<td>—</td>
</tr>
<tr>
<td>Maternal age (years)</td>
<td>28.17 ± 3.22</td>
<td>29.67 ± 3.71</td>
<td>0.05</td>
</tr>
<tr>
<td>Maternal BMI (kg/m²)</td>
<td>21.65 ± 2.67</td>
<td>20.43 ± 2.42</td>
<td>0.02</td>
</tr>
<tr>
<td>GA at blood sample (days)</td>
<td>105.00 ± 6.13</td>
<td>106.49 ± 5.06</td>
<td>0.18</td>
</tr>
<tr>
<td>Maternal peak BP (mmHg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>150.03 ± 8.10</td>
<td>122.33 ± 9.91</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diastolic</td>
<td>97.37 ± 4.64</td>
<td>73.15 ± 6.89</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>GA at delivery (weeks)</td>
<td>36.02 ± 2.14</td>
<td>38.52 ± 1.44</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>2765.00 ± 576.72</td>
<td>3243.55 ± 324.23</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data given as mean ± SD. There were no fetal deaths. BMI, body mass index; BP, blood pressure; GA, gestational age.

![Boxplots of uterine artery PI and inhibin A](image)

**Figure 1** (a) Second-trimester uterine artery pulsatility index (PI) in 93 normal controls and in 31 pregnancies which went on to develop pre-eclampsia. Boxes show median and interquartile range and whiskers represent 2.5th and 97.5th centiles. (b) Receiver–operating characteristics curve showing the clinical discrimination of PI for predicting pre-eclampsia.

**Figure 2** (a) Second-trimester inhibin A levels in 93 normal controls and in 31 pregnancies which went on to develop pre-eclampsia. Boxes show median and interquartile range, whiskers represent 2.5th and 97.5th centiles. (b) Receiver–operating characteristics curve showing the clinical discrimination of inhibin A for predicting pre-eclampsia.

**DISCUSSION**

The sensitivity was 76% with the specificity setting at 80% in detecting pre-eclampsia. It dropped slightly to 57% for a specificity of 90%. Consistent with the literature, our study showed an increased uterine artery PI during the late second trimester in patients who developed pre-eclampsia. Therefore the uterine artery PI is an important marker for the prediction of pre-eclampsia.

Inhibin A has been reported as an early marker in predicting pre-eclampsia. Salomon *et al.* took blood samples at 7–13 weeks’ gestation from 90 pregnant women, 30 who later developed with pre-eclampsia and 60 controls and found that inhibin A was increased five-fold in the women with established pre-eclampsia.

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Another study reported a sensitivity of inhibin A in the prediction of pre-eclampsia of 32% at a specificity of 90%, concluding that the sensitivity of inhibin A was too low for it to be useful as a marker for predicting pre-eclampsia, but that, combined with other markers, it could play an important role. Activin A has also been proposed as a marker for predicting pre-eclampsia. The level was elevated at 10–14 weeks’ gestation in women with established pre-eclampsia.

In a case–control study, the activin MoM was 1.79 ± 0.63 (P < 0.001) and the sensitivity was 82% at a specificity of 91%. Published data regarding PlGF in the prediction of pre-eclampsia is controversial. A sensitivity of 80.4% at a specificity of 78% has been reported. A prospective study documented that PlGF was decreased distinctly in early pregnancy in women who subsequently developed pre-eclampsia, and that inhibition of activin A and uterine artery PI or activin A, PlGF and uterine artery PI provided a test with high sensitivity and specificity that may be useful in predicting pre-eclampsia. Moreover, the combination of all three serum markers with uterine artery PI had an even higher prediction value.

A limitation of our study is that the population studied was all ethnic Chinese; further work is required to determine whether levels of these biochemical markers differ among different ethnic groups.

In conclusion, while it seems that the prediction of pre-eclampsia is technically feasible using inhibin A, activin A, PlGF and uterine artery Doppler, the cost–benefit balance of this screening needs to be evaluated. In order to accommodate the extra scans required to perform uterine artery Doppler, more qualified sonographers would need to be trained.

ACKNOWLEDGMENTS

We thank all colleagues in the hospital for their cooperation and help during this study.

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Table 2 Area under the receiver–operating characteristics curve (AUC) and sensitivity for each marker and combination of markers for the prediction of all cases of pre-eclampsia using logistic regression analysis with specificity set to 90% and 80%.

