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Serum placental growth factor in the three trimesters of pregnancy: effects of maternal characteristics and medical history

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KEYWORDS: first-trimester screening; placental growth factor; pre-eclampsia; pyramid of pregnancy care; second-trimester screening; third-trimester screening; trisomy 21

ABSTRACT

Objective *To define the contribution of maternal variables which influence the measured level of maternal serum placental growth factor (PlGF) in screening for pregnancy complications.*

Methods *Maternal characteristics and medical history were recorded and serum levels of PlGF were measured in women with a singleton pregnancy attending for three routine hospital visits at 11 + 0 to 13 + 6, 19 + 0 to 24 + 6 and 30 + 0 to 34 + 6 or 35 + 0 to 37 + 6 weeks' gestation. For women delivering phenotypically normal live births or stillbirths ≥ 24 weeks' gestation, variables from maternal demographic characteristics and medical history important in the prediction of PlGF were determined from a linear mixed-effects multiple regression.*

Results *Serum levels of PlGF were measured in 38 002 cases in the first trimester, 10 281 in the second trimester and 12 392 in the third trimester. Significant independent contributions to serum PlGF were provided by gestational age, maternal age, weight and racial origin, cigarette smoking, diabetes mellitus, and gestational age at delivery and birth-weight Z-score of the neonate in the previous pregnancy. The machine used to measure serum PlGF was also found to have a significant effect. Allowing for other factors, the effect of maternal age on PlGF changed over the three trimesters, whereas other variables had constant effects over the three trimesters. Random-effects multiple regression analysis was used to define the contribution of maternal variables that influence the measured serum PlGF and express the values as multiples of the median (MoMs). The model was shown to provide an adequate fit of MoM values for all covariates, both in pregnancies that developed pre-eclampsia and in those without this complication.*

Conclusions *A model was fitted to express the measured level of maternal serum PlGF across the three trimesters of pregnancy as MoMs, after adjusting for variables of maternal characteristics and medical history that affect this measurement.* Copyright © 2015 ISUOG. *Published by John Wiley & Sons Ltd.*

INTRODUCTION

Placental growth factor (PlGF) is a member of the vascular endothelial growth factor family and is implicated in angiogenesis and trophoblastic invasion of the maternal spiral arteries[1–3]. Maternal serum levels of PlGF at 11–13 weeks' gestation are decreased in pregnancies with fetal aneuploidy and those with impaired placentation resulting in pre-eclampsia (PE) and delivery of small-for-gestational-age (SGA) neonates[4–9]. Serum levels of PlGF are also reduced in the second and third trimesters of pregnancies that develop PE or deliver SGA neonates[10–15].

Our approach to risk assessment and screening for aneuploidy and pregnancy complications is to apply Bayes' theorem to combine the *a-priori* risk from maternal characteristics and medical history with the results of various combinations of biophysical and biochemical measurements made at different times during pregnancy[16–18]. In normal pregnancies, serum PlGF concentration is affected by gestational age and maternal characteristics, including age, weight, racial origin and cigarette smoking[5,10]. Consequently, for the effective use of serum PlGF in risk assessment, these variables need to be taken into account and this can be achieved by standardizing the measured levels into multiples of the normal median (MoM) values.

The objectives of this study were to first, identify and quantify the effects of variables from maternal

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characteristics and medical history on serum levels of PlGF, second, to present a model for standardizing serum PlGF measurements in all three trimesters as MoM values and third, summarize the distribution of MoM values in pregnancies with normal outcome and those that subsequently develop PE. The main focus of this paper is on pregnancies with normal outcome. Further details of the distribution of PlGF MoM values in pregnancies with PE, SGA and fetal aneuploidy are the subject of other publications.

