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Serum inhibin A and activin A are elevated prior to the onset of pre-eclampsia

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Serum inhibin A and activin A concentrations increase in pre-eclampsia. We investigated the time courses of the changes in relation to the onset of the maternal syndrome and if their measurement could be useful for clinical prediction particularly in relation to early onset disease, the most severe of the clinical presentations. Serial samples were taken from 1496 healthy nulliparae. Changes in activin A and inhibin A were analysed in women with: early onset pre-eclampsia (n = 11), pre-eclampsia delivering at 34–36 weeks (n = 14), term pre-eclampsia (n = 25) and gestational hypertension (n = 25); and in a subset with uncomplicated pregnancies (n = 25). Serum inhibin A and activin A were increased in all groups prior to pre-eclampsia, before 20 weeks in those with early onset pre-eclampsia. Screening efficacy was determined at 15–19 and 21–25 weeks in all women who developed pre-eclampsia (n = 70) and randomly selected controls (n = 240). Predictive sensitivities were low (16–59%) but much better for early onset pre-eclampsia: 67 and 44% at 15–19 weeks and 89 and 89% at 21–25 weeks for inhibin A and activin A respectively. Hence, serum inhibin A and activin A concentrations increase before the onset of pre-eclampsia at gestational ages that depend on when pre-eclampsia develops. On their own such measures are unlikely to prove efficient for screening.

Key words: activin A/gestational hypertension/inhibin A/pre-eclampsia/pregnancy

Introduction

Hypertensive complications develop in one in eight pregnancies. The clinical hallmarks of the most severe form, pre-eclampsia, are hypertension in the second half of pregnancy (gestational hypertension) and proteinuria. Pre-eclampsia may result in substantial maternal and fetal morbidity and mortality, particularly if the condition develops at an early gestational age (Sibai *et al.*, 1990; Roberts and Redman, 1993). However, it may occur at any time after mid-term and progress rapidly. Clinical screening may fail to detect the disease before severe complications have developed (Redman and Roberts, 1993). The requirements for an effective screening test are that it needs to identify women at risk early in pregnancy so that care and outcome can be modified. Currently there are no useful screening tests for pre-eclampsia (North *et al.*, 1994; Suarez *et al.*, 1996; Ashour *et al.*, 1997).

Inhibins (α-β dimers) and activins (β-β dimers) are glycoprotein hormones belonging to the transforming growth factor β superfamily. Concentrations of circulating dimeric inhibin A rise in early pregnancy, fall after 12 weeks gestation, and remain low until 24 weeks (Muttukrishna *et al.*, 1995; Birdsall *et al.*, 1997). Thereafter, concentrations increase gradually but with a marked rise in the third trimester (Muttukrishna *et al.*, 1995). Circulating concentrations of activin A are similar during the first and second trimesters and rise progressively in the third trimester with a steep increase at term (Muttukrishna *et al.*, 1996). The feto–placental unit is an important source of inhibin A and activin A in pregnancy (Birdsall *et al.*, 1997; Muttukrishna *et al.*, 1997a). It has been shown that maternal serum concentrations of inhibin A and activin A are 10-fold higher in women with severe pre-eclampsia compared to gestational age matched controls (Muttukrishna *et al.*, 1997b). Other investigators have also reported elevated inhibin A and activin A concentrations in pre-eclampsia (Petraglia *et al.*, 1995; Fraser *et al.*, 1998). In women who subsequently developed pre-eclampsia, inhibin A concentrations were found to be elevated at 13–18 weeks in a retrospective analysis of a Down's screening programme (Cuckle *et al.*, 1998; Aquilina *et al.*, 1999). Neither of these studies analysed the time courses of the changes in serum measurements or how such changes may vary depending on when pre-eclampsia develops. These studies were not designed to test the ability of activin A and inhibin A to predict pre-eclampsia. In a nested case-control study, elevated inhibin A concentrations were found at 15–22 weeks in women who later developed pre-eclampsia, particularly in those delivering preterm (King *et al.*, 1998).

This study attempted to confirm and extend the results of the earlier reports. There were two objectives. The first was to establish in a longitudinal study whether serum concentrations of inhibin A and activin A were raised prior to the clinical onset of pre-eclampsia and gestational hypertension and, if so, with what time course in relation to the time when clinical pre-eclampsia became manifest. The second was to determine, in a nested case-control study, whether measurements of serum inhibin A or activin A at 15–18 weeks and

21–24 weeks gestation could be clinically effective predictors of pre-eclampsia, in particular of early-onset pre-eclampsia.

