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Maternal serum inhibin-A and activin-A levels in the first trimester of pregnancies developing pre-eclampsia

K. SPENCER*†, N. J. COWANS* and K. H. NICOLAIDES†

**Prenatal Screening Unit, Clinical Biochemistry Department, King George Hospital, Goodmayes and †Harris Birthright Research Centre for Fetal Medicine, King's College Hospital, London, UK*

KEYWORDS: activin-A; inhibin-A; PAPP-A; pregnancy hypertension; screening

ABSTRACT

Objective *To evaluate whether measurement of maternal serum inhibin-A and activin-A at 11 + 0 to 13 + 6 weeks of gestation, alone or in combination with second-trimester uterine artery pulsatility measured by Doppler velocimetry, is useful in predicting those women who will develop pre-eclampsia.*

Methods *This was a nested case-control study of pre-eclampsia cases with controls matched for gestational age and storage time for the maternal serum. Samples were collected as part of a first-trimester prenatal chromosomal anomaly screening program. Activin-A and inhibin-A were measured using a commercial enzyme-linked immunosorbent assay and the clinical outcomes were blinded to the operator. All the patients underwent uterine artery Doppler flow velocimetry to measure the mean pulsatility index at 22–24 weeks' gestation.*

Results *In total there were 64 cases with pre-eclampsia, with 34 delivering prior to 35 weeks of gestation. The control group included 240 cases. In the control group the levels of activin-A and inhibin-A did not change across the narrow gestational window and the median levels were 2.16 ng/mL and 231.13 pg/mL, respectively. In the pre-eclamptic group levels of activin-A and inhibin-A were significantly increased, at 2.52 ng/mL and 286.64 pg/mL (1.24 multiples of the median (MoM) and 1.17 MoM, respectively). There was no difference in the median MoM in those delivering prior to 35 weeks and those delivering later. At cut-offs of the 90th centile of normal, activin-A and inhibin-A levels would have identified 20% and 35%, respectively, of cases that would develop pre-eclampsia. When combined with uterine artery Doppler, activin-A measurement could have increased the detection rate from 55% to 63% and inhibin-A measurement could have increased it to 68% at a 5% false positive rate.*

Conclusion *Although increased in the first trimester, levels of activin-A and inhibin-A are probably too low to make a significant contribution to screening for pre-eclampsia at this time. Copyright © 2008 ISUOG. Published by John Wiley & Sons, Ltd.*

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 characterized by defective trophoblast invasion[1]. The consequent increased resistance in the uteroplacental circulation forms the basis of screening for the condition by uterine artery Doppler velocimetry[2]. Such screening at 20–25 weeks' gestation can identify 45% of pregnancies subsequently developing pre-eclampsia at a 5% false positive rate[2,3]. There is some evidence that the combination of certain biochemical markers measured either in the first trimester (e.g. pregnancy-associated plasma protein-A (PAPP-A[4,5])) or at the time of uterine artery Doppler measurement (e.g. activin or inhibin[6,7]) could potentially enhance the sensitivity of uterine artery Doppler measurement.

Preliminary studies of inhibin-A[8] and activin-A[9] in the first trimester have shown increased levels in pregnancies developing pre-eclampsia in the third trimester. In this study we extend our earlier work with these two markers to a further series of pregnancies developing pre-eclampsia.

METHODS

All women booked for maternity care at Harold Wood Hospital, Essex, UK between October 1999 and

Correspondence to: Prof. K. Spencer, Clinical Biochemistry Department, King George Hospital, Barley Lane, Goodmayes, IG3 8YB, UK (e-mail: KevinSpencer1@aol.com)

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August 2002 were offered screening for trisomy 21 by a combination of fetal nuchal translucency thickness and maternal serum free β-human chorionic gonadotropin (β-hCG) and PAPP-A at 11 to 13 + 6 weeks' gestation[10]. Serum free β-hCG and PAPP-A were measured within 30 min of venepuncture using the Kryptor analyzer (Brahms AG, Berlin, Germany), and the ultrasound scans were carried out by sonographers who had obtained The Fetal Medicine Foundation Certificate of Competence in the 11 to 13 + 6 weeks' scan (www.fetalmedicine.com). After analysis samples were immediately archived to a serum bank held at −20°C. A second ultrasound examination was routinely performed at 22–24 weeks for measurement of fetal growth and examination for fetal defects. In those cases where no major fetal defect was detected, the women were offered the option of participating in a screening study for pre-eclampsia by transvaginal Doppler measurement of the uterine artery pulsatility index (PI)[11]. Each uterine artery was identified using color-flow mapping and pulsed-wave Doppler was then used to obtain three similar consecutive waveforms. The PI was measured and the mean PI of the two uterine arteries was calculated. The Doppler studies were performed by sonographers who had received The Fetal Medicine Foundation Certificate of Competence in Doppler (www.fetalmedicine.com).

