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Prediction of pregnancy complications by first-trimester maternal serum PAPP-A and free β-hCG and with second-trimester uterine artery Doppler

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Background Previous studies have shown an association between low first trimester maternal serum free β-hCG and PAPP-A and subsequent development of pregnancy complications. Similarly, uterine artery Doppler in the late second trimester has shown that high impedance to flow is associated with increased risk for preeclampsia and fetal growth restriction. The objective of this study is to determine whether there is an association between the maternal serum concentration of PAPP-A and free β-hCG at $11-13^{+6}$ weeks with the uterine artery pulsatility index (PI) at 22–24 weeks, and secondly, to compare the screening characteristics of the two methods in the prediction of adverse pregnancy outcome.

Methods Maternal serum PAPP-A and free β-hCG at $11-13^{+6}$ weeks and uterine artery PI at 22–24 weeks were measured in 4390 women with singleton pregnancies. Pregnancies with chromosomal defects or fetal anomalies were excluded. The biochemical and Doppler measurements were compared between those with normal outcome and those resulting in spontaneous preterm delivery, pre-eclampsia and fetal growth restriction (FGR). Detection rates using a combination of the biochemical and Doppler measurements were investigated.

Results In the pregnancies resulting in pre-eclampsia ($n = 64$) and FGR ($n = 172$), the median PAPP-A was lower (0.844 and 0.813 MoM), the median uterine artery mean PI was higher (1.56 and 1.18) but the median free βhCG was not significantly different (0.923 and 0.933 MoM) than in the normal outcome group. In the preterm delivery group ($n = 159$), the median free β-hCG (0.944 MoM) and uterine artery mean PI (1.06) were not significantly different from normal but the median PAPP-A (0.928 MoM) was significantly lower than normal. In screening for pre-eclampsia, the detection rate, for a 5% false-positive rate, was 14.1% for PAPP-A, 54.7% for uterine artery mean PI and 62.1% for a combination of PAPP-A and uterine artery mean PI.

Conclusion Maternal serum PAPP-A at $11-13^{+6}$ of gestation is significantly lower in adverse pregnancy outcomes. The combination of first trimester serum PAPP-A and uterine artery mean PI at 22-24 weeks improves the screening efficacy for the prediction of pre-eclampsia. Copyright © 2005 John Wiley & Sons, Ltd.

KEY WORDS: pre-eclampsia; fetal growth restriction; PAPP-A; free βhCG; uterine artery Doppler; pregnancy complications

INTRODUCTION

Several studies have documented an association between low first-trimester maternal serum free ß-hCG and PAPP-A and subsequent development of pregnancy complications, including pre-eclampsia, fetal growth restriction (FGR) and preterm delivery (Ong *et al*., 2000; Smith *et al*., 2002; Tul *et al*., 2003; Dugoff *et al*., 2004; Krantz *et al*., 2004; Yaron *et al*., 2002a,b). Similarly, studies of uterine artery Doppler, during the second trimester, have reported that high impedance to flow is associated with increased risk for subsequent pre-eclampsia and FGR (Papageorghiou *et al*., 2004).

The aims of this study are, firstly, to examine the possible association between the maternal serum concentration of free ß-hCG and PAPP-A at $11-13^{+6}$ weeks with the uterine artery pulsatility index (PI) at 22–24 weeks, and secondly, to compare the screening characteristics of the two methods in the prediction of adverse pregnancy outcome.

METHODS

All women booked for maternity care at Harold Wood Hospital, Essex (between October 1999 to August 2002) were offered screening for trisomy 21 by a combination of fetal nuchal translucency thickness and maternal serum free ß-hCG and PAPP-A at $11-13^{+6}$ weeks (Spencer *et al*., 1999). Serum free ß-hCG and PAPP-A were measured using the Kryptor analyser (Brahms AG, Berlin, Germany), and the ultrasound scans were

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carried out by sonographers who had obtained the Fetal Medicine Foundation Certificate of competence in the $11-13^{+6}$ weeks scan (www.fetalmedicine.com). A second ultrasound examination was routinely performed at 22–24 weeks for measurement of fetal growth and examination for fetal defects. In the cases where no major fetal defect was detected, women were offered the option of participating in a screening study for pre-eclampsia by Doppler measurement of the uterine artery PI (Papageorghiou *et al*., 2001). Each uterine artery was identified using colour 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).

Demographic characteristics, ultrasound findings and the results of biochemical testing were entered into a computer database at the time of assessment. Data on pregnancy outcome were obtained from examination of individual patient notes and labour ward records.