Materials and methods

In a prospective, longitudinal study, blood samples were collected from 1651 healthy nulliparous women recruited in the community (Schellenberg *et al.*, 1998). Eighty-three women were excluded because of miscarriages, fetal congenital malformations, multiple pregnancy, essential hypertension or aspirin therapy. A further 40 withdrew from the study and 32 did not follow-up, leaving a total of 1496 women. Blood samples were obtained at 8–13, 15–19, 21–25, 27–30 and 35–38 weeks gestation and 5–11 weeks postpartum. Gestational age was determined by last menstrual period or ultrasound scan before 20 weeks. Blood samples were collected in plain tubes and centrifuged at 3000 r.p.m. for 10 min. Serum was separated and stored at –80°C until assayed. The regional ethics committee approved the study and informed consent was obtained from all women.

Gestational hypertension was defined as systolic blood pressure >140 mm Hg with a rise >30 mm Hg or diastolic blood pressure >90 mm Hg with a rise >15 mm Hg on two or more occasions after 20 weeks gestation, but before the onset of labour. Pre-eclampsia was defined as gestational hypertension with proteinuria (>300 mg/24 h or if no formal quantitation available >1 g/l on spot urinalysis).

Study 1: time course of changes

Samples from five groups of women were studied. The method of selection from the total serum bank was as follows. (i) 223 women were identified by random number selection (Paradox 4.5, Borland, Scott Valley, California, USA) from the total cohort of normotensive women who delivered after 37 weeks. The first 25 of these women with complete sets of specimens were selected (normal pregnant control women). (ii) The first 25 women, with the most complete sets of specimens, of the 46, who developed pre-eclampsia and delivered at >37 weeks were taken (term pre-eclampsia). Samples from (iii) all women with pre-eclampsia who delivered at 34–36 weeks (n = 14) (pre-term pre-eclampsia) and (iv) all women with pre-eclampsia who delivered before 34 weeks (early onset pre-eclampsia; n = 11) were selected. (v) The first 25 of the 117 women who developed gestational hypertension, who delivered after 37 weeks and who had the most complete sets of specimens were included.

Study 2: clinical prediction

Serum samples taken at 15–19 weeks and 21–25 weeks were analysed using a nested case control design. A control group (n = 244) was randomly selected by computer from women remaining in the cohort after exclusion of women with pre-eclampsia. A serum sample was not available at one time point in some women either because they had failed to present for sampling or because insufficient volume was stored. Forty-two of the 244 women in the control group developed other pregnancy complications. Twenty (8.2%) developed gestational hypertension (one with iatrogenic preterm birth), nine (3.7%) had spontaneous preterm birth and 13 (5.3%) had idiopathic fetal growth retardation (birthweight <5th centile). These women were excluded when the reference ranges were established, but included when the predictive characteristics of the tests were calculated.

Hormone assays

Samples from women who developed pre-eclampsia and from controls were included in each assay plate.

Inhibin A

Serum concentrations of dimeric inhibin A were measured in duplicate 5 µl aliquots as described elsewhere (Groome *et al.*, 1994). Samples were oxidized with 6% hydrogen peroxide and plated on to 96 well plates coated with a mouse monoclonal antibody (E4) against the βA subunit. After overnight incubation, the plates were washed and incubated for 2 h with a second mouse monoclonal antibody (against the alpha subunit) conjugated to alkaline phosphatase. Bound alkaline phosphatase was quantified using a commercially available enzyme-linked immunosorbent assay (ELISA) amplification kit (Immuno-Select ELISA amplification system, Life Technologies, Uxbridge, UK). Minimum detection limit of the assay for human recombinant inhibin A (National Institute for Biological Standards and Controls, South Mimms, UK) was 2 pg/ml. The mean intra- and inter-assay coefficients of variation were 4.3 and 5.1% respectively. The intra- and inter-assay variations were calculated at three points on the standard curve [low concentration (0.4–1.0 pg/well), middle concentration (3.13–6.25 pg/well) and high concentration (12.5–25 pg/well)] using three quality controls (n = 12 assays).

