Original Article

Screening for pre-eclampsia by using maternal serum inhibin A, activin A, human chorionic gonadotropin, unconjugated estriol, and alpha-fetoprotein levels and uterine artery Doppler in the second trimester of pregnancy

Emine AY, Zehra Nese KAVAK, Koray ELTER, Husnu GOKASLAN and Tanju PEKIN

Department of Obstetrics and Gyneacology, Marmara University School of Medicine, Istanbul, Turkey

Abstract

Aims: To analyse the predictive power of maternal serum inhibin A, activin A, human chorionic gonadotropin (hCG), unconjugated estriol (uE$_3$), alpha-fetoprotein (AFP) levels and uterine artery Doppler in the second trimester of pregnancy in screening for pre-eclampsia.

Methods: Maternal serum inhibin A, activin A, hCG, uE$_3$, and AFP levels and uterine artery Doppler were determined in 178 healthy, pregnant women in the second trimester of pregnancy. Serum samples were collected between the 16th and 18th weeks of gestation, and Doppler investigation was performed between the 24th and 26th weeks of gestation. Receiver operating characteristic curves were created to analyse the predictive powers of the above parameters in screening for pre-eclampsia. Different combinations also were analysed.

Results: The rate of pre-eclampsia was 7.9% (14/178). Maternal serum inhibin A, activin A, hCG, AFP levels, the rate of presence of the prediastolic notch and uterine artery resistance index (RI) values in pre-eclamptic pregnancies were significantly higher than those in healthy pregnancies. Presence of the prediastolic notch, uterine artery RI, maternal serum activin A and inhibin A levels had high predictive efficacy, and each had a sensitivity between 70 and 93% and a specificity between 87% and 98%. The addition of inhibin A or activin A measurement to the Doppler velocimetry improved the specificity to 99–100%.

Conclusions: Maternal serum inhibin A and activin A levels and uterine artery Doppler appear to be useful screening tests during the second trimester for pre-eclampsia. However, addition of these hormonal markers to Doppler velocimetry only slightly improves the predictive efficacy, which appears clinically insignificant.

Key words: Activin A; alpha-fetoprotein; Doppler; hCG; inhibin A; pre-eclampsia; screening; uterine artery.

Introduction

Pre-eclampsia and eclampsia are associated with significant maternal and fetal morbidity and mortality and are unpredictable in onset and progression.[1] A major aim of early identification of a high-risk group of women is the reduction of associated morbidity and mortality.

There is a known association between elevated maternal serum human chorionic gonadotropin (hCG) levels and established pre-eclampsia.[2] Inhibin A, activin A, alpha-fetoprotein (AFP) also have been demonstrated to be elevated in women with established pre-eclampsia.[3–6] Following these studies, maternal levels of these markers have been proposed as possible endocrine markers of preclinical disease.[6–11] Lower unconjugated estriol (uE$_3$) level has also been reported to be associated with the subsequent development of pre-eclampsia.[12,13]

One of the pathophysiological mechanisms associated with the development of pre-eclampsia is the partial or complete failure of trophoblastic invasion.[14] In the past 25 years, Doppler ultrasonographic studies of uteroplacental circulation have confirmed the original observation that high impedance to flow in these vessels is associated with an increased risk for subsequent development of pre-eclampsia.[15,16] However, the value of Doppler findings have been analysed in combination with any of the above hormonal parameters in only a limited number of studies.[17,18] In the present study, we aimed to analyse the predictive power of maternal serum inhibin A,

activin A, hCG, uE$_3$, AFP levels and uterine artery Doppler, either alone or in combination, in the second trimester of pregnancy in screening for pre-eclampsia.

Materials and methods

Subjects

One hundred and eighty three consecutive pregnant women at 16–18 weeks' gestation who presented to the outpatient antenatal clinic of the Marmara University Hospital for a routine follow-up between February 2003 and January 2004, were included in the present study. Gestational ages were estimated from the last menstrual periods for women who had regular (21–35 days) menstrual cycles or from ultrasonographic scans in the first trimester for women who had irregular menstrual cycles. Women with multiple pregnancy, hypertension diagnosed before 26 weeks' gestation, diabetes, or pregnancy with a prenatal or postnatal diagnosis of a chromosomal or structural abnormality, were excluded from the study. Women with a history of previous pregnancy complicated by pre-eclampsia were also excluded.

