First-trimester biochemical markers of aneuploidy and the prediction of small-for-gestational age fetuses

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ABSTRACT

Objectives *To examine the clinical utility of the first-trimester biochemical markers of aneuploidy in their ability to predict subsequent delivery of a small-for-gestational age (SGA) infant.*

Methods *We examined singleton pregnancies with no chromosomal abnormality and with complete outcome data that had undergone screening for trisomy 21 by a combination of fetal nuchal translucency (NT) thickness and maternal serum free β-human chorionic gonadotropin (β-hCG) and pregnancy-associated plasma protein-A (PAPP-A) at 11 + 0 and 13 + 6 weeks' gestation. The biochemical markers were converted to multiples of the expected normal median (MoM) for a pregnancy of the same gestation. The association between free β-hCG and PAPP-A and the incidence of SGA were assessed by comparing the relative incidence at MoM cut-offs and birth-weight centile cut-offs. At various marker levels the likelihood ratios (LR) for SGA were also calculated after excluding other adverse pregnancy complications.*

Results *There were 46 262 pregnancies resulting in live births with birth weight at or above the 10th centile, and 3539 below the 10th centile for gestation (SGA). There was a significant inverse association between the risk for SGA and maternal serum PAPP-A MoM but not free β-hCG MoM. At the 5th centile of the normal outcome group for PAPP-A (0.415 MoM) the odds ratios for SGA below the 10th, 5th and 3rd centiles of normal were 2.70, 3.21 and 3.66 and the respective detection rates for SGA were 12.0%, 14.0% and 16.0%.*

Conclusions *Low levels of maternal serum PAPP-A are associated, in the absence of an abnormal karyotype, with an increased risk for subsequent delivery of an SGA infant.*

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INTRODUCTION

In the first trimester of pregnancy the placentally-derived biochemical markers pregnancy-associated plasma protein-A (PAPP-A) and free β-human chorionic gonadotropin (β-hCG) are increasingly being used in conjunction with the ultrasound measurement of nuchal translucency thickness (NT) as part of screening programs for trisomy 21 and other aneuploidies in which approximately 90% of such anomalies can be identified[1–7] for a false positive rate of 5%. Preliminary studies[8] have shown that reduced levels of these biochemical markers – particularly PAPP-A – may be of potential value in identifying those pregnancies that may result in adverse outcome, including the delivery of a small-for-gestational age (SGA) infant.

In this study we examine the clinical utility of free β-hCG and PAPP-A measurements in their ability to predict SGA in a large cohort of women prospectively screened during the first trimester.

METHODS

All the women booked for maternity care at the following UK hospitals were offered screening for trisomy 21 by a combination of fetal NT and maternal serum free β-hCG and PAPP-A at 11 + 0 to 13 + 6 weeks' gestation: Harold Wood Hospital, Romford (between June 1998 and December 2003); King George Hospital, Goodmayes (between July 2001 and December 2003); Kent and Canterbury Hospital, Canterbury (between July 2002 and December 2003); William Harvey Hospital,

ORIGINAL PAPER

Ashford (between July 2002 and December 2003); Queen Elizabeth The Queen Mother's Hospital, Margate (between July 2002 and December 2003); King's College Hospital, London (between January 1999 and February 2000); and those attending The Fetal Medicine Centre, London (between July 1999 and December 2003). They received an information leaflet about the service and gave details about their demographic characteristics and medical history, which were entered into a computer database (PIA-Fetal Database, ViewPoint, Webling, Germany).

Maternal serum free β-hCG and PAPP-A were measured using the Kryptor analyzer (Brahms AG, Berlin, Germany) as previously described[1] and an ultrasound examination was carried out to measure fetal NT and crown–rump length (CRL) and to diagnose any major fetal abnormalities. All scans were carried out by sonographers who had obtained The Fetal Medicine Foundation Certificate of Competence in the $11 + 0$ to $13 + 6$-week scan (www.fetalmedicine.com). Patient-specific risks were calculated by a multivariate approach using biochemical population parameters as outlined in a previous study[1], likelihood ratios based on delta NT – the difference from the normal median NT at the measured CRL – as outlined in a previous study[9] and the gestational age-related risk of trisomy 21 at the time of screening[10]. Data on pregnancy outcomes were obtained from the cytogenetics laboratories, the National Chromosomal Anomaly Register, the patients themselves, their general practitioners, or the maternity units in which they delivered.

The measured free β-hCG and PAPP-A were converted to multiples of the expected normal median (MoM) for a pregnancy of the same gestational age using values established in a previous study[8] corrected for maternal weight[11,12], self-recorded smoking status[13,14] and ethnicity[11,15].