Activin A

Serum concentrations of total activin A were measured in duplicate 10 µl aliquots using an enzyme immunoassay (EIA) specific for total activin A, as described previously (Knight *et al.*, 1996). Samples were treated with 1:1 volume of 15% (w/v) sodium dodecyl sulphate (SDS) and incubated in a boiling water bath for 10 min. SDS treated samples were oxidized with 2.5% (v/v final concentration) hydrogen peroxide and 100 µl/well transferred to 96 well plates coated with E4 antibodies. After 10 min incubation, biotinylated E4 antibody (25 µl/well) was added and the plates were incubated overnight. On day 2, they were washed and 50 µl/well of extravidin (Sigma Chemical Co., St Louis, MO, USA) conjugated with alkaline phosphatase (1:10 000 v/v) was added to all wells. After 2 h, the plates were washed and bound alkaline phosphatase was quantified as described above. The minimum detection limit of the assay for human recombinant activin A (Genentech, San Francisco, CA, USA) was 50 pg/ml. The mean intra- and inter-assay coefficients of variation were 6.5 and 7.7% respectively. The intra and interassay variations were calculated at three points on the standard curve [low concentration (5–10 pg/well), middle concentration (62.5–125 pg/well) and high concentration (250–500 pg/well)] using three quality controls (n = 12 assays).

Table I. Reference ranges of inhibin A and activin A concentrations in maternal serum

	90th centile	95th centile
Inhibin A (pg/ml)		
15–19 weeks	320.8	375.7
21–25 weeks	543.1	712.4
Activin A (ng/ml)		
15–19 weeks	7.3	10.3
21–25 weeks	9.7	12.5

Statistics

Analyses of time courses of changes

As the raw data were not normally distributed, they were log transformed (base 10) to obtain normal distribution. General estimating equations (GEE) statistical analysis was used to analyse the data (STATA statistical software, STATA Corporation, Houston, Texas, USA).

Analyses of predictive efficacy

The reference ranges at 15–19 and 21–25 weeks gestation were generated from women (n = 202) who remained normotensive and delivered a normally grown (birthweight >5th centile) infant at term.

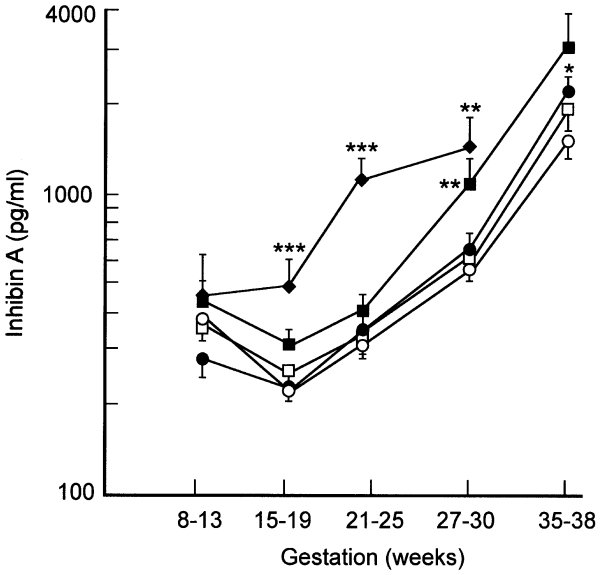

Figure 1. Mean (SEM) $\log_{10}$ concentrations of inhibin A in peripheral serum in normal pregnancy (○), women who developed pre-eclampsia at <34 weeks (◆), women who developed pre-eclampsia at 34–36 weeks (■), women who developed pre-eclampsia at >37 weeks (●) and women who developed gestational hypertension (□). Statistical difference in means compared with controls analysed by general estimating equations (GEE) analysis are expressed as ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$.

