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Prediction of Small-for-Gestation Neonates from Biophysical and Biochemical Markers at 11–13 Weeks

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Key Words
First-trimester screening · Fetal growth restriction · Small-for-gestational age · Nuchal translucency · Uterine artery Doppler · Serum biochemistry

Abstract
Objective: To develop a model for prediction of small-for-gestational age (SGA) neonates in the absence of preeclampsia (PE) based on maternal factors and biophysical and biochemical markers at 11–13 weeks' gestation. ***Methods:*** Screening study in 1,536 SGA and 31,314 non-SGA pregnancies based on maternal characteristics, fetal nuchal translucency (NT) thickness, serum pregnancy-associated plasma protein-A (PAPP-A) and free β-human chorionic gonadotrophin (β-hCG). We also measured mean arterial pressure (MAP), uterine artery pulsatility index (PI) and performed case-control studies for measurement of maternal serum concentration of placental growth factor (PLGF), placental protein 13 (PP13) and A Disintegrin And Metalloprotease (ADAM12). Regression analysis was used to develop a model for the prediction of SGA. ***Results:*** In the SGA group, uterine artery PI and MAP were increased and serum PAPP-A, free β-hCG, PLGF, PP13, and ADAM12 and fetal NT were decreased. At a false positive rate of 10%, the estimated detection rate by a combination of maternal factors and biophysical and biochemical markers at 11–13 weeks was 73% for SGA requiring delivery before 37 weeks and 46% for those delivering at term. ***Conclusions:*** Half of pregnancies with SGA neonates in the absence of PE could potentially be identified at 11–13 weeks.

Introduction

Small-for-gestational age (SGA) fetuses are at increased risk of perinatal death and handicap. These risks are substantially reduced in cases of SGA identified antenatally, compared to those detected after birth [1].

Histological studies reported that in pregnancies complicated by preeclampsia (PE) and SGA without PE there is evidence of impaired placentation characterized by inadequate trophoblastic invasion of the maternal spiral arteries [2–4]. PE, which is commonly associated with SGA, can now be predicted effectively at 11–13 weeks' gestation by algorithms combining maternal characteristics with uterine artery pulsatility index (PI), mean arterial pressure (MAP) and serum concentrations of various placental products, including pregnancy-associated plasma protein-A (PAPP-A) and placental growth factor (PLGF) [5].

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A screening study involving more than 30,000 singleton pregnancies attending for routine care at 11–13 weeks established an algorithm for the prediction of SGA in the absence of PE from maternal characteristics and the measurements of fetal nuchal translucency (NT) thickness and maternal serum concentrations of free β-human chorionic gonadotrophin (β-hCG) and PAPP-A [6]. The risk for SGA was inversely related to maternal weight and height, and increased with maternal age and in cigarette smokers, in women of African and Asian racial origin, in those with a medical history of chronic hypertension, in women with a previous SGA neonate and in those who had assisted conception. Additionally, the risk was inversely related to NT, free β-hCG and PAPP-A. The estimated detection rate of SGA neonates, at a false positive rate of 10%, in screening by maternal characteristics was 34% and this was improved to 37% by the addition of fetal NT and serum PAPP-A and free β-hCG.

Case-control studies have reported that the first-trimester maternal serum concentration of PLGF, placental protein 13 (PP13) and A Disintegrin And Metalloprotease (ADAM12) is reduced in pregnancies delivering SGA neonates in the absence of PE [7–15]. All three are placental products thought to be involved in fetal and placental growth and development.

The aim of this study is to investigate whether improved early screening for SGA in the absence of PE can be provided by uterine artery PI, MAP and serum PLGF, PP13 and ADAM12 at 11–13 weeks in addition to maternal characteristics and fetal NT, PAPP-A and free β-hCG.

Methods

Study Population

The data for this study were derived from prospective screening for adverse obstetric outcomes in women attending for their routine first hospital visit in pregnancy. In this visit, which is held at 11^{+0} to 13^{+6} weeks of gestation, we record maternal characteristics and medical history and perform combined screening for aneuploidies by measurement of the fetal crown-rump length (CRL) and NT thickness and maternal serum PAPP-A and free β-hCG [16–18]. The women were screened between March 2006 and September 2009. In the second part of the study period, we also measured the maternal MAP by automated devices [19] and used transabdominal colour Doppler ultrasound to visualise the left and right uterine artery, measure the PI in each vessel and calculate the mean PI [20]. Samples of serum and plasma are stored at –80°C for subsequent biochemical analysis. Written informed consent was obtained from the women agreeing to participate in the study, which was approved by King's College Hospital Ethics Committee.