The following outcome measures were examined: spontaneous preterm delivery, pre-eclampsia and FGR. Preterm delivery and early preterm delivery were defined by spontaneous delivery before 37 and 34 completed weeks respectively. Pre-eclampsia was defined according to the guidelines of the International Society for the Study of Hypertension in Pregnancy. This requires two recordings of diastolic blood pressure of >90 mmHg at least 4 h apart in previously normotensive women, and proteinuria of 300 mg or more in 24 h, or two readings of at least ++ on dipstick analysis of midstream or catheter urine specimens if no 24-h collection is available (Brown *et al*., 2001). FGR was defined as birthweight below the 5th centile for gestational age (Yudkin *et al*., 1987).

Statistical analysis

Free β-hCG and PAPP-A results were expressed as a multiple of the median (MoM) in normal pregnancies, using previously derived parameters (Spencer *et al*., 1999) and adjusted for maternal weight (Spencer *et al*., 2003), smoking status (Spencer *et al*., 2004) and ethnicity. (Spencer *et al*., 2000; Spencer *et al*., 2005) Regression analysis was used to determine the significance of the association between the adjusted serum free β-hCG and PAPP-A in MoM and uterine artery mean PI. The significance of differences in serum free β-hCG and PAPP-A and uterine artery mean PI between the pregnancies with a normal outcome (pregnancies without pre-eclampsia resulting in the delivery after 37 weeks of liveborn infant with birthweight above the 5th centile) and each adverse pregnancy outcome group was determined by an unpaired t-test of unequal variance after log 10 transformation of the uterine artery mean PI and MoM values to achieve a Gaussian distribution of the data. The detection rates of each adverse pregnancy outcome and false-positive rates for different cut-offs in free β-hCG, PAPP-A and mean uterine artery PI were calculated and receiver operating characteristic curves (ROC) were constructed. A multivariate Gaussian model combining PAPP-A MoM and mean uterine artery PI was developed using standard statistical techniques, and its performance in discriminating cases with pre-eclampsia compared to either measure alone was also assessed by ROC analysis. All statistical analysis was performed using Analyse-It (Smart Software, Leeds) and Microsoft Excel or SPSS 12 (SPSS, Woking).

RESULTS

The study population comprised 4390 singleton pregnancies that had both measurement of free β-hCG and PAPP-A at $11-13^{+6}$ (median 12) weeks and measurement of uterine artery mean PI at 22–24 (median 23) weeks. The median maternal age was 30 (16–47) years; 4183 (95.3%) of the women were Caucasian, 117 (2.7%) were of Afro-Caribbean origin and 89 (2.0%) were Asian; 1,746 (39.8%) were nulliparous and 668 (15.2%) were cigarette smokers.

In 3999 (91.1%) pregnancies, there was no pre-eclampsia and the outcome was delivery after 37 weeks of liveborn infants with birthweight above the 5th centile (normal group). Five cases resulted in fetal death at 24–40 (median 36) weeks. Pre-eclampsia developed in 64 (1.5%) pregnancies with livebirths and 23 (35.9%) of these deliveries occurred before 34 weeks (pre-eclampsia group). FGR occurred in 172 (3.9%) pregnancies with livebirths, including 21 cases complicated by pre-eclampsia and 7 with spontaneous preterm delivery (FGR group). Spontaneous preterm delivery occurred in 159 (3.6%) pregnancies with livebirths, including 1 case complicated by pre-eclampsia and 7 with FGR.

In the normal outcome group, the median and 5th centile for serum free β-hCG were 1.000 MoM and 0.400 MoM respectively, for serum PAPP-A were 1.000 MoM and 0.422 MoM and for the uterine artery mean PI the median and 95th centile were 1.02 and 1.48 respectively. The normal and adverse outcome groups are compared for median maternal serum free-βhCG, serum PAPP-A and uterine artery mean PI and for the incidence of low free-βhCG and PAPP-A and high uterine artery mean PI in Table 1.

In the pre-eclampsia and FGR groups, the median PAPP-A was significantly lower ($p = 0.039$ and <0.001 respectively) and median uterine artery mean PI was significantly higher than normal ($p < 0.001$ in both cases). In both pre-eclampsia and FGR groups, the median free βhCG MoM was not significantly different from normal ($p = 0.736$ and 0.451 respectively). In the preterm delivery group, the median free β-hCG MoM was not significantly different from normal ($p = 0.266$) but the median PAPP-A MoM was just significantly lower than normal ($p = 0.050$).