The fetal database was searched to identify singleton pregnancies resulting in live births with no features of a chromosomal abnormality and with complete data on maternal ethnic origin, maternal weight, cigarette smoking status, gestational age at delivery and birth weight. The women were assigned to two groups – the normal pregnancy group was defined as those pregnancies in which a live baby was delivered after 37 complete weeks of gestation with a birth weight at or above the 10th centile for gestational age[16]. The SGA group was classified into three subgroups according to birth weight below the 10th, 5th and 3rd centile for gestational age[16]. Cases included in this study were not part of our previous study[8] but were part of those included in a recent study of PAPP-A and ADAM12[17].

The association between serum metabolites and the incidence of SGA was assessed by comparing the relative incidence at a number of MoM cut-offs and at various birth-weight centile cut-offs. Likelihood ratios (LR) for SGA were also calculated.

Regression analysis was used to determine the significant independent contributors in the prediction of SGA from the following variables: maternal serum PAPP-A and free β-hCG as MoMs, maternal weight as kg, cigarette smoking (yes or no) and maternal ethnic origin (Caucasian, Afro-Caribbean, Oriental (Chinese or Japanese) and Asian (Indian or Pakistani)). Statistical analysis was performed with Analyse-It (Analyse-It Software Ltd, Leeds, UK), SPSS for Windows (v.13, Chicago, IL, USA) and Excel for Windows 2003 (Microsoft Corp., Redmond, WA, USA). A *P*-value of less than 0.05 was considered statistically significant.

RESULTS

The normal group comprised 46 262 pregnancies resulting in live births at term with no features of chromosomal abnormality. The SGA group comprised 3539 pregnancies resulting in live births at any gestation with no features of chromosomal abnormality. In 1605 cases the birth weight was below the 5th centile and in 979 cases it was below the 3rd centile. The characteristics of the normal pregnancy group and the SGA group are summarized in Table 1.

Multiple regression analysis demonstrated significant independent contributions in predicting SGA from PAPP-A, as well as maternal weight, smoking and ethnic origin (Table 2).

In SGA pregnancies the median PAPP-A MoM was significantly lower than that in the normal group, but the median free β-hCG was not statistically significantly different from normal (Table 3). At the 5th centile of the normal outcome group for PAPP-A (0.415 MoM) the odds ratios for SGA below the 10th, 5th and 3rd centiles were 2.70, 3.21 and 3.6574 (Table 3) and the respective detection rates for SGA were 12.0%, 14.0% and 16.0%. For free β-hCG at the 5th centile of normal (0.41 MoM) the odds were only marginally increased.

The individual likelihood ratios for SGA at each specific MoM level of PAPP-A are shown in Figure 1. The best-fit equations for PAPP-A, using a power regression data fit,

Table 1 Demographic and pregnancy characteristics of the study groups

Parameter	Appropriate for gestation	Small for gestation (< 10th centile)
n	46 262	3539
Maternal age (years)	31.5 (5.5)	30.4 (5.9)
Maternal weight (kg)	66.5 (13.2)	62.7 (13.0)
Cigarette smoker (%)	10.8	21.2
Ethnicity (%)		
Caucasian	85.9	74.4
Afro-Caribbean	3.5	4.6
Indian/Pakistani	6.1	14.9
Other	4.5	6.0
Crown–rump length (mm)	63.2 (8.6)	61.7 (8.8)
Gestational age at delivery (weeks)	39.6 (1.2)	37.5 (3.1)
Birth weight (kg)	3.5 (0.4)	2.3 (0.4)

Values are either mean (SD) or percentage.

Table 2 Multiple regression analysis in the prediction of small-for-gestational age (SGA) fetuses ($n = 49\,801$)

Variable	SGA < 3rd centile		SGA < 5th centile		SGA < 10th centile	
	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
PAPP-A MoM	0.427 (0.373–0.489)	< 0.0001	0.485 (0.438–0.537)	< 0.0001	0.535 (0.500–0.573)	< 0.0001
Maternal weight (kg)	0.984 (0.978–0.989)	< 0.0001	0.976 (0.972–0.981)	< 0.0001	0.975 (0.972–0.978)	< 0.0001
Smoker	2.791 (2.394–3.255)	< 0.0001	2.640 (2.332–2.988)	< 0.0001	2.596 (2.376–2.836)	< 0.0001
Caucasian	1		1		1	
Afro-Caribbean	2.460 (1.800–3.362)	< 0.0001	2.305 (1.791–2.968)	< 0.0001	2.500 (2.106–2.969)	< 0.0001
Indian/Pakistani	2.970 (2.457–3.590)	< 0.0001	2.866 (2.468–3.329)	< 0.0001	3.013 (2.712–3.346)	< 0.0001
Other ethnic group	1.535 (1.186–1.986)	< 0.001	1.490 (1.212–1.832)	< 0.0001	1.415 (1.218–1.643)	< 0.0001

OR, odds ratio; PAPP-A MoM, pregnancy-associated plasma protein-A multiples of the median.