Maternal serum samples were obtained at the 16th to 18th weeks of gestation, and Doppler measurements were performed at the 24th to 26th weeks of gestation. Pre-eclampsia was diagnosed when there was hypertension, which was defined as a blood pressure of ≥ 140/90 mmHg and first diagnosed after 20 weeks' gestation, in association with ≥ 300 mg of urinary protein excretion per 24 h.[19] The study was approved by the institutional review board at the Marmara University, and written informed consent was obtained from each subject.

Assays and ultrasonographic measurements

Maternal venous blood samples were obtained between the 16th to 18th weeks of gestation, and stored at −20°C for the determination of biochemical markers. Serum samples were analysed at the end of the study in the laboratory by personnel who were blind to subjects and outcome. Serum activin A and inhibin A concentrations were measured by using specific two-site enzyme immunoassays (Serotec, Oxford, UK). Serum HCG and uE$_3$ measurements were performed by using a two-site immunometric assay (Immulite, Diagnostic Products Corporation, Los Angeles, USA). Serum AFP concentrations were measured by using a solid-phase, two-site sequential chemiluminescent immunometric assay (Immulite, Diagnostic Products Corporation, Los Angeles, USA).

Uterine artery flow velocity waveforms were obtained using a real-time ultrasound machine (Logiq 500, GE Medical Systems, Milwaukee, WI, USA) with a 3.5-MHz convex probe. The transducer was placed in the lower lateral quadrant of the abdomen, angled medially. Colour flow pulsed Doppler was employed to identify the uterine artery, where it crossed the external iliac artery. The range gate was placed over the entire diameter of the uterine artery, approximately one centimetre distal to the crossover point. In a small proportion of cases, where the uterine arteries branched before intersecting the external iliac vessels, assessment was performed on the main artery. The quality of the flow velocity waveforms was maximised by using the smallest possible angle of insonation (range: 15–50) and accepting only those waveforms with a clear and sharp outline. When four consecutive waveforms of satisfactory quality were obtained, the RI was calculated from the mean of the four waveforms, and the presence or absence of a prediastolic notch was noted. The procedure was then repeated on the other side. The presence of a notch in any of the right or left uterine arteries was accepted as positive. All the measurements were performed by the same operator (E.A.). Doppler findings were not kept in clinical case files, but instead, kept secret in a separate file. They were revealed only after the study has ended, except those of women who developed pre-eclampsia, in which case Doppler findings were revealed at the time of diagnosis.

Statistical analysis

For each serum marker, the median values in the unaffected pregnancies at each week of gestation were determined. Curve estimation was performed to determine the optimal relationship between these median values and gestational weeks. For the curve estimation procedure, curve estimation regression statistics were performed for 11 different regression models, including linear, logarithmic, inverse, quadratic, cubic, power, compound, S-curve, logistic, growth, and exponential models. The model with the highest coefficient of determination (r^2) was accepted as the most optimal model for the relationship, and an optimal regression line was fitted. Coefficient of determination is the amount of the scatter in one variable that can be explained by another.

The normal median value for each marker at each week of gestation was estimated from the regression line. Serum levels of markers were expressed as the multiples of this median (MoM) value.

Mann–Whitney *U* and Fisher's exact tests were performed to compare the characteristics of normal and pre-eclamptic pregnancies in Table 1, where appropriate.

Receiver operating characteristic (ROC) analysis was performed to analyse the predictive value of markers for pre-eclampsia. Diagnostic sensitivity and specificity were calculated, and the ROC curve was constructed by plotting the sensitivity against the false-positive rate (1-specificity) of various cut-off values for predicting pre-eclampsia. The value with the optimal combination of sensitivity and specificity was chosen as the optimum cut-off value. Area under each ROC curve (AUC$_{ROC}$), which indicates the predictive power of the parameter, was calculated. The *P*-value of the ROC analysis indicates the significance of the difference between the relevant variable and the coin test, which has an AUC of 0.5. Since the presence of notch was a categorical variable, ROC analysis could not be performed for that variable and only predictive values were calculated.