Data on pregnancy outcome were obtained from the maternity computerised records or the general medical practitioners of the women and were recorded in our database. The neonate was considered to be SGA if the birth weight was less than the 5th percentile for gestation at delivery, using a reference range derived from our population [6]. Neonates with birth weight at or above the 5th percentile were classified as non-SGA. We excluded pregnancies with major fetal abnormalities, those ending in termination, miscarriage or fetal death before 24 weeks, those with PE and cases with no pregnancy follow-up.

In this study we compare uterine artery PI, MAP and serum PLGF, PP13 and ADAM12 in the SGA and non-SGA pregnancies. Data from these investigations were included in previous publications [9, 12, 15, 20], but in this study we combine all data to develop an integrated algorithm for the early prediction of SGA in the absence of PE. In the case of uterine artery PI and MAP, we used data from the whole screened population in which these biophysical measurements were recorded, whereas PLGF, PP13 and ADAM12 were examined in case-control studies.

Case-Control Study for Biochemical Markers

The case-control study involved measurement of maternal serum concentration of PLGF, PP13 and ADAM12 at 11–13 weeks' gestation in pregnancies complicated by the birth of SGA and non-SGA neonates. The cases were drawn from the screening study population on the basis of availability of stored serum. The non-SGA cases were from pregnancies with no complications and normal outcome matched to the SGA cases for storage time.

None of the samples were previously thawed and refrozen. Serum PLGF was measured by a quantitative enzyme-linked immunoassay (ELISA) technique using Quantikine® human PLGF immunoassay (R&D Systems Europe Ltd, Abingdon, UK). Serum PP13 was measured by DELFIA® (Dissociation-Enhanced Lanthanide Fluorescent Immunoassay) using research reagents (PerkinElmer Life and Analytical Sciences, Turku, Finland). Serum ADAM12 was measured by a heterogeneous time-resolved fluorescent immunoassay (DELFIA/AutoDELFIA ADAM12 research kit, PerkinElmer Life and Analytical Sciences).

Statistical Analysis

In each patient in the SGA and non-SGA groups the measured free β-hCG, PAPP-A, uterine artery PI and MAP in the screening study and the measured serum PLGF, PP13 and ADAM12 in the case-control study were converted to multiples of the expected normal median (MoM) corrected for fetal CRL, maternal age, weight, smoking, parity, racial origin and method of conception as previously described [5, 21]. The measured NT was expressed as a difference from the expected normal mean for gestation (Δ value) [22]. Comparisons between the SGA and non-SGA groups were conducted by χ^2 or Fisher's exact test for categorical variables and by Mann-Whitney U test for continuous variables.

The following steps were used to develop a model for predicting the birth of SGA neonates. First, in the total screened population there were 1,536 SGA and 31,314 non-SGA pregnancies. In each patient the risk for SGA was calculated using multivariate logistic regression analysis of maternal factors as previously described [6]. Secondly, gaussian distributions of markers in SGA and non-SGA pregnancies were fitted. These fitted distributions define the likelihood ratios for the screening tests that can be combined with the prior risk to produce a posterior risk. Third,

Table 1. Median and interquartile range (IQR) of uterine artery PI, MAP, Δ NT thickness, PAPP-A, free β-hCG, PLGF, PP13 and ADAM12 in the non-SGA group and in those delivering SGA neonates

Biophysical and biochemical marker	Non-SGA		All SGA		SGA <37 weeks		SGA ≥37 weeks	
	n	MoM	n	MoM	n	MoM	n	MoM
Uterine artery PI	19,957	1.02 (0.84–1.22)	1,133	1.14 (0.90–1.34)*	126	1.27 (0.96–1.53)*	1,007	1.12 (0.89–1.32)*
MAP	12,854	1.00 (0.95–1.06)	661	1.01 (0.96–1.07)*	68	1.00 (0.96–1.07)	593	1.02 (0.96–1.07)*
Δ NT	31,314	0.12 (–0.08–0.34)	1,536	0.10 (–0.12–0.30)*	163	0.11 (–0.09–0.31)	1,373	0.10 (–0.12–0.30)*
PAPP-A	31,314	1.03 (0.71–1.45)	1,536	0.82 (0.55–1.12)*	163	0.71 (0.40–0.95)*	1,373	0.83 (0.58–1.40)*
Free β-hCG	31,314	0.97 (0.66–1.47)	1,536	0.89 (0.58–1.40)*	163	0.93 (0.54–1.47)	1,373	0.89 (0.58–1.40)*
PLGF	1,869	1.00 (0.78–1.28)	274	0.90 (0.63–1.24)*	37	0.79 (0.50–1.14)†	237	0.93 (0.64–1.26)†
PP13	877	1.00 (0.76–1.34)	173	0.82 (0.62–1.07)*	20	0.79 (0.70–1.00)‡	153	0.85 (0.60–1.08)*
ADAM12	830	0.99 (0.81–1.20)	168	0.86 (0.69–1.08)*	19	0.80 (0.56–0.92)†	149	0.87 (0.70–1.08)*