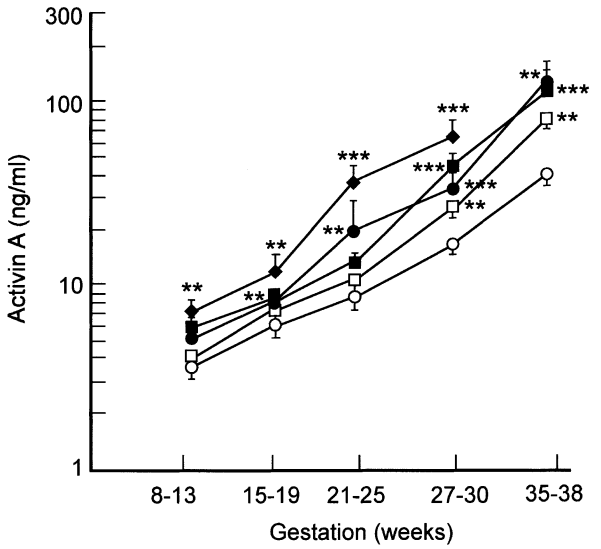

Figure 2. Mean (SEM) $\log_{10}$ concentrations of 'total' activin A in peripheral serum in normal pregnancy (○), women who developed pre-eclampsia at <34 weeks (◆), women who developed pre-eclampsia at 34–36 weeks (■), women who developed pre-eclampsia at >37 weeks (●) and women who developed gestational hypertension (□). Statistical difference in means compared with controls analysed by general estimating equations (GEE) analysis are expressed as ***$P < 0.001$, **$P < 0.01$.

The 90th and 95th centiles for activin A and inhibin A are shown in Table I. These threshold values were then used to calculate the sensitivity, specificity, likelihood ratio and post-test probability (post-test odds = pre-test odds×likelihood ratio; probability = odds/1 + odds) (Deeks and Morris, 1996). The test characteristics were then calculated separately for early onset pre-eclampsia (delivered before 34 weeks) and compared with those of the control group.

Results

Time courses of changes in relation to clinically evident pre-eclampsia

Inhibin A

Inhibin A was detected in all samples taken during pregnancy. Concentrations of inhibin A in sera of healthy women fell between 8–13 weeks and 15–19 weeks, thereafter gradually rising to term (Figure 1, $P < 0.001$). Inhibin A concentrations in women with early onset pre-eclampsia were significantly higher than those in healthy women at 15–19 weeks ($P < 0.001$), 21–25 weeks ($P < 0.001$) and 27–30 weeks ($P = 0.002$). In women with pre-eclampsia who delivered at 34–36 weeks, serum inhibin A concentrations were higher than in healthy women at 27–30 weeks ($P = 0.003$). Serum inhibin A concentrations in women with term pre-eclampsia were increased only at 35–38 weeks ($P < 0.05$). Inhibin A concentrations in women who developed gestational hypertension did not vary significantly from those in healthy women. Postpartum, serum concentrations of inhibin A were low in all groups. Inhibin A concentrations postpartum ranged between <2 pg/ml ($n = 43$) and 42 pg/ml, with no difference between groups.

Table II. Maternal and fetal characteristics: predictive study. Continuous variables are mean (SD) and categorical variables are percentage

	Pre-eclampsia $n = 70$	Controls $n = 240$	*P* value
Age	27.6 (4.3)	28.3 (4.7)	NS
Ethnicity (%)			
European	85.7	88.8	NS
Maori	4.3	2.5	
Pacific Islander	4.3	2.9	
Other	5.7	5.8	
Booking BP (mm Hg)			
Systolic	117 (12)	113 (12)	0.03
Diastolic	69 (9) $n = 233$	67 (9)	NS
Maximum BP (mm Hg)			
Systolic	158 (14)	122 (13)	0.0001
Diastolic	104 (9)	77 (10)	0.0001
Caesarean section (%)	42.9	24.2	0.002
Gestation at delivery (weeks)	36.9 (4.2)	39.8 (1.8)	0.0001
Preterm delivery (%)			
<34 weeks	15.7	1.7	0.001
34–36 weeks	20.0	2.5	
Birthweight (g)	2785 (947)	3390 (517)	0.0001
Small for gestational age <10th percentile	25.7	13.8	0.02

BP = blood pressure, NS = not significant.

Activin A

Activin A was detected in all serum samples. Activin A concentrations in women with early onset pre-eclampsia were higher than in the healthy women at each gestational period (Figure 2, $P < 0.01$). Activin A concentrations in the term pre-eclampsia group were elevated throughout pregnancy from 15–19 weeks ($P < 0.01$). In women with pre-eclampsia who

Table III. Test characteristics of maternal serum inhibin A and activin A at 15–19 weeks and 21–25 weeks to predict pre-eclampsia (prevalence of 4.8%). Percentage (95% confidence intervals)