Predictive values of different combinations were also determined. 'And' type combinations indicate that the test is positive when both of the components of the combination

Table 1 Demographic and clinical characteristics of subjects

	Normal pregnancies (n = 164)	Pre-eclamptic pregnancies (n = 14)	P
Maternal age (years)	28.8 ± 5.1	30.6 ± 4.3	NS
Incidence of nulliparous women (%)	58	43	NS
BMI at biochemical screening (kg/m^2)	24.6 ± 3.4	24.8 ± 2.7	NS
Gestational age (weeks) at delivery	39.2 ± 1.0	35.3 ± 5.2	< 0.001
Birth weight (g)	3402 ± 367	2567 ± 395	< 0.001
Mean diastolic blood pressure at delivery (mmHg)	73.9 ± 8.1	106.1 ± 11.5	< 0.001
Mean serum activin A value (MoM)	1.00 (0.15–3.35)	12.33 (7.72–18.17)	< 0.001
Mean serum inhibin A value (MoM)	0.99 (0.76–1.49)	3.36 (1.74–6.95)	< 0.001
Mean serum AFP value (MoM)	0.94 (0.76–1.20)	1.40 (0.75–1.63)	0.03
Mean serum hCG value (MoM)	1.20 (0.73–1.63)	1.76 (0.93–2.54)	0.04
Mean serum estriol value (MoM)	1.10 (0.81–1.38)	1.09 (0.67–1.22)	NS
Uterine artery Doppler (RI)	0.54 ± 0.08	0.69 ± 0.12	< 0.001
Presence of prediastolic notch (%)	2.4	85.7	< 0.001

NS, not significant.

were higher than the optimum cut-off value. 'Or' type combinations indicate that the test is positive when either of the components of the combination were higher than the optimum cut-off value. SPSS, Release 11.5 (SPSS, Inc, Chicago, IL, USA) was used for the statistical analysis. Since MoM values have a log-Gaussian distribution, instead of a normal distribution, these values were expressed as median with their interquartile range, that is, the range between the 25th and 75th percentiles. Other values were expressed as mean ± SD, and a P-value of < 0.05 was considered significant.

Results

One hundred and eighty three consecutive pregnancies were included into the present study and 178 women completed the study. One woman was excluded as she was hypertensive before 26 weeks' gestation and four women did not return for follow-up. Fourteen women (7.9%) developed pre-eclampsia.

The mean (± SD) for gestation at diagnosis was 32.0 ± 2.4 weeks. Demographic characteristics of women are shown in Table 1. Maternal serum activin A, inhibin A, hCG and AFP levels were significantly higher in women with subsequent development of pre-eclampsia than those who had an uneventful pregnancy (Table 1). Maternal serum uE$_3$ values were comparable between groups. In Doppler findings, RI values of pre-eclamptic pregnancies were significantly higher than those of normal pregnancies (Table 1). Presence of notch was also more commonly observed in women with subsequent development of pre-eclampsia than those with an uneventful pregnancy (Table 1).

ROC analysis revealed that maternal serum levels of activin A, inhibin A, hCG, AFP and uterine artery Doppler in the second trimester have significant predictive values for pre-eclampsia (Table 2, Fig. 1). As a single marker, the presence of notch had the highest combination of sensitivity and specificity, and predicted 96.6% of the cases correctly (Table 2). The odds ratio was 240 (95% CI = 40–1446). Analysis of different combinations revealed that the addition

Table 2 Results of the ROC analysis. Optimum cut-off values for significantly different parameters and predictive values for these parameters

	AUC ± SE	P*	95% CI	Cut-off	LR	Incidence of + test (%)	Sn (%)	Sp (%)	PPV (%)	NPV (%)	Predicted correctly (%)
Presence of notch	NA	NA	NA	NA	58.5	9.0	85.7	97.6	75.0	98.8	96.6
Serum inhibin A	0.87 ± 0.07	< 0.001	0.74–1.00	2.79	39.4	9.0	71.4	96.3	62.5	97.5	94.4
Serum activin A	0.94 ± 0.02	< 0.001	0.90–0.98	6.58	42.9	18.0	92.9	88.4	40.6	99.3	88.8
Uterine artery Doppler (RI)	0.86 ± 0.06	< 0.001	0.74–0.99	0.62	26.9	18.5	78.6	86.6	33.3	97.9	86.0
Serum hCG	0.67 ± 0.08	0.04	0.50–0.83	1.75	10.2	20.2	57.1	82.9	22.2	95.8	80.9
Serum AFP	0.68 ± 0.09	0.03	0.49–0.86	1.28	12.0	23.0	64.3	80.5	22.0	96.4	80.3
Serum estriol	0.43 ± 0.08	NS	0.27–0.59	NA	NA	NA	NA	NA	NA	NA	NA