Table 3 Median multiples of the median (MoM) for free β-human chorionic gonadotropin (β-hCG) and pregnancy-associated plasma protein-A (PAPP-A) in pregnancies with small-for-gestational age (SGA) infants, probability of difference from the appropriately grown group and the odds ratio (OR) for SGA at the 5th centile of normal (corresponding to 0.41 MoM free β-hCG and 0.415 MoM PAPP-A)

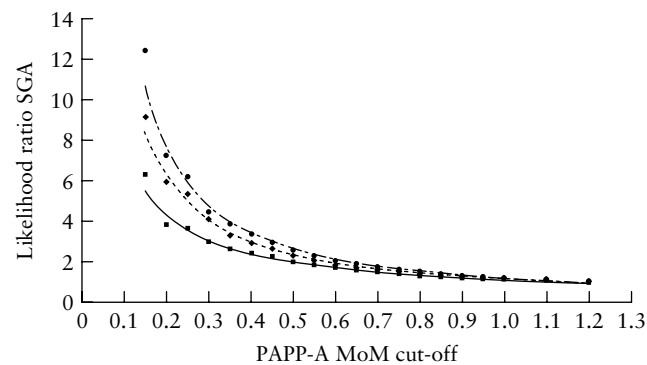| Small for gestation	Free β-hCG			PAPP-A		
	Median MoM	P	OR	Median MoM	P	OR
< 10th centile	0.979	0.159	1.43	0.823	< 0.001	2.70
< 5th centile	0.992	0.665	1.60	0.790	< 0.001	3.21
< 3rd centile	0.978	0.410	1.71	0.748	< 0.001	3.66

Figure 1 Likelihood ratio for small-for-gestational age (SGA) fetuses based on the pregnancy-associated plasma protein-A multiples of the median (PAPP-A MoM). Symbols represent individual data points, while the smoothed lines represent the best fit to the data. ▪, SGA < 10th centile; ◆, SGA < 5th centile; ●, SGA < 3rd centile.

were described by:

$$\text{LR SGA} < 10^{th}\ \text{centile} = 1.091 \times (\text{PAPP-A MoM} - 0.8543)$$

$$\text{LR SGA} < 5^{th}\ \text{centile} = 1.139 \times (\text{PAPP-A MoM} - 1.053)$$

$$\text{LR SGA} < 3^{rd}\ \text{centile} = 1.170 \times (\text{PAPP-A MoM} - 1.161)$$

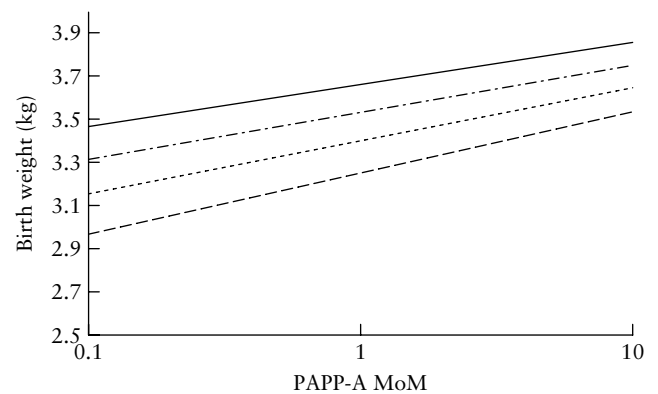The corresponding regression coefficients for goodness of fit were 0.995, 0.994 and 0.996.

Figure 2 Correlation of maternal serum pregnancy-associated plasma protein-A multiples of the median (PAPP-A MoM) with birth weight for pregnancies delivering at 38 (– – –), 39 (·······), 40 (–·–·) and 41 (——) weeks' gestation.