	Sensitivity (%)	Specificity (%)	Likelihood ratio	Post-test probability (%)
Inhibin A				
15–19 weeks				
>90th centile	28 (23–33)	88 (84–92)	2.3 (1.4–4.0)	9.9
>95th centile	23 (19–28)	93 (90–96)	3.4 (1.8–6.5)	14.5
21–25 weeks				
>90th centile	27 (21–32)	89 (85–93)	2.4 (1.4–4.2)	10.6
>95th centile	16 (11–20)	94 (91–97)	2.5 (1.2–5.5)	11.0
Activin A				
15–19 weeks				
>90th centile	41 (35–46)	89 (86–93)	3.8 (2.4–6.1)	15.7
>95th centile	25 (20–30)	96 (93–98)	5.8 (2.8–12.2)	22.1
21–25 weeks				
>90th centile	59 (54–65)	87 (83–91)	4.5 (3.1–6.7)	18.1
>95th centile	38 (32–43)	93 (90–96)	5.0 (2.9–8.8)	19.7

delivered at 34–36 weeks and the gestational hypertension group, concentrations of activin A were higher from 27–30 weeks onwards compared with healthy women ($P < 0.01$). Post partum activin A concentrations were low, ranging from 0.05 to 8.7 ng/ml, and similar in all groups.

Screening efficacy for pre-eclampsia

Pre-eclampsia developed in 71 (4.8%) women and gestational hypertension in 117 (7.8%) women. Eleven women had early onset pre-eclampsia (0.74%), 14 (0.94%) women with pre-eclampsia delivered between 34 and 36 weeks and 46 (3.1%) had term pre-eclampsia. In one woman with pre-eclampsia and in four women in the control group, both the 15–19 week and 21–25 week blood specimens were unavailable. The clinical characteristics of the remaining 70 women with pre-eclampsia and 240 controls are shown in Table II. There were 65 (92%) and 64 (90%) samples in the pre-eclampsia group and 232 (95%) and 227 (93%) samples being assayed in the controls at 15–19 weeks and 21–25 weeks respectively. The normal range of inhibin A at 15–19 weeks was 41.4–690 pg/ml and at 21–25 weeks was 47.6–1719 pg/ml. The normal ranges of activin A at 15–19 weeks and 21–25 weeks were 0.64–15.71 and 0.86–28.74 ng/ml. The sensitivities, specificities, likelihood ratios and post-test probabilities for the prediction of pre-eclampsia and early onset pre-eclampsia by inhibin A and activin A are shown in Tables III and IV. Serum activin A appears to be a better marker than inhibin A in predicting all pre-eclampsia (Table III). Sensitivities and likelihood ratios were higher for both activin A and inhibin A in the prediction of early onset pre-eclampsia than for all pre-eclampsia.

Discussion

This study adds new information to current knowledge of changes prior to the clinical onset of pre-eclampsia. This was achieved by using a large bank of prospectively collected samples from a cohort of primiparae to study changes in the circulating concentrations of inhibin A and activin A. It was demonstrated, for the first time, that there are different time courses of changes. Women with the earliest onset disease manifest biochemical changes at the earliest gestational age. Apart from revealing factors that may be relevant to pathogenesis this study highlights that studies of screening tests for pre-eclampsia need to be in relation to the very variable times at which it may present. The findings of the current study support and extend previous, less rigorously designed studies (Cuckle *et al.*, 1998; King *et al.*, 1998; Aquilina *et al.*, 1999).

As far as is known, trophoblast dysfunction is the primary problem in pre-eclampsia (Redman, 1991). It is thought that the maternal syndrome in some way may be caused by oxidative stress in the placenta (Wang and Walsh, 1998) associated with spiral artery disease. It is not known why inhibin A and activin A are increased and whether they contribute to the aetiology of the disease. Inhibin α subunit, inhibin/activin β subunit and immunoreactive inhibin (ir-inhibin) are synthesized in trophoblast throughout pregnancy (McLachlan *et al.*, 1986; de Kretser *et al.*, 1994; Qu and Thomas, 1995; Petraglia, 1997). Other endocrine products of trophoblast, namely human chorionic gonadotrophin (HCG) (Ashour *et al.*, 1997) and corticotrophin releasing hormone (Perkins *et al.*, 1995), are also increased during or before the onset of pre-eclampsia, indicating dysfunction that affects several secreted syncytiotrophoblast hormones. Activin A stimulates production of HCG by first-trimester trophoblast (Caniggia *et al.*, 1997; Song *et al.*, 1996) and HCG increases secretion of ir-inhibin production by cultured placental cells (Qu and Thomas, 1995). This may be an explanation for the concomitant increase in activin A, HCG and inhibin A (Keelan *et al.*, 1998). Inflammatory cells (Yu *et al.*, 1996) also produce activin A. Since the maternal syndrome of pre-eclampsia is characterized by an intense systemic inflammatory response (Redman *et al.*, 1999), circulating activin A may be derived from circulating inflammatory cells rather than, or as well as, from trophoblast. This may explain why circulating activin A is a more sensitive marker of the disease than inhibin A. Follistatin is a high affinity binding protein for activin A. At 38–39 weeks gestation, activin A and follistatin concentrations both rise to a peak and their concentrations are highly correl-