*Significance of the difference from a coin test, which has an AUC of 0.5.
AUC, area under the curve; CI, confidence interval; LR, likelihood ratio; NA, not applicable; NPV, negative predictive value; PPV, positive predictive value; ROC, receiver operating characteristic; SE, standard error; Sn, sensitivity; Sp, specificity.
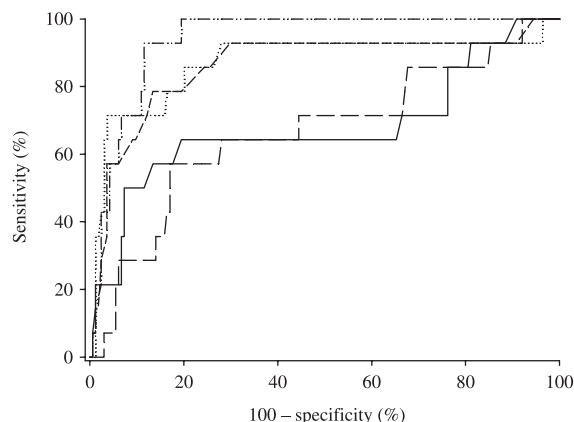
Figure 1 Receiver operating characteristic (ROC) curves for serum markers and uterine artery Doppler. (—) AFP; (–) hCG; (⋯) Inhibin; (- -) RI; (·-) Activin.

of any of the activin A or inhibin A measurements slightly improved the predictive value of the presence of notch (Table 3). The addition of activin A and inhibin A measurement to the presence of notch predicted 98.3 and 97.8% of the cases correctly, respectively (Table 3). The combination of RI and serum inhibin level also had a comparable predictive value to the above combinations (Table 3). Other optimum combinations are shown in Table 3.

Discussion

The present study shows that maternal serum activin A, inhibin A, hCG, and AFP levels and uterine artery Doppler measurements have a value in predicting pre-eclampsia. As a single parameter, the presence of the prediastolic notch had the highest levels of sensitivity and specificity. This parameter also had the lowest rate of positive test (9%). The rate of positivity is important since any positive screening test might need an intervention or a close follow-up, and this could increase the cost-effectiveness of a test.

Maternal levels of these serum markers have been proposed as early endocrine markers for subsequent development of pre-eclampsia. There are accumulating data from studies that evaluated whether a single elevated hCG value between 14 and 24 weeks' gestation is predictive of pre-eclampsia,[8,10,20–27] and these studies are convergent in suggesting that women with elevated hCG levels in the second trimester are at increased risk for pre-eclampsia. While small-sample studies failed to detect any precocious elevation of serum inhibin A and activin A levels in pre-eclampsia,[9,28] larger studies have indicated that inhibin A and activin A are elevated several weeks before the onset of clinical signs of pre-eclampsia.[11,18,29–33] The results on the value of serum AFP level for the prediction of pre-eclampsia have been inconsistent.[3,7,10,12,26,30,34,35] Low uE_3 concentration has been suggested to be significantly associated with severe pre-eclampsia.[12] The results in the present study confirm most of these observations in the literature. However, we did not observe any predictive value of serum estriol level for the prediction of pre-eclampsia. Interestingly, although Stamilio *et al.*[12] suggested an association between low unconjugated estriol concentration and subsequent development of pre-eclampsia with a relative risk of 1.7, the 95% confidence interval (0.9–3.4) was intersecting unity in their study.