Comparisons between the SGA and the non-SGA groups by Mann-Whitney U test.
Significance level * $p < 0.00001$, † $p < 0.01$, ‡ $p < 0.025$.

the maternal factors-related a priori risks and $\log_{10}$ MoM values of the biophysical and biochemical markers were simulated for 500,000 pregnancies from the SGA populations and the non-SGA distributions. For the markers this involved sampling from the fitted multivariate gaussian distributions. For the prior risks, this involved drawing samples, with replacement, 500,000 records from the screening samples of SGA and non-SGA pregnancies. These records were then used to define maternal factors-related a priori risks for SGA that were multiplied by the likelihood ratios of the biophysical and biochemical markers to derive the a posterior risks in simulated samples of 500,000 SGA and 500,000 non-SGA pregnancies. Fifth, the a priori and a posteriori risks in the SGA and non-SGA groups were used to calculate the detection rates at fixed false positive rates of 5 and 10%. The process of sampling with replacement from the SGA and non-SGA screening data means that the modelled screening performance reflects the screening population. The samples of 500,000 were chosen to make the error resulting from the simulation negligible.

The statistical software package SPSS 16.0 (SPSS Inc., Chicago, Ill., USA) was used for data analyses. Monte-Carlo simulations were programmed in R (The R Foundation for Statistical Computing, R version 2.11.0, ISBN 3–900051–070–0, http://www.r-project.org).

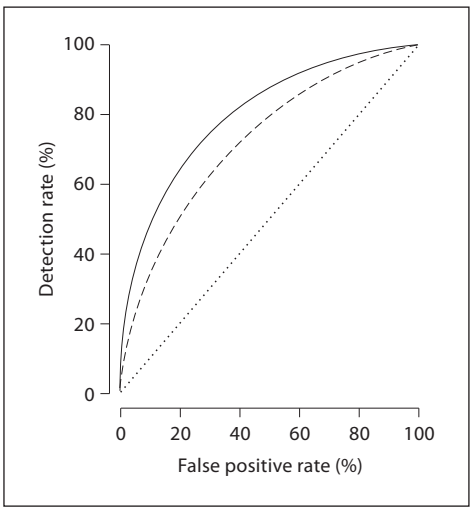

Fig. 1. Receiver operating characteristics curves in the prediction of SGA neonates in the absence of PE by maternal factors only (– – –) and by a combination of maternal factors, uterine artery PI, MAP, fetal NT, maternal serum PAPP-A, free β-hCG, PLGF, PP13 and ADAM (——).

Results

During the study period (March 2006 to September 2009) first-trimester combined screening for aneuploidies was carried out in 36,743 singleton pregnancies. We excluded 3,893 cases because they had missing outcome data (n = 2,005), the pregnancies resulted in miscarriage, termination or the birth of babies with major defects (n = 1,136), or they were complicated by PE (n = 752). In the remaining 32,850 cases there were 1,536 (4.7%) SGA and 31,314 (95.3%) non-SGA pregnancies. In the SGA group the maternal weight and height were significantly lower, and there was a higher prevalence of women of African and Asian racial origin, cigarette smokers and those who had assisted conception, a history of chronic hypertension and previous pregnancies with SGA neonates [6].