From this previously published cohort of 4390 singleton pregnancies 64 developed pre-eclampsia. Pre-eclampsia was defined according to the guidelines of the International Society for the Study of Hypertension in Pregnancy[12]. The pre-eclamptic group were further classified as early onset ($n = 34$) if delivery was required before 35 weeks or late onset ($n = 30$) if delivery took place after 35 weeks. From amongst those cases delivering a normal term infant with a birth weight greater than the 10th centile for gestational age[13], 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 analyzer. Table 1 summarizes the maternal characteristics and birth characteristics of the two groups. All the samples had been stored as aliquots and had not previously been thawed.

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

Characteristic	*Control group* (n = *240*)	*Pre-eclampsia group* (n = *64*)
Mean maternal age (years)	30	29
Caucasian (%)	95	86
Cigarette smoker (%)	15	16
Nulliparous (%)	40	50
Median free β-hCG MoM	1.00	0.92
Median PAPP-A MoM	1.00	0.84
Uterine artery mean pulsatility index	1.02	1.56
Median GA at delivery (weeks)	39.1	36.2
Median birth weight (g)	3418	2025

β-hCG, β-human chorionic gonadotropin; GA, gestational age; MoM, multiples of the median; PAPP-A, pregnancy-associated plasma protein-A.

Maternal serum concentrations of inhibin-A and activin-A were measured in duplicate in a series of eight assays over a 2-week period using quantitative commercial enzyme-linked immunosorbent assay kits (Oxford Bio-Innovation Ltd, UK). Duplicates that differed by more than 10% were repeated in a subsequent assay.

Statistical analysis

Regression analysis was carried out to examine any relationship between inhibin-A or activin-A concentration and gestational age. The median inhibin-A and activin-A concentrations were established across the gestational window 11 + 0 to 13 + 6 weeks. All measurements were then expressed as multiples of the median (MoM) for gestation. The Mann–Whitney *U*-test was used to assess the significance of any differences between the control group and the pre-eclamptic group in the individual marker concentrations, both as raw values and as MoMs. A multivariate Gaussian model combining inhibin-A or activin-A with mean uterine artery PI was developed using standard statistical modeling techniques, and their performance in discriminating cases that would develop pre-eclampsia compared to either measure alone was also assessed by receiver–operating characteristics (ROC) curve analysis. All statistical analysis was performed using Analyse-It (Analyse-It Software, Leeds) a statistical add-in for Microsoft Excel or SPSS 14 (SPSS, Woking, UK).

RESULTS

In the control group inhibin-A and activin-A did not appear to change with gestational age across the narrow window under investigation ($r = 0.0436$ and 0.1034, respectively). Figure 1 shows the distribution of results with gestational age. The median inhibin-A concentration was 231.13 pg/mL in the control group and the median activin-A concentration was 2.16 ng/mL. When the pre-eclamptic group was compared with the control group there were significant increases in both inhibin-A and activin-A concentrations, with levels of 286.64 pg/mL and 2.522 ng/mL, respectively (Figure 2). When corrected for gestational age the median inhibin-A MoM in the pre-eclamptic group was increased (1.24 MoM; 95% CI, 0.988–1.644). Comparing the medians using the Mann–Whitney test confirmed a significant difference ($U = 10073$, two-tailed $P = 0.0006$). For activin-A the median was also increased (1.17 MoM; 95% CI, 0.922–1.314). Comparing the medians using the Mann–Whitney test confirmed a significant difference ($U = 9302$; two-tailed $P = 0.0276$) (Figure 3). In the group delivering prior to 35 weeks there was no significant difference when compared to those delivering at 35 weeks or later for either marker.