METHODS

Study population

The data for this study were derived from prospective screening for adverse obstetric outcomes in women attending for three routine hospital visits at King's College Hospital, University College London Hospital and Medway Maritime Hospital, UK, between January 2006 and March 2014. In the first visit, at 11 + 0 to 13 + 6 weeks' gestation, maternal characteristics and medical history were recorded and combined screening for aneuploidies was performed[19]. The second visit, at 19 + 0 to 24 + 6 weeks' gestation, and third visit, initially at 30 + 0 to 34 + 6 weeks and subsequently at 35 + 0 to 37 + 6 weeks, included ultrasound examination of the fetal anatomy and estimation of fetal size from measurement of fetal head circumference, abdominal circumference and femur length and maternal blood sampling for biochemical testing. Gestational age was determined by the measurement of fetal crown–rump length (CRL) at 11–13 weeks or the fetal head circumference at 20–24 weeks[20,21].

Written informed consent was obtained from the women agreeing to participate in a study on adverse pregnancy outcome, which was approved by the Ethics Committee of each participating hospital. The inclusion criteria for this study were a singleton pregnancy delivering a phenotypically normal live birth or stillbirth ≥ 24 weeks' gestation. We excluded pregnancies with aneuploidies or major fetal abnormalities and those ending in termination, miscarriage or fetal death before 24 weeks.

Patient characteristics

Patient characteristics that were recorded included maternal age, racial origin (Caucasian, Afro-Caribbean, South Asian, East Asian and mixed), method of conception (spontaneous/assisted conception requiring the use of ovulation drugs/*in-vitro* fertilization), cigarette smoking during pregnancy (yes/no), medical history of chronic hypertension (yes/no), diabetes mellitus (yes/no), systemic lupus erythematosus (SLE) or antiphospholipid syndrome (APS), family history of PE in the mother of the patient (yes/no) and obstetric history including parity (parous/nulliparous if no previous pregnancy ≥ 24 weeks), previous pregnancy with PE (yes/no), gestational age at delivery and birth weight of the neonate in the last pregnancy and interval in years between birth of the last child and estimated date of conception of the current pregnancy. Maternal height was measured in the first visit only and maternal weight in each visit.

Measurement of maternal serum placental growth factor

Of the patients included in the study, maternal serum PlGF was measured at each visit by automated biochemical analyzers within 10 min of blood sampling. In 35 069 cases, the sample was analyzed using the DELFIA Xpress system (PerkinElmer Life and Analytical Sciences, Waltham, USA) and, in 23 612 cases, the analysis was performed by the Cobas e411 system (Roche Diagnostics, Penzberg, Germany).

Outcome measures

Data on pregnancy outcome were collected from the hospital maternity records or the general medical practitioners of the women. The obstetric records of all women with pre-existing or pregnancy-associated hypertension were examined to determine if the condition was chronic hypertension, PE or non-proteinuric gestational hypertension (GH).

The definitions of GH and PE were those of the International Society for the Study of Hypertension in Pregnancy[22]. GH was defined as systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg on at least two occasions 4 h apart, developing after 20 weeks of gestation in previously normotensive women. PE was defined as GH with proteinuria ≥ 300 mg in 24 h or two readings of at least ++ on dipstick analysis of midstream or catheter urine specimens if no 24-h collection was available. PE superimposed on chronic hypertension was defined as significant proteinuria (as defined above) after 20 weeks of gestation in women with known chronic hypertension (history of hypertension before conception or the presence of hypertension at the booking visit before 20 weeks' gestation in the absence of trophoblastic disease). The birth-weight *Z*-score of the neonate in the last pregnancy was derived from our reference range of birth weight for gestational age at delivery[23].

Statistical analysis

The effect of the following variables from maternal characteristics and medical history on serum levels of PlGF were examined: age, weight, height, racial origin, family history of PE in the mother of the patient, history of chronic hypertension, diabetes mellitus Type 1 or Type 2, SLE or APS, parity, previous pregnancy with PE, gestational age at delivery and birth weight of the neonate in the last pregnancy and interpregnancy interval, method of conception, smoking during pregnancy and gestational age at assessment.