Table IV. Test characteristics of maternal serum inhibin A and activin A at 15–19 weeks and 21–25 weeks to predict early onset pre-eclampsia (delivered at <34 weeks) with a prevalence of 0.74. Percentage (95% confidence intervals)

	Sensitivity (%)	Specificity (%)	Likelihood ratio	Post-test probability (%)
Inhibin A				
15–19 weeks				
>90th centile	67 (61–73)	88 (84–92)	5.6 (3.1–9.9)	4.0
>95th centile	56 (50–61)	93 (90–96)	8.1 (3.8–17.2)	5.7
21–25 weeks				
>90th centile	89 (85–93)	89 (85–93)	8.1 (5.2–12.5)	5.7
>95th centile	67 (61–73)	94 (91–97)	10.86 (5.5–21.6)	7.6
Activin A				
15–19 weeks				
>90th centile	44 (38–50)	89 (85–93)	4.14 (1.8–9.4)	3.0
>95th centile	33 (27–39)	96 (93–98)	7.8 (2.6–23.4)	5.5
21–25 weeks				
>90th centile	89 (85–93)	87 (83–91)	6.8 (4.5–10.1)	4.9
>95th centile	89 (85–93)	93 (89–96)	11.93 (7.1–19.9)	8.2

ated. The parallel increase throughout pregnancy probably reflects feto–placental secretion (O'Connor *et al.*, 1999). There are different molecular forms of follistatin in the circulation and FS315 is the predominant form in humans. The assay used in the above stated study measures FS285 and has a 10% cross-reaction with FS315. Therefore, the concentrations measured by using this ELISA do not give an accurate measure of 'total' follistatin, making it difficult to evaluate precisely the follistatin:activin ratio. This ratio would indicate the availability of 'free' activin A that is biologically active.

It is possible that elevated serum concentrations of activin A and inhibin A in pre-eclampsia result from reduced clearance. In the first trimester, inhibin A and activin A clear from the circulation within 6 h of pregnancy termination (Muttukrishna *et al.*, 1997a). The rates and modes of clearance in pre-eclampsia are not known. However, the early rise before the onset of pre-eclampsia cannot be attributed to impaired renal function, which is not established before 20 weeks in patients who later develop pre-eclampsia.

Although activin A was better than inhibin A for predicting pre-eclampsia, 41–75% of women who subsequently developed pre-eclampsia would still not be identified. This low risk nulliparous population had an incidence of pre-eclampsia of 4.8%. The post-test probabilities of disease for activin A were 22.1 and 19.7% at 15–19 weeks and 21–25 weeks respectively. These are comparable to probabilities of 20–30% in women identified as at high risk on clinical criteria alone (Caritis *et al.*, 1998).

The majority of women with early onset pre-eclampsia, the subset at greatest risk of adverse maternal and fetal outcome, were detected by measures of serum inhibin A and activin A at 21–25 weeks. However, the incidence of early onset disease was only 0.74%. Consequently, despite likelihood ratios of 5.6–10.9 for inhibin A and 4.1–11.9 for activin A, the post-test probabilities ranged from 4.0–7.6 and 3–8.2% respectively (Table IV). The interpretation of these low values needs to take account of the rarity of the endpoint. Hence a serum activin A or inhibin A concentration above the 95th centile increases the odds of developing early onset pre-eclampsia from 1 in 135 to 1 in 10 or 11. The study was deliberately underpowered to estimate accurately the test characteristics for early onset pre-eclampsia, and larger studies are necessary.

We (Redman *et al.*, 1999) and others (Ness and Roberts, 1996) have emphasized the heterogeneity of pre-eclampsia in relation to the interaction between multiple factors specific to the pregnancy or to the mother. From such considerations, it was predicted that the search for a single predictive test would never succeed, but that some tests might predict some subtypes of pre-eclampsia (Redman *et al.*, 1999). The results of this study are entirely consistent with this viewpoint, as are those of all previous searches for early biochemical or haematological markers.