At cut-offs of the 90th centile of normal, inhibin-A (1.70 MoM) and activin-A (1.75 MoM) levels would have identified 35% and 20%, respectively, of cases

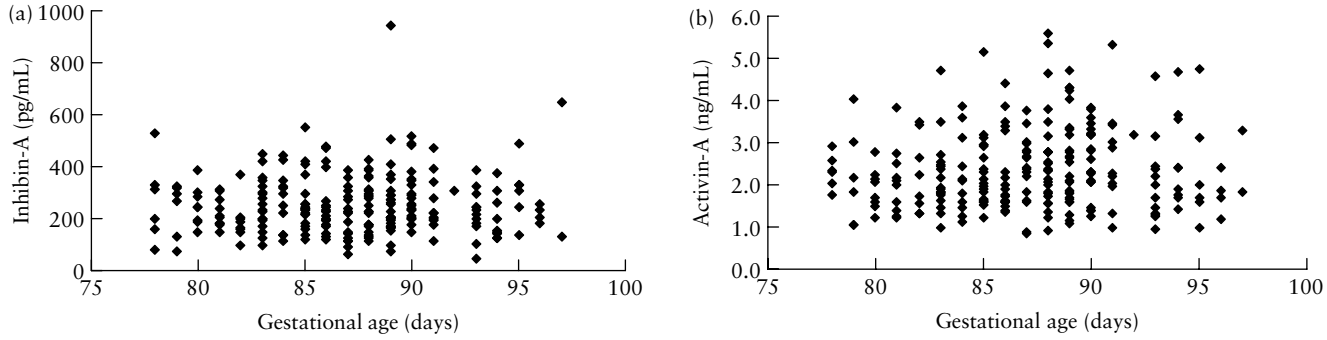

Figure 1 Variation of inhibin-A (a) and activin-A (b) concentrations with gestational age.