Multiple linear regression models were fitted to $\log_{10}$ PlGF values within each trimester. Continuous variables were coded initially into groups and represented as factors

Table 1 Maternal and pregnancy characteristics in women with singleton pregnancy attending for routine visits between January 2006 and March 2014, according to trimester of pregnancy

Characteristic	*11 + 0 to 13 + 6 weeks* (n = *38 002*)	*19 + 0 to 24 + 6 weeks* (n = *10 281*)	*30 + 0 to 37 + 6 weeks* (n = *12 392*)
Maternal age (years)	31.1 (26.6–34.9)	31.1 (26.6–34.8)	31.1 (26.7–34.8)
Maternal weight (kg)	66.7 (59.0–77.3)	71.0 (63.3–82.0)	77.0 (68.8–88.0)
Maternal height (cm)	164.5 (160.0–169.0)	165.0 (160.0–169.0)	165.0 (160.0–169.0)
GA at examination (weeks)	12.7 (12.3–13.1)	21.9 (21.2–22.1)	32.3 (32.0–32.9)
Racial origin			
Caucasian	27 778 (73.1)	7522 (73.2)	8990 (72.5)
Afro-Caribbean	6710 (17.7)	1896 (18.4)	2380 (19.2)
South Asian	1713 (4.5)	435 (4.2)	464 (3.7)
East Asian	884 (2.3)	203 (2.0)	251 (2.0)
Mixed	917 (2.4)	225 (2.2)	307 (2.5)
Medical history			
Chronic hypertension	585 (1.5)	145 (1.4)	167 (1.3)
Diabetes mellitus	334 (0.9)	109 (1.1)	123 (1.0)
SLE/APS	59 (0.2)	18 (0.2)	20 (0.2)
Cigarette smoker	3714 (9.8)	1042 (10.1)	1198 (9.7)
Family history of PE	2920 (3.9)	1090 (3.6)	398 (3.2)
Obstetric history			
Nulliparous	18 191 (47.9)	4924 (47.9)	6012 (48.5)
Parous with no previous PE	18 444 (48.5)	4981 (48.4)	5953 (48.0)
Parous with previous PE	1367 (3.6)	376 (3.7)	427 (3.4)
Interpregnancy interval (years)	3.0 (2.0–5.0)	3.0 (1.9–5.0)	3.2 (2.1–5.2)
GA of previous pregnancy (weeks)	40 (39–40)	40 (39–40)	40 (39–40)
Birth weight of previous pregnancy (g)	3435 (3090–3770)	3450 (3090–3780)	3377 (3030–3708)
Mode of conception			
Spontaneous	36 735 (96.7)	9920 (96.5)	11 994 (96.8)
Ovulation induction	379 (1.0)	101 (1.0)	113 (1.0)
In-vitro fertilization	888 (2.3)	260 (2.5)	285 (2.3)
Pregnancy outcome			
PE	1167 (3.1)	334 (3.2)	319 (2.6)
No PE	36 835 (96.9)	9947 (96.8)	12 073 (97.4)

Data are given as median (interquartile range) or *n* (%). APS, antiphospholipid syndrome; GA, gestational age; PE, pre-eclampsia; SLE, systemic lupus erythematosus.

to identify suitable parametric forms. Backward elimination was used to identify potentially important terms in the model by sequentially removing non-significant ($P > 0.05$) variables. Effect sizes were assessed relative to the error standard deviation (SD) and a criterion of 0.1 SD was used to identify terms that had little substantive impact in model predictions. Residual analyses were used to assess the adequacy of the model. Graphical displays of the relationship between gestational age and PlGF levels and the effects of maternal age, weight, height and other characteristics on PlGF MoM values were produced for the final model. Having identified potential models for each trimester, a parsimonious model was selected to cover the data for the three trimesters combined. This model was fitted using a linear mixed model with random effects to represent random effects between women. A smooth relationship for gestational age that provided an adequate fit across all trimesters was achieved after applying equal weights to each trimester. The unweighted model was dominated by the first trimester and provided a relatively poor fit to the second and third trimesters. A full analysis of residuals, including an investigation of interactions, was used to check the model fit and, on the basis of this, model refinements were made.

The statistical software package R was used for data analyses[24].