The distributions of $\log_{10}$ PAPP-A MoM and $\log_{10}$ uterine artery mean PI were a reasonable Gaussian fit in both the normal and the pre-eclampsia groups as

Table 1—Maternal characteristics and the median maternal free β-hCG (MoM), PAPP-A (MoM) and uterine artery mean PI in each pregnancy outcome group

	Normal	Pre-eclampsia	Pre-term	FGR
	(n = 3,999)	(n = 64)[a]	(n = 159)[b]	(172)[c]
Maternal age (yr)	30 (16–47)	29 (17–44)	31 (17–42)	30 (17–43)
Caucasian	3,816 (95.4%)	55 (85.9%)	155 (97.5%)	159 (92.4%)
Cigarette smoker	566 (14.8%)	10 (15.6%)	30 (18.9%)	64 (37.2%)
Nulliparous	1592 (39.8%)	32 (50.0%)	57 (35.8%)	74 (42.0%)
Free β-hCG (MoM)				
Median	1.000	0.923	0.944	0.933
95% Confidence interval	0.979–1.024	0.763–1.327	0.829–1.078	0.796–1.138
Range	0.115–10.477	0.16–12.86	0.22–4.83	0.12–12.86
≤5th centile (0.400 MoM)	198 (5.0%)	5 (7.8%)	11 (6.9%)	11 (6.4%)
PAPP-A (MoM)				
Median	1.000	0.844	0.928	0.813
95% Confidence interval	0.978–1.019	0.646–1.035	0.826–1.008	0.743–0.900
Range	0.172–8.876	0.26–3.40	0.23–3.98	0.06–2.89
≤5th centile (0.422 MoM)	200 (5.0%)	9 (14.1%)	11 (6.9%)	16 (9.3%)
Uterine mean PI				
Median	1,02	1.56	1.06	1.18
Range	0.49–3.2	0.60–3.0	0.6–2.05	0.62–3.0
≥95th centile (1.48)	201 (5.0%)	35 (54.7%)	13 (8.2%)	38 (22.1%)

[a] Includes one spontaneous preterm delivery and 25 with FGR.
[b] Includes one with pre-eclampsia and seven with FGR.
[c] Includes 21 with pre-eclampsia and seven with spontaneous preterm delivery.

assessed by the Shapiro–Wilk tests with a probability of $p < 0.05$. There was a small but significant association between $\log_{10}$ PAPP-A MoM and $\log_{10}$ mean PI in the control group ($p < 0.001$; $r = -0.177$) but not so in the pre-eclamptic group ($p = 0.695$; $r = -0.066$). The mean and one standard deviation (SD) of the distribution of $\log_{10}$ PAPP-A MoM in the normal group were −0.009 and 0.2177 respectively, and in the pre-eclampsia group they were −0.0801 and 0.2655 respectively. Similarly, the mean and SD of the distribution of $\log_{10}$ uterine artery mean PI in the normal group were 0.000 and 0.0982 respectively and in the pre-eclampsia group they were 0.1830 and 0.1481, respectively.

The detection rates of pre-eclampsia and false-positive rates for different cut-offs in $\log_{10}$ PAPP-A MoM and $\log_{10}$ uterine artery mean PI are shown in Figure 1. In the case of $\log_{10}$ PAPP-A MoM, the area under the ROC curve was 0.588, and the detection rate for a 5% false-positive rate was 14.1%. The respective values for uterine artery mean PI were 0.819 and 54.7%. In the multivariate Gaussian model, combining PAPP-A MoM and uterine artery mean PI, the area under the curve was improved compared with either marker alone (0.853), and the detection rate for pre-eclampsia was 62.1% for a 5% false-positive rate. The proportion of cases with FGR, which would also be identified by such a combined protocol, was approximately 16%.

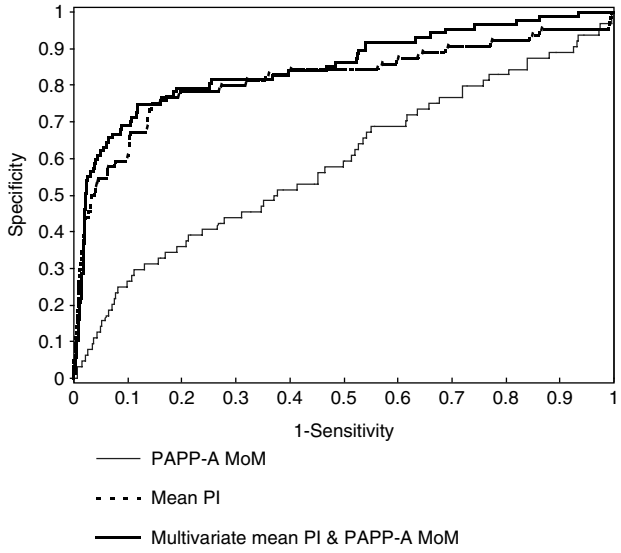

Figure 1—Receiver operator characteristic curves for $\log_{10}$ PAPP-A MoM (solid line), $\log_{10}$ uterine artery mean PI (dotted line) and the multivariate combination of the two (interrupted line)