In the SGA group, compared to the non-SGA group, the median MoM uterine artery PI and MAP were increased and serum PAPP-A, free β-hCG, PLGF, PP13 and ADAM12 as well as fetal NT were decreased (table 1). The

Table 2. Inter-correlation between $\log_{10}$ MoM values of uterine artery PI, MAP, PAPP-A, free β-hCG, PLGF and PP13 and square root ADAM12 MoM and Δ NT thickness, in pregnancies delivering non-SGA and SGA neonates

Variable	Uterine artery PI		MAP		NT		PAPP-A	
	r	p	r	p	r	p	r	p
Non-SGA pregnancies								
Uterine artery PI	1.000	–	−0.044	<0.0001	−0.015	0.038	−0.146	<0.0001
MAP	−0.044	<0.0001	1.000	–	0.011	0.233	−0.014	0.121
NT	−0.015	0.038	0.011	0.233	1.000	–	0.010	0.082
PAPP-A	−0.146	<0.0001	−0.014	0.121	0.010	0.082	1.000	–
Free β-hCG	−0.014	0.055	−0.006	0.464	−0.024	<0.0001	0.215	<0.0001
PLGF	−0.116	<0.0001	0.019	0.408	0.043	0.064	0.317	<0.0001
PP13	−0.089	0.009	0.035	0.312	0.090	0.008	0.300	<0.0001
ADAM12	−0.005	0.881	0.077	0.029	0.054	0.121	0.414	<0.0001
SGA pregnancies								
Uterine artery PI	1.000	–	−0.152	<0.0001	−0.040	0.182	−0.184	<0.0001
MAP	−0.152	<0.0001	1.000	–	−0.079	0.041	0.001	0.979
NT	−0.040	0.182	−0.079	0.041	1.000	–	0.016	0.536
PAPP-A	−0.184	<0.0001	0.001	0.979	0.016	0.536	1.000	–
Free β-hCG	−0.015	0.606	0.055	0.157	−0.023	0.370	0.174	<0.0001
PLGF	−0.261	<0.0001	−0.073	0.288	0.082	0.178	0.438	<0.0001
PP13	−0.254	0.001	0.148	0.053	0.026	0.733	0.381	<0.0001
ADAM12	−0.030	0.696	0.043	0.577	−0.028	0.715	0.513	<0.0001

r = Pearson correlation coefficient, p = significance value.

differences between the SGA and non-SGA groups were greater for the subgroup of SGA delivering before 37 weeks (n = 163) than those delivering at or after 37 weeks (n = 1,373) for most markers apart from MAP, fetal NT and serum free β-hCG. The inter-correlations between biophysical and biochemical markers in the SGA and non-SGA pregnancies are shown in table 2.

The estimated detection rates at fixed false positive rates of 5 and 10% in screening by maternal factors only and by combinations of maternal factors with biophysical and biochemical markers are given in table 3. Receiver operating characteristic curves are given in figure 1.

Discussion

This study has demonstrated that an algorithm combining maternal characteristics and biophysical and biochemical tests at 11–13 weeks could potentially identify half of pregnancies that deliver SGA neonates in the absence of PE, at a 10% false positive rate. We used logistic regression analysis to derive the a priori risk for SGA from maternal characteristics in a prospective screening study involving more than 30,000 pregnancies. The patient-specific a posteriori odds for SGA were then calculated by multiplying the a priori odds with the likelihood ratio of a series of biophysical and biochemical markers after appropriate adjustments for the inter-correlations of all markers.

The data confirm the results of previous studies and indicate that, as in the case of PE, in pregnancies with SGA in the absence of PE there is evidence of impaired placental perfusion and function from the first trimester of pregnancy. However, the magnitude of such impairment is considerably less than in PE [5]. This is not surprising because, unlike PE which is a pathological disorder, SGA is a heterogeneous condition which includes constitutionally small fetuses, at no or minimally increased risk of perinatal death and handicap, and growth-restricted fetuses (FGR) due to impaired placentation, genetic disease or environmental damage. In our study we excluded fetal abnormalities but did not distinguish between constitutional SGA and FGR by such measures as performing Doppler studies in the third trimester of pregnancy [23] because the diagnosis of SGA was made retrospectively. Nevertheless, we found that the differ-