RESULTS

Characteristics of the study population

The maternal characteristics and medical history in women that fulfilled the entry criteria are presented in Table 1. Serum levels of PlGF were measured in 38 002 cases in the first trimester, 10 281 in the second trimester

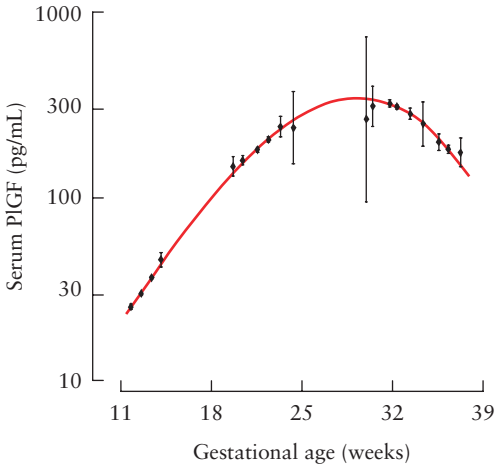

Figure 1 Relationship between median (95% CI) levels of serum placental growth factor (PlGF) and gestational age across the three trimesters of pregnancy.

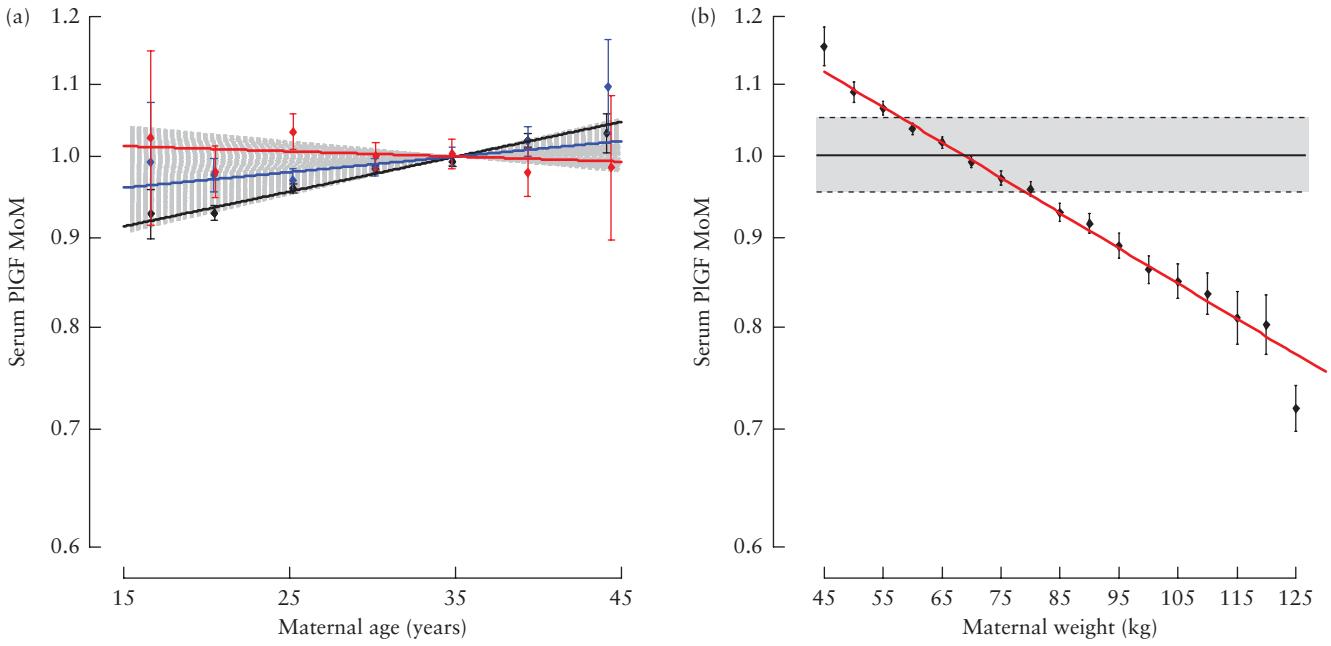

Figure 2 (a) Effect of maternal age on median serum placental growth factor (PlGF) (with 95% CI) at 11 + 0 to 13 + 6 weeks' gestation (black), 19 + 0 to 24 + 6 weeks (blue) and 30 + 0 to 37 + 6 weeks (red), plotted on multiples of the median (MoM) scale after correcting for other factors. Shaded areas show range of possible relationships between maternal age and serum PlGF for gestational ages 11–37 weeks. (b) Relationship between serum PlGF and maternal weight. Fitted effect (—), median MoM of 1.0 (—) and median MoM ± 0.1 SD (----) are indicated (log MoM scale).