DISCUSSION

This study has demonstrated that low maternal serum concentration of PAPP-A at $11–13^{+6}$ weeks and high uterine artery PI at $22–23^{+6}$ weeks can predict the subsequent development of pre-eclampsia. Furthermore, the prediction of this pregnancy complication is improved by combining the biochemical and sonographic markers. Thus, for a false-positive rate of 5%, the detection rate of pre-eclampsia was 14% using PAPP-A, 55% with uterine artery PI and 62% with the combination of the two.

The finding that maternal serum PAPP-A at $11–13^{+6}$ of gestation is significantly lower in those pregnancies that subsequently develop pre-eclampsia, FGR and, to a lesser degree, preterm delivery, are in general agreement with the results of previous reports. (Ong *et al*., 2000; Smith *et al*., 2002; Tul *et al*., 2003; Dugoff *et al*., 2004; Krantz *et al*., 2004; Yaron *et al*., 2002a) In one

recent study, women with pre-eclamptic symptoms in the third trimester were found to have raised levels of PAPP-A compared with women with normal pregnancies (median MoM 2.05) (Bersinger *et al*., 2003); this confirmed earlier observations of elevated PAPP-A post onset of symptoms (Hughes *et al*., 1980; Toop and Klopper, 1981). Placental extracts of normal pregnant women and pre-eclamptic patients at term also confirmed the presence of high tissue levels of PAPP-A in those affected by pre-eclampsia (Bersinger *et al*., 2002). However, we and others have found that PAPP-A levels are reduced at 11–14 weeks in pregnancies that develop pre-eclampsia in the third trimester (Ong *et al*., 2000; Smith *et al*., 2002; Dugoff *et al*., 2004; Krantz *et al*., 2004). Bersinger *et al*. (Bersinger and Odegard, 2004) have further compared PAPP-A levels at 17, 25 and 33 weeks in cases developing pre-eclampsia and found that at 17 weeks the levels were reduced, while at 25 and 33 weeks the levels were not significantly different from normal controls. These findings indicate that altered placental function and regulation of synthesis and/or secretion of PAPP-A during the first trimester is a marker of subsequent development of those pregnancy complications that are attributed to impaired placental perfusion. The underlying mechanism by which PAPP-A may affect placental function is through its action on insulin-like growth factors (IGFs), because PAPP-A is a protease for IGF binding protein 4. (Lawrence *et al*., 1999) The IGF binding proteins (IGFBP) inhibit the action of IGFs (Clemmons, 1998), which are thought to play a key role in regulating fetal growth and trophoblast invasion of the decidua. (van Kleffens *et al*., 1998; Conover *et al*., 2004) Despite this being the most plausible mechanism for the reduced PAPP-A, the literature with respect to IGF1 and IGFBP is a little confusing with regard to pre-eclampsia. In the 2nd trimester, some studies (Grobman and Kazer, 2001) find increased levels of IGF1 and reduced levels of IGFBP1 in cases that developed pre-eclampsia, whilst others (Hubinette *et al*., 2003) found no difference in levels of IGF1 of IGFBP3 at 17 or 33 weeks despite the reduced levels found in other studies over the same time period (Halhali *et al*., 2004). Clearly more studies are required in order to provide evidence to support such a mechanism and to see how it relates to changing levels of PAPP-A as the pregnancy progresses.

The association between increased uterine artery PI and subsequent development of pre-eclampsia is thought to be the consequence of impaired trophoblastic invasion of the maternal spiral arteries. (Sagol *et al*., 1999) Pre-eclampsia, which is genetically and immunologically governed, constitutes a disease of circulatory maladaptation to this defective trophoblastic invasion. (Wilson *et al*., 2002) In normal pregnancy, the luminal diameter of the spiral arteries is greatly increased and the vascular smooth muscle is replaced by trophoblast cells. In pre-eclampsia, this process is deficient with a consequent decrease in vascular capacitance and increased resistance in the uteroplacental circulation. (Lyall, 2002b).