<table>
<thead>
<tr>
<th>Markers</th>
<th>AUC (95% CI)</th>
<th>P</th>
<th>Specificity 90%</th>
<th>Specificity 80%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inhibin A</td>
<td>0.796 (0.712–0.880)</td>
<td>&lt; 0.001</td>
<td>46</td>
<td>53</td>
</tr>
<tr>
<td>Activin A</td>
<td>0.823 (0.746–0.899)</td>
<td>&lt; 0.001</td>
<td>51</td>
<td>61</td>
</tr>
<tr>
<td>PlGF</td>
<td>0.831 (0.752–0.910)</td>
<td>&lt; 0.001</td>
<td>58</td>
<td>73</td>
</tr>
<tr>
<td>Uterine artery PI</td>
<td>0.851 (0.783–0.920)</td>
<td>&lt; 0.001</td>
<td>57</td>
<td>76</td>
</tr>
<tr>
<td>Activin A + uterine artery PI</td>
<td>0.852 (0.766–0.939)</td>
<td>&lt; 0.001</td>
<td>57</td>
<td>77</td>
</tr>
<tr>
<td>Inhibin A + uterine artery PI</td>
<td>0.813 (0.726–0.900)</td>
<td>&lt; 0.001</td>
<td>47</td>
<td>63</td>
</tr>
<tr>
<td>PlGF + uterine artery PI</td>
<td>0.880 (0.794–0.920)</td>
<td>&lt; 0.001</td>
<td>73</td>
<td>80</td>
</tr>
<tr>
<td>Activin A + inhibin A + uterine artery PI</td>
<td>0.907 (0.830–0.938)</td>
<td>&lt; 0.001</td>
<td>83</td>
<td>87</td>
</tr>
<tr>
<td>Activin A + PlGF + uterine artery PI</td>
<td>0.925 (0.852–0.978)</td>
<td>&lt; 0.001</td>
<td>84</td>
<td>90</td>
</tr>
<tr>
<td>Inhibin A + PlGF + uterine artery PI</td>
<td>0.840 (0.749–0.932)</td>
<td>&lt; 0.001</td>
<td>66</td>
<td>76</td>
</tr>
<tr>
<td>Activin A + inhibin A + PlGF + uterine artery PI</td>
<td>0.941 (0.891–0.990)</td>
<td>&lt; 0.001</td>
<td>90</td>
<td>93</td>
</tr>
</tbody>
</table>

Figure 5 Receiver–operating characteristics curves showing the clinical discrimination of different combinations of markers in predicting pre-eclampsia: activin A plus uterine artery PI (---); inhibin A plus uterine artery PI (----); placental growth factor (PlGF) plus uterine artery PI (_____); activin A plus inhibin A plus uterine artery PI (_____); activin A plus PlGF plus uterine artery PI (_____); inhibin A plus PlGF plus uterine artery PI (______); activin A plus inhibin A plus PlGF plus uterine artery PI (______). Areas under the curves are given in Table 2.

REFERENCES


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AQ3 Can I say instead that you enrolled Chinese women or do you want to specify the men were Chinese also? If so we can leave it as it is.

AQ4 ‘participate in’ changed to ‘undergo an additional’ – OK?

AQ5 ‘uterine artery’ added

AQ6 ‘later withdrew’ changed to ‘did not deliver at our hospital and were lost to follow-up’ – is this OK?

AQ7 Most figure legends: I’ve added numbers (93 and 30, following Table 1 – OK?) Also, ‘pregnancies which went on to develop pre-eclampsia’ added – OK? Also, should the y-axis have a unit of just MoM (is it just the line within the box that is the median?) or of Median MoM? Also, please define what the circle and * in the figures are.

AQ8 Note during the editing process there have been a lot of changes to the Discussion. Please read through the whole thing very carefully now it’s in proof

AQ9 ‘with’ changed to ‘who later developed’ – is this better?

AQ10 You missed a couple of my original queries here … Please could you clarify what was related to the severity of disease – the degree of decrease? Also, is ref 17 ok here – should it be just ref 18? These seem to be two different studies and ref 17 was mentioned in the previous sentence, which seems to be about a different study from this sentence.

AQ11 I’ve changed ‘a higher’ to ‘an even higher’ – are you happy with this?

AQ12 Is this the best place for the limitation? I wonder if it could go earlier somewhere.

AQ13 Page numbers changed from 641–642 to 672–683. Please check.

AQ14 References 19 to 33 have not been cited in the text. Please indicate where it should be cited; or delete from the Reference List and renumber the References in the text and Reference List.
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