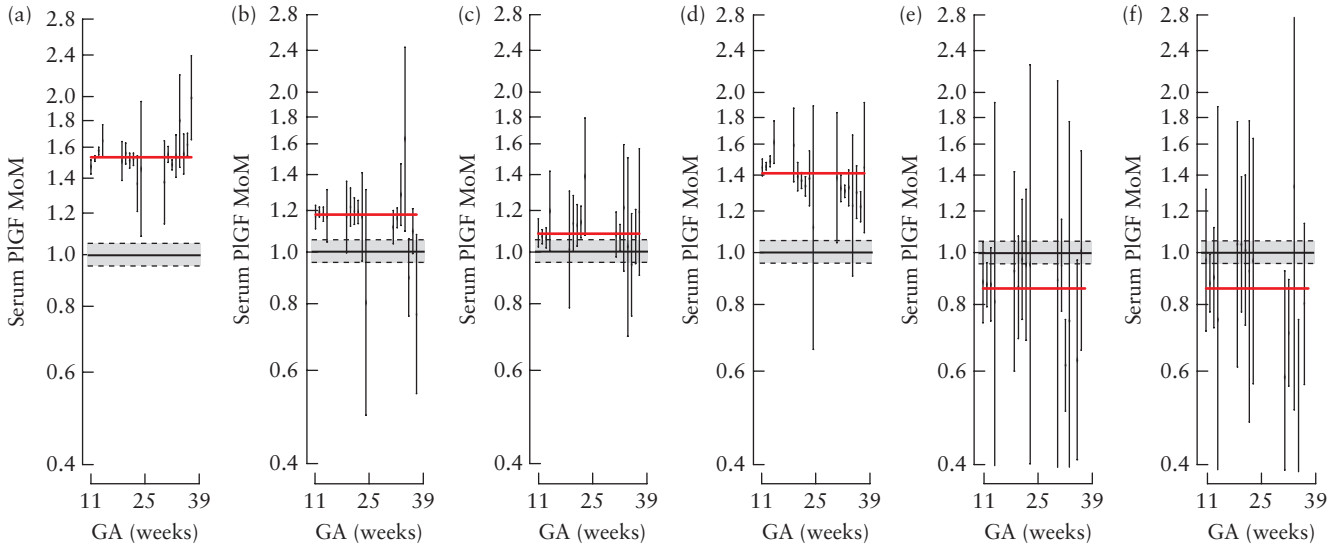

Figure 3 Effects of Afro-Caribbean (a), South Asian (b) and East Asian (c) racial origin, cigarette smoking (d) and diabetes mellitus Type 1 (e) and Type 2 requiring treatment with insulin (f) on median serum placental growth factor (PlGF) (with 95% CI), plotted on multiples of the median (MoM) scale after correcting for other factors. Fitted effects (—), median MoM of 1.0 (—) and median MoM ± 0.1 SD (----) are indicated. GA, gestational age.

and 12 392 in the third trimester. In the first phase of the study the serum PlGF was measured only in the first-trimester visit but this was subsequently extended to the second- and then the third-trimester visits. There were 3908 cases with measurements in all three trimesters, 2567 with measurements in the first and second trimesters, 4614 in the first and third trimesters, 1497 in the second and third trimesters, 26 913 in the first trimester only, 2309 in the second trimester only and 2373 in the third trimester only.