These results are only applicable to healthy nulliparous women. Studies in high risk women are required to test if activin A or inhibin A are useful predictors in these populations. It will be important to assess whether the measurement of other indices combined with activin A or inhibin A can produce a test with greater sensitivity for pre-eclampsia as occurs with triple or quadruple blood tests for Down's syndrome. If this was possible, an effective predictive test before 20 weeks would allow antenatal care to be tailored appropriately to the individual's level of risk. It would also identify women suitable for recruitment into randomized trials testing prophylactic therapy.

In conclusion, serum concentrations of activin A and inhibin A were elevated prior to the onset of pre-eclampsia and may have a clinical application in identifying women at risk of early onset pre-eclampsia, although larger studies are needed to confirm this point. The time course of changes is consistent with the view that there is placental dysfunction prior to the appearance of the clinical signs of pre-eclampsia.

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References

Aquilina, J., Barnet, A., Thompson, O. and Harrington, K. (1999) Second trimester maternal serum inhibin A concentrations as an early marker for pre-eclampsia. *Am. J. Obstet. Gynecol.*, **181**, 131–136.

Ashour, A.M., Lieberman, E.S., Haug, L.E. and Repke, J.T. (1997) The value of elevated second-trimester beta-human chorionic gonadotropin in predicting development of pre-eclampsia. *Am. J. Obstet. Gynecol.*, **176**, 438–442.

Birdsall, M., Ledger, W., Groome, N. *et al.* (1997) Inhibin A and activin A in the first trimester of human pregnancy. *J. Clin. Endocrinol. Metab.*, **82**, 1557–1560.

Caniggia, I., Lye, S.J. and Cross, J.C. (1997) Activin is a local regulator of human cytotrophoblast cell differentiation. *Endocrinology*, **138**, 3976–3986.

Caritis, S., Sibai, B., Hauth, J. *et al.* (1998) Low-dose aspirin to prevent pre-eclampsia in women at high risk. National Institute of Child Health and Human Development Network of Maternal-Fetal Medicine Units. *N. Engl. J. Med.*, **338**, 701–705.

Cuckle, H., Sehmi, I. and Jones, R. (1998) Maternal serum inhibin A can predict pre-eclampsia. *Br. J. Obstet. Gynaecol.*, **105**, 1101–1103.

Deeks, J. and Morris, J. (1996) Evaluating diagnostic tests. *Balliere's Clin. Obstet. Gynecol.*, **10**, 613–630.

de Kretser, D.M., Foulds, L.M., Hancock, M. and Robertson, D.M. (1994) Partial characterization of inhibin, activin, and follistatin in the term human placenta. *J. Clin. Endocrinol. Metab.*, **79**, 502–507.

Fraser, R.F., McAsey, M.E. and Coney, P. (1998) Inhibin-A and pro-alpha C are elevated in preeclamptic pregnancy and correlate with human chorionic gonadotropin. *Am. J. Reprod. Immunol.*, **40**, 37–42.

Groome, N.P., Illingworth, P.J., O'Brien, M. *et al.* (1994) Detection of dimeric inhibin throughout the human menstrual cycle by two-site enzyme immunoassay. *Clin. Endocrinol.*, **40**, 717–723.

Keelan, J.A., Groome, N.P. and Mitchell, M.D. (1998) Regulation of activin A production by amnion, decidua, and placenta *in vitro* by proinflammatory cytokine. *Placenta*, **19**, 429–434.

King, I., Williams, M., Sorensen, T. and Luthy, D. (1998) Inhibin A and activin A levels in the second trimester as predictors of pre-eclampsia. *Am. J. Obstet. Gynaecol.*, **178**, S115.

Knight, P.G., Muttukrishna, S. and Groome, N.P. (1996) Development and application of a two-site enzyme immunoassay for the determination of 'total' activin-A concentrations in serum and follicular fluid. *J. Endocrinol.*, **148**, 267–279.

McLachlan, R.I., Healy, D.L., Robertson, D.M. *et al.* (1986) The human placenta: a novel source of inhibin. *Biochem. Biophys. Res. Commun.*, **140**, 485–490.

Muttukrishna, S., George, L., Fowler, P.A. *et al.* (1995) Measurement of serum concentrations of inhibin-A (alpha-beta A dimer) during human pregnancy. *Clin. Endocrinol.*, **42**, 391–397.