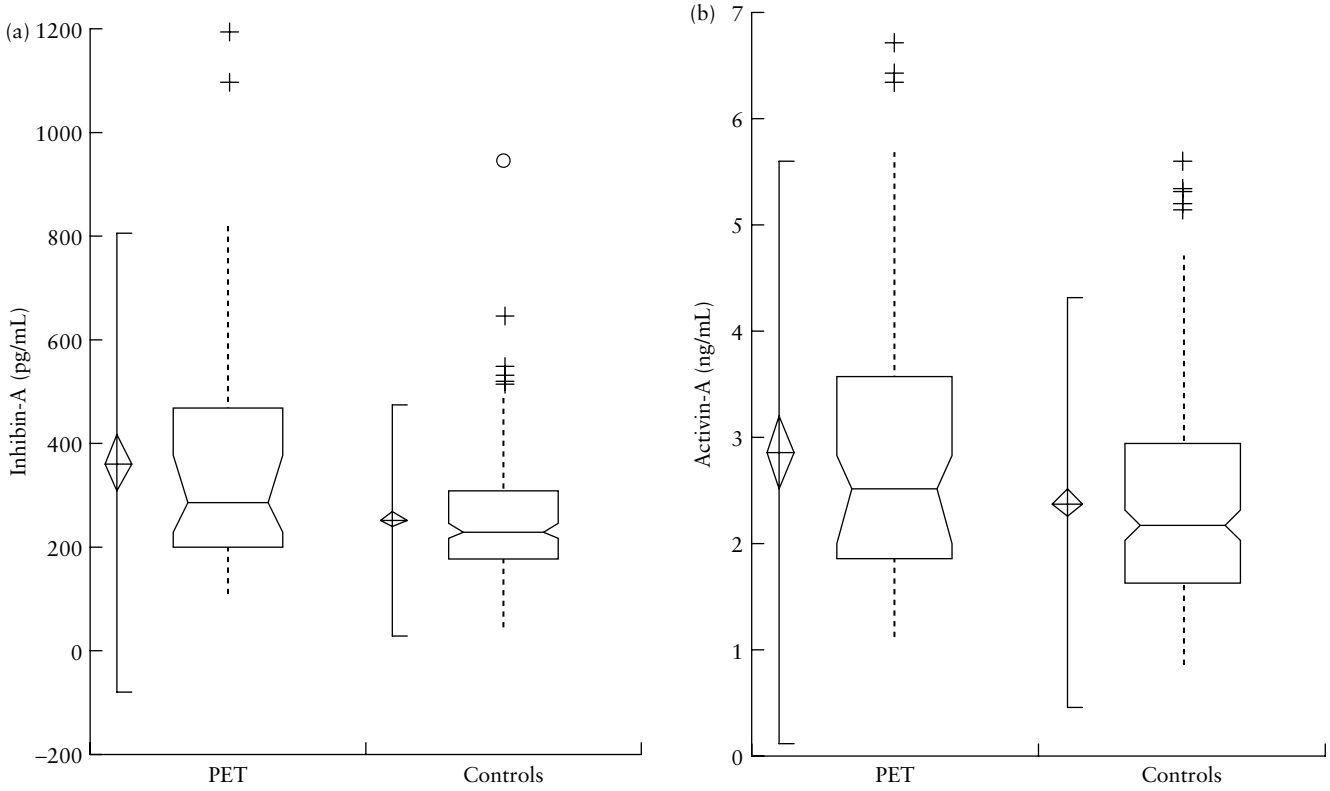

Figure 2 Box-and-whisker plots showing the difference in inhibin-A (a) and activin-A (b) concentrations between cases of pre-eclampsia (PET) and controls. The diamonds show the mean and the 95% confidence interval around the mean. The notched lines show the parametric 95% percentile range. The notched boxes show the median, lower and upper quartiles, and confidence interval around the median. The dotted lines connect the nearest observations within 1.5 interquartile ranges (IQRs) of the lower and upper quartiles. The crosses (+) and circles (○) indicate possible outliers: observations more than 1.5 IQRs (near outliers) and 3.0 IQRs (far outliers) from the quartiles.

that would develop pre-eclampsia. At cut-offs of the 95th centile, inhibin-A (2.00 MoM) and activin-A (2.00 MoM) levels would have given detection rates of 28% and 15%, respectively. Figure 4 shows the ROC curve for each marker. The area under the curve was 0.638 (95% CI, 0.555–0.720) for inhibin-A and 0.589 (95% CI, 0.510–0.668) for activin-A.

In the multivariate Gaussian model, for a combination of mean PI and inhibin-A, at a 5% false positive rate, the detection rate was 67.5%, compared with 54.7% for mean PI alone. For a combination of mean PI and activin-A the detection rate was 63.2%. The performance characteristics are summarized in Table 2 along with data for free β-hCG and PAPP-A, alone and in combination with mean PI, from our previous study with this same cohort[4]. When the data were analyzed comparing early- and late-onset disease, the predictive model was no better at identifying early- from late-onset pre-eclampsia.

DISCUSSION

This study has confirmed that levels of maternal serum inhibin-A and activin-A were increased in the first trimester of pregnancies that subsequently developed pre-eclampsia. However the size of the increase is unlikely, on its own, to be of significant clinical value. When combined with second-trimester uterine artery Doppler

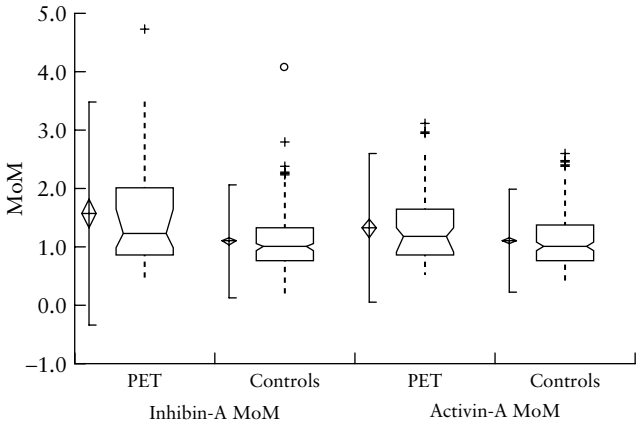

Figure 3 Box-and-whisker plots showing the difference in inhibin-A and activin-A expressed as multiples of the median (MoM) between cases of pre-eclampsia (PET) and controls. The diamonds show the mean and the 95% confidence interval around the mean. The notched lines show the parametric 95% percentile range. The notched boxes show the median, lower and upper quartiles, and confidence interval around the median. The dotted line connects the nearest observations within 1.5 interquartile ranges (IQRs) of the lower and upper quartiles. The crosses (+) and circles (∘) indicate possible outliers: observations more than 1.5 IQRs (near outliers) and 3.0 IQRs (far outliers) from the quartiles.