Free β-hCG		PLGF		PP13		ADAM12	
r	p	r	p	r	p	r	p
–0.014	0.055	–0.116	<0.0001	–0.089	0.009	–0.005	0.881
–0.006	0.464	0.019	0.408	0.035	0.312	0.077	0.029
–0.024	<0.0001	0.043	0.064	0.090	0.008	0.054	0.121
0.215	<0.0001	0.317	<0.0001	0.300	<0.0001	0.414	<0.0001
1.000	–	0.138	<0.0001	0.378	<0.0001	0.238	<0.0001
0.138	<0.0001	1	–	0.037	0.290	0.259	<0.0001
0.378	<0.0001	0.037	0.290	1	–	0.413	<0.0001
0.238	<0.0001	0.259	<0.0001	0.413	<0.0001	1.000	–
–0.015	0.606	–0.261	<0.0001	–0.254	0.001	–0.030	0.696
0.055	0.157	–0.073	0.288	0.148	0.053	0.043	0.577
–0.023	0.370	0.082	0.178	0.026	0.733	–0.028	0.715
0.174	<0.0001	0.438	<0.0001	0.381	<0.0001	0.513	<0.0001
1.000	–	0.049	0.421	0.444	<0.0001	0.207	<0.0001
0.049	0.421	1.000	–	0.054	0.490	0.303	<0.0001
0.444	<0.0001	0.054	0.490	1.000	–	0.404	<0.0001
0.207	0.007	0.303	<0.0001	0.404	<0.0001	1.000	–

Table 3. Performance of screening for delivery of SGA neonates by maternal factors only and maternal factors with uterine artery pulsatility index, mean arterial pressure, fetal nuchal translucency thickness, maternal serum pregnancy-associated plasma protein-A, free β-human chorionic gonadotrophin, placental growth factor, placental protein 13, A Disintegrin And Metalloprotease and their combinations

Method of screening	All SGA		SGA <37 weeks		SGA >37 weeks	
	DR for FPR 5%	DR for FPR 10%	DR for FPR 5%	DR for FPR 10%	DR for FPR 5%	DR for FPR 10%
Maternal factors	21.0	34.0	23.3	35.0	20.8	33.9
Maternal factors plus						
uterine artery PI	23.9	35.9	32.0	44.5	23.1	34.9
MAP	22.5	34.5	22.6	34.6	22.6	34.6
NT	21.8	33.9	22.5	34.5	22.0	33.6
PAPP-A	25.5	37.5	35.5	47.8	24.5	36.6
free β-hCG	22.4	34.1	22.9	34.8	22.4	34.1
PLGF	25.1	37.0	34.3	46.2	23.9	35.7
PP 13	24.7	37.0	25.9	38.6	24.8	37.1
ADAM12	25.1	37.3	29.5	42.6	24.7	37.1
biophysical markers	25.5	37.7	33.7	46.8	24.3	36.8
biochemical markers	30.4	42.7	50.1	63.0	29.0	41.7
all markers	34.3	47.3	60.7	73.2	32.5	45.8

DR = Detection rate; FPR = false positive rate.

ences in uterine artery PI and serum PAPP-A, PLGF, PP13 and ADAM12 between the SGA and non-SGA groups were greater for the subgroup of SGA delivering before 37 weeks than those delivering at or after 37 weeks. Since the proportion of FGR to constitutional SGA is likely to be higher in the preterm rather than term SGA, our findings imply that the early biophysical and biochemical markers could be identifying the FGR subgroup amongst the SGA. This should be the subject of prospective studies involving follow-up assessment in the late second and third trimesters of fetuses identified in early pregnancy as being at high risk for subsequent development of SGA.

Assessment of the patient-specific risk for SGA at 11–13 weeks is a by-product of early screening for aneuploidies. In this hospital visit a series of maternal characteristics are recorded because these are essential for the correct interpretation of the measured free β-hCG and PAPP-A [21]. Additionally, an ultrasound scan is performed for examination of the fetal anatomy and assessment of markers of aneuploidy. We have shown that the use of an algorithm derived from multiple regression analysis of a series of basic maternal characteristics and the results of the combined test for aneuploidies can identify 37% of pregnancies that will deliver SGA neonates, at a false positive rate of 10% [6]. We have previously advocated that the same ultrasound examination at 11–13 weeks should include measurement of uterine artery PI because this in combination with maternal characteristics, MAP and biomarkers could identify about 90% of pregnancies that will develop PE [5, 24]. This study has shown that inclusion of such additional biochemical and biophysical markers could improve the early detection of SGA in the absence of PE to 73% for those requiring delivery before 37 weeks and 46% for those delivering at term.

Early estimation of patient-specific risks for SGA could potentially improve pregnancy outcome by shifting antenatal care from a series of routine visits to a more individualized approach both in terms of the schedule and content of such visits. There is also evidence that the prophylactic use of low-dose aspirin started in early pregnancy can potentially halve the incidence of FGR [25].

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