Although Doppler ultrasonography is widely used in screening for adverse pregnancy outcome, the use of above hormonal markers in combination with Doppler ultrasonography in the prediction of pre-eclampsia has only been analysed in a limited number of studies.[17,18] Florio *et al.* prospectively analysed 58 asymptomatic pregnant women, in whom a diastolic notch of the uterine artery waveform was noted at routine Doppler examination at 24 weeks' gestation, and observed that maternal serum activin A and inhibin A levels were higher in patients who developed pre-eclampsia than in those who did not present with pre-eclampsia at follow-up.[18] Activin A achieved a sensitivity of 61% and a specificity of 89% at the cut-off value of 1.7 MoM, and inhibin A achieved a sensitivity of 39% and a specificity of 92% at the cut-off value of 1.8 MoM for prediction of pre-eclampsia.[18] The authors concluded that the measurement of serum activin A and inhibin A levels might add significant prognostic information for predicting pre-eclampsia in pregnant women showing specific Doppler alterations in the late second trimester.[18] Aquilina *et al.* measured inhibin-A

Table 3 Predictive values for different combinations

	Incidence of + test (%)	Sn (%)	Sp (%)	PPV (%)	NPV (%)	Predicted correctly (%)
Presence of notch and high serum activin	6.2	78.6	100	100	98.2	98.3
Presence of notch and high serum inhibin	5.6	71.4	100	100	97.6	97.8
High UA RI and high serum inhibin	5.6	71.4	100	100	97.6	97.8
High serum activin and high serum inhibin	5.1	64.3	100	100	97.0	97.2
Presence of notch or high serum inhibin	12.4	85.7	93.9	54.5	98.7	93.3
Presence of notch or high serum activin	20.8	100	86.0	100	37.8	87.1
Presence of notch or High UA RI	19.1	85.7	86.6	35.3	98.6	86.0
High UA RI or high serum inhibin	21.9	78.6	82.9	28.2	97.8	82.6
High serum inhibin or high serum hCG	26.4	100	79.9	29.8	100	81.5

NPV, negative predictive value; PPV, positive predictive value; RI, resistance index; Sn, sensitivity; Sp, specificity; UA, uterine artery.

levels of 689 consecutive unselected women between 15 and 19 weeks' gestation and subsequently performed colour flow pulsed Doppler of uterine arteries between 18 and 22 weeks' gestation.[17] They have reported that when Doppler findings were analysed in combination with serum inhibin A levels for the prediction of pre-eclampsia, the sensitivity improved from 60 to 71%.[17]

The results in the present study show that Doppler ultrasonography is the single best method for the screening of pre-eclampsia, and addition of maternal serum hormone levels, that is, activin A or inhibin A, slightly improves the predictive efficacy. However, this improvement does not seem to be clinically important. The predictive efficacy of the combined use of Doppler and inhibin A for the prediction of pre-eclampsia in the present study seems comparable to that reported in the study by Aquilina *et al.*[17] However, the sensitivity of Doppler findings seems high in the present study, when compared to that in their study[17] as well as to those in other studies.[15] This could be because of the relatively late (24–26 weeks) application of Doppler screening compared to other studies examining midtrimester prediction or to the limited number of subjects in the present study. Doppler findings in the present study should be cautiously interpreted since the screening interval is later than the period, which has been studied previously.[36]

It should be mentioned that our aim was not to suggest cut-offs in the present study. These cut-offs helped us to compare the predictive efficacy of parameters in the study population. Cut-offs should be validated in larger groups. Also, some median values for MoM values in normal pregnancies are slightly different from 1.0 MoM (Table 1). This is because of the separate calculation of median values for each week between the 16th and 18th weeks of gestation.

Among the screening modalities in pre-eclampsia, only two, that is, placental perfusion-related and fetoplacental unit endocrinology dysfunction-related tests, were analysed in the present study. Predictive values of these parameters in the present study appear to be higher than those of other modalities, that is, renal dysfunction-related and oxidative stress dysfunction-related tests.[36]

In conclusion, the present study shows that the presence of a prediastolic notch has the highest predictive efficacy amongst the markers which were analysed. Also, the results indicated that serum inhibin A and activin A are sufficiently efficacious to provide reliable second trimester screening for pre-eclampsia. Serum AFP and hCG levels have modest efficacy in the prediction of pre-eclampsia. However, addition of these hormonal measurements to uterine artery Doppler velocimetry does not cause a clinically significant improvement in the prediction of pre-eclampsia over the use of Doppler velocimetry alone.