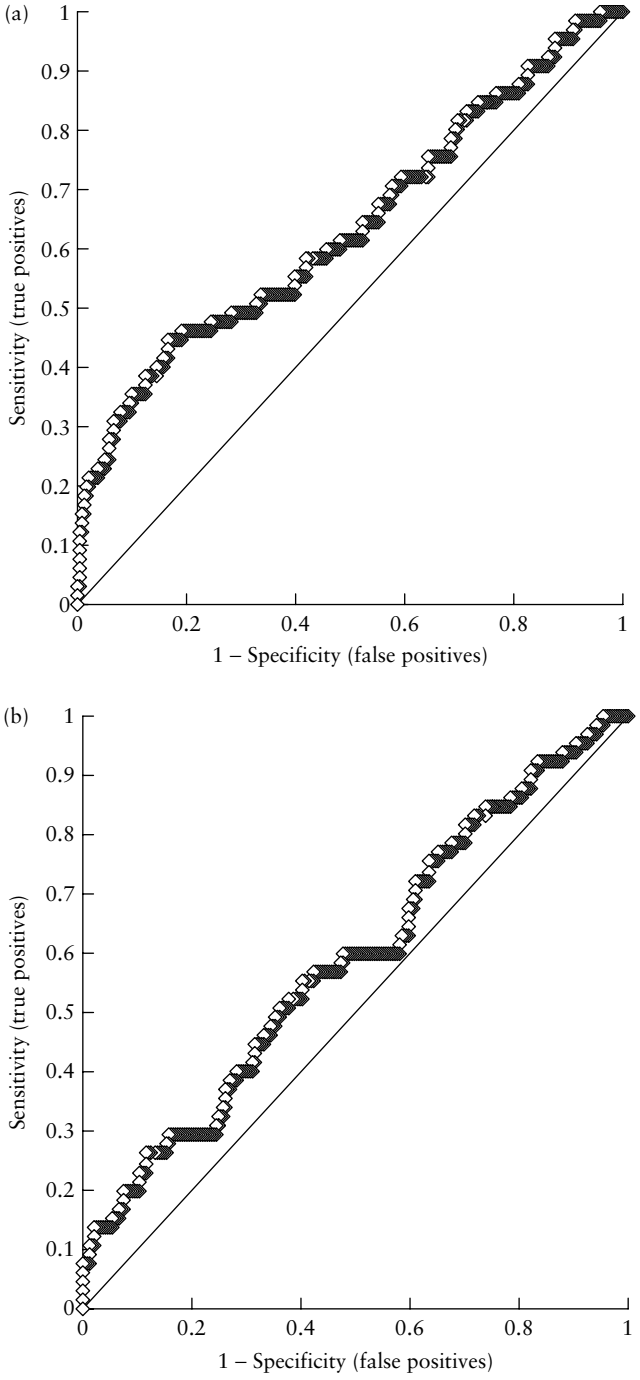

Figure 4 Receiver–operating characteristics curve for identification of pre-eclampsia cases using inhibin-A (a) and activin-A (b).

mean PI some improvement in the prediction of pre-eclampsia could be observed. A previous small study of inhibin-A in the first trimester has shown a modest elevation (1.4 MoM) in women who subsequently develop pre-eclampsia, allowing 33% detection at a 5% false positive rate[8]. In another study of 30 cases taken at around 10 weeks[14], raised levels of inhibin-A were also observed. Although the study did not quote the results in a conventional way, close inspection of the controls and cases plotted graphically in the paper would suggest that the median MoM was also approaching 1.4. A further study conducted at around 12 weeks of gestation in 52 cases[15] reported increased levels of both inhibin-A and activin-A, but again unfortunately absolute values or MoMs were not quoted in the study and the graphical displays could not be used to make an accurate estimate. The authors concluded that the most potent first-trimester serum markers associated with the risk of developing pre-eclampsia later were inhibin-A and activin-A, followed by PAPP-A and placental growth factor (PlGF). Our own study of PlGF, however, found levels of PlGF to be not significantly different from those of controls[16]. A recently published study[17] of 56 cases, however, has confirmed our previous observation[9] of increased levels of activin-A in the first trimester of pregnancies that later developed pre-eclampsia. The median activin-A in the study of Banzola *et al.*[17] was 1.79 MoM compared with 1.49 in our previous study.

It is evident from many studies that the further one gets away from the clinical manifestation of the disease the more the value of the biochemical markers appears to decline. Thus while activin-A and inhibin-A have been shown to be extremely elevated at the time of clinical presentation, in the second trimester the median values (although increased) had not yet built up to very high levels, and certainly during the first trimester (again although increased) their clinical value is probably limited[18–21]. Combinations of markers, along with the best clinical tool (uterine artery Doppler mean PI) may improve clinical discrimination, but the search still goes on for a truly clinically useful early first-trimester biochemical marker of pre-eclampsia.

Table 2 Summary of detection rates of the individual markers and their combinations at a 5% false-positive rate

Marker	*Detection rate (%)*
Inhibin-A	28
Activin-A	15
Mean PI	55
PAPP-A[4]	14
Free β-hCG[4]	8
Inhibin-A plus mean PI	68
Activin-A plus mean PI	63
PAPP-A plus mean PI[4]	62

β-hCG, β-human chorionic gonadotropin; PAPP-A, pregnancy-associated plasma protein-A; PI, pulsatility index.

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