Muttukrishna, S., Fowler, P.A., George, L. *et al.* (1996) Changes in peripheral serum levels of total activin A during the human menstrual cycle and pregnancy. *J. Clin. Endocrinol. Metab.*, **81**, 3328–3334.

Muttukrishna, S., Child, T.J., Groome, N.P. and Ledger, W.L. (1997a) Source of circulating levels of inhibin A, pro alpha C-containing inhibins and activin A in early pregnancy. *Hum. Reprod.*, **12**, 1089–1093.

Muttukrishna, S., Knight, P.G., Groome, N.P. *et al.* (1997b) Activin A and inhibin A as possible endocrine markers for pre-eclampsia. *Lancet*, **349**, 1285–1288.

Ness, R.B. and Roberts, J.M. (1996) Heterogeneous causes constituting the single syndrome of pre-eclampsia: a hypothesis and its implications. *Am. J. Obstet. Gynecol.*, **175**, 1365–1370.

North, R.A., Ferrier, C., Long, D. *et al.* (1994) Uterine artery Doppler flow velocity waveforms in the second trimester for the prediction of pre-eclampsia and fetal growth retardation. *Obstet. Gynaecol.*, **83**, 378–386.

O'Connor, A.E., McFarlane, J.R., Hayward, S. *et al.* (1999) Serum activin A and follistatin concentrations during human pregnancy: a cross-sectional and longitudinal study. *Hum. Reprod.*, **14**, 827–832.

Perkins, A.V., Linton, E.A., Eben, F. *et al.* (1995) Corticotrophin-releasing hormone and corticotrophin-releasing hormone binding protein in normal and pre-eclamptic human pregnancies. *Br. J. Obstet. Gynaecol.*, **102**, 118–122.

Petraglia, F. (1997) Inhibin, activin and follistatin in the human placenta – a new family of regulatory proteins. *Placenta*, **18**, 3–8.

Petraglia, F., Aguzzoli, L., Gallinelli, A. *et al.* (1995) Hypertension in pregnancy: changes in activin A maternal serum concentration. *Placenta*, **16**, 447–454.

Qu, J. and Thomas, K. (1995) Inhibin and activin production in human placenta. *Endocrinol. Rev.*, **16**, 485–507.

Redman, C.W.G. (1991) Current topic, pre-eclampsia and the placenta. *Placenta*, **12**, 301–308.

Redman, C.W.G. and Roberts, J.M. (1993) Management of pre-eclampsia. *Lancet*, **341**, 1451–1454.

Redman, C.W.G., Sacks, G.P. and Sargent, I.L. (1999) Pre-eclampsia, an excessive maternal inflammatory response to pregnancy. *Am. J. Obstet. Gynecol.*, **180**, 499–506.

Roberts, J.M. and Redman, C.W.G. (1993) Pre-eclampsia, more than pregnancy-induced hypertension. *Lancet*, **341**, 1447–1451.

Schellenberg, J.C., North, R.A., Taylor, R. and Zhou, R.L. (1998) Secretory component of immunoglobulin A in maternal serum and the prediction of preterm delivery. *Am. J. Obstet. Gynecol.*, **178**, 535–539.

Sibai, B.M., Akl, S., Fairlie, F. and Moretti, M. (1990) A protocol for managing severe pre-eclampsia in the second trimester. *Am. J. Obstet. Gynecol.*, **163**, 733–738.

Song, Y., Keelan, J. and France, J.T. (1996) Activin-A stimulates, while transforming growth factor beta 1 inhibits, chorionic gonadotrophin production and aromatase activity in cultured human placental trophoblasts. *Placenta*, **17**, 603–610.

Suarez, V.R., Trelles, J.G. and Miyahira, J.M. (1996) Urinary calcium in asymptomatic primigravidas who later developed pre-eclampsia. *Obstet. Gynaecol.*, **87**, 79–82.

Wang, Y. and Walsh, S.W. (1998) Placental mitochondria as a source of oxidative stress in pre-eclampsia. *Placenta*, **19**, 581–586.

Yu, J., Shao, L.E., Frigon, N.L.J. *et al.* (1996) Induced expression of the new cytokine, activin A, in human monocytes: inhibition by glucocorticoids and retinoic acid. *Immunology*, **88**, 368–374.

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