References

1 Walker JJ. Pre-eclampsia. *Lancet* 2000; **356**: 1260–1265.
2 Hsu CD, Chan DW, Iriye B, Johnson TR, Hong SF, Repke JT. Elevated serum human chorionic gonadotropin as evidence of secretory response in severe preeclampsia. *Am J Obstet Gynecol.* 1994; **170**: 1135–1138.
3 Walters BN, Lao T, Smith V, De Swiet M. Alpha-Fetoprotein elevation and proteinuric pre-eclampsia. *Br J Obstet Gynaecol.* 1985; **92**: 341–344.
4 Fraser RF III, McAsey ME, Coney P. Inhibin-A and pro-alpha C are elevated in preeclamptic pregnancy and correlate with human chorionic gonadotropin. *Am J Reprod Immunol.* 1998; **40**: 37–42.
5 Silver HM, Lambert-Messerlian GM, Star JA, Hogan J, Canick JA. Comparison of maternal serum total activin A and inhibin A in normal, preeclamptic, and nonproteinuric gestationally hypertensive pregnancies. *Am J Obstet Gynecol.* 1999; **180**: 1131–1137.
6 Muttukrishna S, Knight PG, Groome NP, Redman CW, Ledger WL. Activin A and inhibin A as possible endocrine markers for pre-eclampsia. *Lancet* 1997; **349**: 1285–1288.
7 Leung TN, Chung TK, Madsen G *et al.* Analysis of mid-trimester corticotrophin-releasing hormone and alpha-fetoprotein concentrations for predicting pre-eclampsia. *Hum Reprod* 2000; **15**: 1813–1818.
8 Onderoglu LS, Kabukcu A. Elevated second trimester human chorionic gonadotropin level associated with adverse pregnancy outcome. *Int J Gynecol. Obstet* 1997; **56**: 245–249.
9 Raty R, Koskinen P, Alanen A, Irjala K, Matinlauri I, Ekblad U. Prediction of pre-eclampsia with maternal mid-trimester total renin, inhibin A, AFP and free beta-hCG levels. *Prenat Diagn* 1999; **19**: 122–127.
10 Pouta AM, Hartikainen AL, Vuolteenaho OJ, Ruokonen AO, Laatikainen TJ. Midtrimester N-terminal proatrial natriuretic peptide, free beta hCG, and alpha-fetoprotein in predicting preeclampsia. *Obstet Gynecol.* 1998; **91**: 940–944.
11 Muttukrishna S, North RA, Morris J *et al.* Serum inhibin A and activin A are elevated prior to the onset of pre-eclampsia. *Hum Reprod* 2000; **15**: 1640–1645.
12 Stamilio DM, Sehdev HM, Morgan MA, Propert K, Macones GA. Can antenatal clinical and biochemical markers predict the development of severe preeclampsia? *Am J Obstet Gynecol.* 2000; **182**: 589–594.
13 Wald NJ, Morris JK. Multiple marker second trimester serum screening for pre-eclampsia. *J Med Screen* 2001; **8**: 65–68.
14 Meekins JW, Pijnenborg R, Hanssens M, McFadyen IR, van Asshe A. A study of placental bed spiral arteries and trophoblast invasion in normal and severe pre-eclamptic pregnancies. *Br J Obstet Gynaecol.* 1994; **101**: 669–674.
15 Albaiges G, Missfelder-Lobos H, Lees C, Parra M, Nicolaides KH. One-stage screening for pregnancy complications by color Doppler assessment of the uterine arteries at 23 weeks' gestation. *Obstet Gynecol.* 2000; **96**: 559–564.
16 Campbell S, Pearce JM, Hackett G, Cohen-Overbeek T, Hernandez C. Qualitative assessment of uteroplacental blood flow: early screening test for high-risk pregnancies. *Obstet Gynecol.* 1986; **68**: 649–653.
17 Aquilina J, Thompson O, Thilaganathan B, Harrington K. Improved early prediction of pre-eclampsia by combining second-trimester maternal serum inhibin-A and uterine artery Doppler. *Ultrasound Obstet Gynecol.* 2001; **17**: 477–484.
18 Florio P, Reis FM, Pezzani I, Luisi S, Severi FM, Petraglia F. The addition of activin A and inhibin A measurement to uterine artery Doppler velocimetry to improve the early prediction of pre-eclampsia. *Ultrasound Obstet Gynecol.* 2003; **21**: 165–169.