In contrast to PAPP-A, maternal serum levels of free β-hCG were not significantly altered in pregnancies that developed complications. This lack of significance of free β-hCG levels is in contrast to our previous findings of a reduced level in the study of 135 cases that developed pre-eclampsia (Ong *et al*., 2000) but is in agreement with a smaller series (Sebire *et al*., 2000) and the data of Smith *et al*. (Smith *et al*., 2002) and Yaron *et al*. (2002b).

Low maternal serum PAPP-A at $11-13^{+6}$ weeks is associated with subsequent development of pre-eclampsia, FGR and spontaneous preterm delivery. The detection rate of pre-eclampsia in screening by second-trimester uterine artery Doppler is improved by the inclusion of first-trimester maternal serum PAPP-A. Although the use of pharmacological therapy to reverse the disease process is unlikely to be beneficial at 22–24 weeks, it may be advantageous to select a large screen positive group of say 10%, which would include 70% of the affected cases, and to follow these women with much closer surveillance throughout the rest of their pregnancy.

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REFERENCES

Bersinger NA, Odegard RA. 2004. Second and third trimester serum levels of placental proteins in pre-eclampsia and small for gestational age pregnancies. *Acta Obstet Gynecol Scand* **83**: 37–45.

Bersinger NA, Groome N, Muttukrishna S. 2002. Pregnancy associated and placental proteins in the placental tissue of normal pregnant women and patients with pre-eclampsia. *Eur J Endocrinol* **147**: 1785–1793.

Bersinger NA, Smarason AK, Muttukrishna S, Groome NP, Redman CW. 2003. Women with pre-eclampsia have increased serum levels of pregnancy associated plasma protein A (PAPP-A), inhibin A, activin A and soluble E-selectin. *Hypertens Pregnancy* **23**: 45–55.

Brown MA, Lindheimer MD, de Swiet M, Van Assche A, Moutquin JM. 2001. The classification and diagnosis of the hypertensive disorders of pregnancy: statement from the International Society for the Study of Hypertension in Pregnancy (ISSHP). *Hypertens Pregnancy* **20**: IX–XIV.

Clemmons DR. 1998. Role of insulin-like growth factor binding proteins in controlling IGF actions. *Mol Cell Endocrinol* **140**: 19–24.

Conover CA, Bale LK, Overgaard MT, *et al*. 2004. Metalloproteinases pregnancy associated plasma protein A is a critical growth factor during fetal development. *Development* **131**: 1187–1194.

Dugoff L, Hobbins JC, Malone FD, *et al*. First-trimester maternal serum PAPP-A and free-beta subunit human chorionic gonadotropin concentrations and nuchal translucency are associated with obstetric complications: a population-based screening study (the FASTER Trial). *Am J Obstet Gynecol* 2004. **191**: 1446–1451.

Grobman WA, Kazer RR. 2001. Serum insulin, insulin-like growth factor-I, and insulin-like growth factor binding protein-I in women who develop preeclampsia. *Obstet Gynecol* **97**: 521–526.

Halhali A, Villa AR, Madrazo E, *et al*. 2004. Longitudinal changes in maternal serum 1,25 di-hydroxyvitamin D and insulin like growth factor I levels in pregnant women who developed preeclampsia: comparison with normotensive pregnant women. *J Steroid Biochem Mol Biol* **89**: 553–556.

Hubinette A, Lichstein P, Brismar K, *et al*. 2003. Serum insulin-like growth factors in normal pregnancy and in pregnancies complicated by preeclampsia. *Acta Obstet Gynecol Scand* **82**: 1004–1009.

Hughes G, Bischof P, Wilson G, Smith R, Klopper A. 1980. Assay of placental proteins to determine fetal risk. *Br Med J* **280**: 671–673.

Krantz D, Goetzl L, Simpson JL, *et al*. Association of extreme first-trimester free human chorionic gonadotropin-beta, pregnancy-associated plasma protein A, and nuchal translucency with intrauterine growth restriction and other adverse pregnancy outcomes. *Am J Obstet Gynecol* 2004. **191**: 1452–1458.

Lawrence JB, Oxvig C, Overgaard MT, *et al*. The insulin-like growth factor (IGF)-dependent IGF binding protein-4 protease secreted by human fibroblasts is pregnancy-associated plasma protein-A. *Proc Natl Acad Sci U S A* 1999. **96**: 3149–3153.