Table 4 Correlation of pregnancy-associated plasma protein-A (PAPP-A MoM) (as log$_{10}$) with birth weight at various gestational ages (GA)

GA at delivery (weeks)	Correlation coefficient	P	n
38	0.1457	< 0.001	8030
39	0.1288	< 0.001	11 740
40	0.1144	< 0.001	16 042
41	0.0937	< 0.001	10 570

Smith *et al.*[18] showed a significant positive correlation between first-trimester levels of PAPP-A and eventual birth weight at 38 to 41 weeks' gestation. Analysis of our data confirms this positive correlation with first-trimester levels of PAPP-A (Figure 2 and Table 4).

DISCUSSION

The data from this study confirm that the incidence of SGA is higher in women of Afro-Caribbean and Indian or Pakistani origin than in Caucasians, in cigarette smokers than in non-smokers and is inversely associated with maternal weight. Additionally, there is an association between low maternal serum PAPP-A at $11 + 0$ to $13 + 6$ weeks and subsequent delivery of an SGA infant.

Table 5 Studies reporting on the association between first-trimester maternal serum pregnancy-associated plasma protein-A (PAPP-A) and free β-human chorionic gonadotropin (β-hCG), and delivery of small-for-gestational age (SGA) infants

| | | | | Serum PAPP-A | | | | Serum free β-hCG | | | |
| | | SGA | | *Median* | < 5th centile MoM | | OR or RR | *Median* | < 5th centile MoM | | OR or RR |
Reference	Total n	Centile	n	*MoM*	OR or RR	DR	< 0.5 MoM	*MoM*	OR or RR	DR	< 0.2 MoM
Ong et al.[8]	4692	< 10th	395	0.967	1.47	7.8		1.033	1.36	6.1	
		< 5th	171	0.900	2.43	12.9		1.017	1.56	7.0	
		< 3rd	103	0.819	2.75	14.6		1.038	1.73	7.8	
Smith et al.[22]	8469	< 5th	370	0.830	2.90	12.4		0.900	1.30	8.1	
Yaron et al.[28]	1622	< 5th	49				3.30				NS
De Leon et al.[29]	145	—	10	0.760				1.700			
Krantz et al.[24]	8012	< 5th	384		2.70	9.7			1.30	5.1	
Dugoff et al.[23]	33 395	< 10th	2994		2.47	10.5			1.55		
		≤ 5th	1300		2.81	12.2			NS		
Morssink et al.[30]	800	< 5th	73	0.830				0.950			
Tul et al.[31]	1136	< 10th	51	0.760			2.36				
This study	49 801	< 10th	3539	0.823	2.70	12.0		0.979	1.43	6.5	
		< 5th	1605	0.790	3.21	14.0		0.992	1.60	7.4	
		< 3rd	979	0.748	3.66	16.0		0.978	1.71	7.9	

DR, detection rate; NS, not significant; OR, odds ratio; RR, relative risk.

Low serum PAPP-A presumably indicates impaired placentation. Certainly a plausible hypothesis to explain how low PAPP-A can reflect poor placental function and lead to potential SGA is the role of PAPP-A as an insulin-like growth factor binding protein (IGFBP)-4 and -5 protease[19,20]. Lowered levels of PAPP-A would have less of a protease effect on IGFBPs leading to higher levels of bound (biologically inactive) IGF-I and -II and thus reduced fetal growth. One recent study has shown a significant correlation of PAPP-A levels with second-trimester ultrasound measures of fetal growth such as femur length and abdominal circumference[21].

The data on biochemical markers in relation to SGA essentially confirm the initial observations we made in our earlier smaller study[8] and the data presented recently from other studies[22–24]. Since the introduction of first-trimester screening for aneuploidy using maternal serum PAPP-A and free β-hCG in conjunction with fetal NT thickness, more data in limited studies have become available, but the clinical usefulness of the data provided is often obscured by the wide range of presentation of the data. Table 5 summarizes the published data with respect to the various biochemical marker levels, odds ratios and detection rates. The presentation of our data in terms of likelihood ratios for SGA at any marker level for PAPP-A should enable clinicians to better assess an individual's risk and to use this information when counseling the patient about invasive testing, or when the results of invasive testing have identified a normal karyotype.

There is accumulating evidence that increased surveillance of high-risk fetuses reduces perinatal morbidity and mortality, with a systematic review showing that the use of umbilical artery Doppler is advantageous in monitoring such fetuses[25] but not in unselected populations[26]. Lindqvist and Molin[27] found that an awareness of SGA before delivery, in combination with a structured program of surveillance for those identified as SGA, was related to a four-fold lowered risk of adverse fetal outcome. We believe that there is now sufficient evidence to use lowered PAPP-A as part of this structured approach to identifying SGA-affected pregnancies.

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