19 Report of the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy. *Am J Obstet Gynecol.* 2000; **183**: S1–S22.

20 Lee IS, Chung DY, Cole LA, Copel JA, Isozaki T, Hsu CD. Elevated serum nicked and urinary beta-core fragment hCG in preeclamptic pregnancies. *Obstet Gynecol.* 1997; **90**: 889–892.

21 Muller F, Savey L, Le Fiblec B *et al.* Maternal serum human chorionic gonadotropin level at fifteen weeks is a predictor for preeclampsia. *Am J Obstet Gynecol.* 1996; **175**: 37–40.

22 Bahado-Singh RO, Oz U, Isozaki T *et al.* Midtrimester urine human chorionic gonadotropin beta-subunit core fragment levels and the subsequent development of pre-eclampsia. *Am J Obstet Gynecol.* 1998; **179**: 738–741.

23 Ashour AM, Lieberman ES, Haug LE, Repke JT. The value of elevated second-trimester beta-human chorionic gonadotropin in predicting development of preeclampsia. *Am J Obstet Gynecol.* 1997; **176**: 438–442.

24 Vaillant P, David E, Constant I *et al.* Validity in nulliparas of increased beta-human chorionic gonadotrophin at mid-term for predicting pregnancy-induced hypertension complicated with proteinuria and intrauterine growth retardation. *Nephron* 1996; **72**: 557–563.

25 Luckas M, Hawe J, Meekins J, Neilson J, Walkinshaw S. Second trimester serum free beta human chorionic gonadotrophin levels as a predictor of pre-eclampsia. *Acta Obstet Gynecol. Scand.* 1998; **77**: 381–384.

26 Morssink LP, Heringa MP, Beekhuis JR, De Wolf BT, Mantingh A. The association between hypertensive disorders of pregnancy and abnormal second-trimester maternal serum levels of hCG and alpha-fetoprotein. *Obstet Gynecol.* 1997; **89**: 666–670.

27 Heinonen S, Ryynanen M, Kirkinen P, Saarikoski S. Elevated midtrimester maternal serum hCG in chromosomally normal pregnancies is associated with preeclampsia and velamentous umbilical cord insertion. *Am. J. Perinatol.* 1996; **13**: 437–441.

28 Grobman WA, Wang EY. Serum levels of activin A and inhibin A and the subsequent development of preeclampsia. *Obstet Gynecol.* 2000; **96**: 390–394.

29 Lambert-Messerlian GM, Silver HM, Petraglia F *et al.* Second-trimester levels of maternal serum human chorionic gonadotropin and inhibin a as predictors of preeclampsia in the third trimester of pregnancy. *J Soc Gynecol Invest* 2000; **7**: 170–174.

30 Davidson EJ, Riley SC, Roberts SA, Shearing CH, Groome NP, Martin CW. Maternal serum activin, inhibin, human chorionic gonadotrophin and alpha-fetoprotein as second trimester predictors of pre-eclampsia. *Bjog* 2003; **110**: 46–52.

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

32 Aquilina J, Barnett A, Thompson O, Harrington K. Second-trimester maternal serum inhibin A concentration as an early marker for preeclampsia. *Am J Obstet Gynecol.* 1999; **181**: 131–136.

33 Sebire NJ, Roberts L, Noble P, Wallace E, Nicolaides KH. Raised maternal serum inhibin A concentration at 10–14 weeks of gestation is associated with pre-eclampsia. *Bjog* 2000; **107**: 795–797.

34 Brazerol WF, Grover S, Donnenfeld AE. Unexplained elevated maternal serum alpha-fetoprotein levels and perinatal outcome in an urban clinic population. *Am J Obstet Gynecol.* 1994; **171**: 1030–1035.

35 Wenstrom KD, Owen J, Davis RO, Brumfield CG. Prognostic significance of unexplained elevated amniotic fluid alpha-fetoprotein. *Obstet Gynecol.* 1996; **87**: 213–216.

36 Conde-Agudelo A, Villar J, Lindheimer M. World Health Organization systematic review of screening tests for preeclampsia. *Obstet Gynecol.* 2004; **104**: 1367–1391.