Lyall F. 2002. The human placental bed revisited. *Placenta* **23**: 555–562.

Ong CY, Liao AW, Spencer K, Munim S, Nicolaides KH. 2000. First trimester maternal serum free beta human chorionic gonadotrophin and pregnancy associated plasma protein A as predictors of pregnancy complications. *BJOG* **107**: 1265–1270.

Papageorghiou AT, Yu CK, Nicolaides KH. 2004. The role of uterine artery Doppler in predicting adverse pregnancy outcome. *Best Pract Res Clin Obstet Gynaecol* **18**: 383–396.

Papageorghiou AT, Yu CKH, Bindra R, Pandis G, Nicolaides KN. 2001. Multicentre screening for pre-eclampsia and fetal growth restriction by transvaginal uterine artery Doppler at 23 weeks of gestation. *Ultrasound Obstet Gynecol* **18**: 441–449.

Sagol S, Ozkinay E, Oztekin K, Ozdemir N. 1999. The comparison of uterine artery Doppler velocimetry with the histopathology of the placental bed. *Aust N Z J Obstet Gynaecol* **39**: 324–329.

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

Smith GC, Stenhouse EJ, Crossley JA, Aitken DA, Cameron AD, Connor JM. 2002. Early pregnancy levels of pregnancy-associated plasma protein a and the risk of intrauterine growth restriction, premature birth, preeclampsia, and stillbirth. *J Clin Endocrinol Metab* **87**: 1762–1767.

Spencer K, Bindra R, Nicolaides KH. 2003. Maternal weight correction of maternal serum PAPP-A and free beta-hCG MoM when screening for trisomy 21 in the first trimester of pregnancy. *Prenat Diagn* **23**: 851–855.

Spencer K, Ong CY, Liao AW, Nicolaides KH. 2000. The influence of ethnic origin on first trimester biochemical markers of chromosomal abnormalities. *Prenat Diagn* **20**: 491–494.

Spencer K, Bindra R, Cacho AM, Nicolaides KH. 2004. The impact of correcting for smoking status when screening for chromosomal anomalies using maternal serum biochemistry and fetal nuchal translucency thickness in the first trimester of pregnancy. *Prenat Diagn* **24**: 169–173.

Spencer K, Souter V, Tul N, Snijders R, Nicolaides KH. 1999. A screening program for trisomy 21 at 10–14 weeks using fetal nuchal translucency, maternal serum free β human chorionic gonadotropin and pregnancy-associated plasma protein-A. *Ultrasound Obstet Gynecol* **13**: 231–237.

Spencer K, Heath V, El-Sheikhah A, Ong CYT, Nicolaides KH. 2005. Ethnicity and the need for correction of biochemical and ultrasound markers of chromosomal anomalies in the first trimester: a study of Oriental, Asian and Afro-Caribbean populations. *Prenat Diagn* **25**: 365–369.

Toop K, Klopper A. 1981. Concentration of Pregnancy Associated Plasma Protein—A (PAPP-A) in patients with pre-eclamptic toxaemia. *Placenta* **4**: 167–1747.

Tul N, Pusenjak S, Osredkar J, Spencer K, Novak-Antolic Z. 2003. Predicting complications of pregnancy with first-trimester maternal serum free-betahCG, PAPP-A and inhibin-A. *Prenat Diagn* **23**: 990–996.

van Kleffens M, Groffen C, Lindenbergh-Kortleve DJ, *et al*. 1998. The IGF system during fetal-placental development of the mouse. *Mol Cell Endocrinol* **140**: 129–135.

Wilson ML, Goodwin TM, Pan VL, Ingles SA. 2002. Molecular epidemiology of pre-eclampsia. *Obstet Gynecol Surv* **58**: 39–66.

Yaron Y, Heifetz S, Ochshorn Y, Lehavi O, Orr-Urtreger A. 2002a. Decreased first trimester PAPP-A is a predictor of adverse pregnancy outcome. *Prenat Diagn* **22**: 778–782.

Yaron Y, Oschshorn Y, Heifetz S, Lehavi O, Sapir Y, Orr-Urtreger A. 2002b. First trimester maternal serum free human chorionic gonadotropin as a predictor of adverse pregnancy outcome. *Fetal Diagn Ther* **17**: 352–356.

Yudkin PL, Aboualfa M, Eyre JA, Redman CW, Wilkinson AR. 1987. New birthweight and head circumference centiles for gestational ages 24 to 42 weeks. *Early Hum Dev* **15**: 45–52.