Variables affecting serum PlGF

Variables with substantial effect on serum levels of PlGF were the machine used for the measurements, gestational age at assessment, maternal age, weight, racial origin, cigarette smoking, diabetes mellitus, and gestational age at delivery and birth-weight *Z*-score of the neonate in the previous pregnancy. Median levels of serum PlGF showed a curvilinear relationship with gestational age with increase in the first and second trimester, reaching

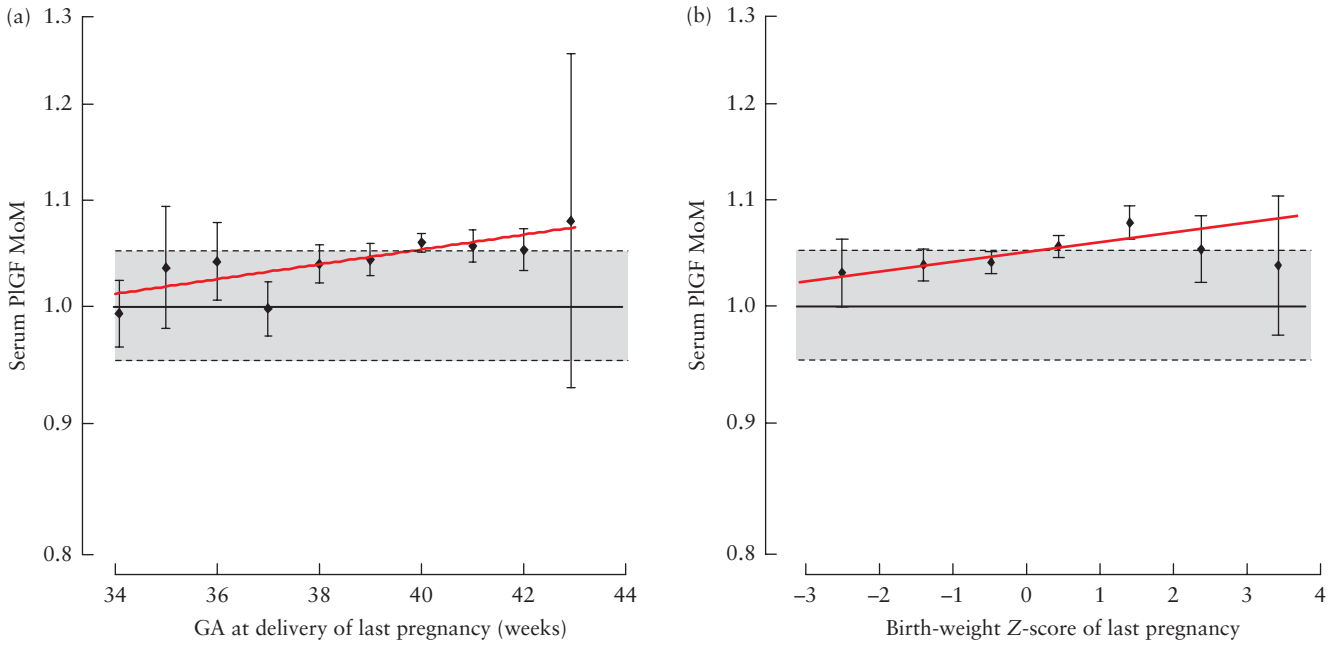

Figure 4 Effects of gestational age at delivery (a) and birth-weight *Z*-score (b) of neonate in last pregnancy on median serum placental growth factor (PlGF) (with 95% CI), plotted on multiples of the median (MoM) scale after correcting for other factors. Fitted effects (——), MoM of 1.0 (——) and median MoM ± 0.1 SD (----) are indicated (log MoM scale).

Table 2 Linear mixed model with random effects for variables from maternal characteristics and history that contribute substantively to the serum levels of placental growth factor

Term	*Estimate*	*95% CI*	*SE*	P
Intercept	1.3192346424	1.3076913630 to 1.3307779217	0.0058894282	< 0.0001
Trimester-dependent effects				
Trimester 2				
Constant	−0.0119410235	−0.0172585170 to −0.0066235300	0.0027130069	< 0.0001
Trimester 3				
Constant	0.0363138987	0.0304916087 to 0.0421361887	0.0029705561	< 0.0001
Trimester-independent effects				
Gestational age				
Gestational age (−77)*	0.0150600000	0.0142663960 to 0.0158536040	0.0004049000	< 0.0001
(Gestational age (−77))^{2*}	−0.0000136300	−0.0000273128 to 0.0000000528	0.0000069810	0.05084
(Gestational age (−77))^{3*}	−0.0000002336	−0.0000002829 to −0.0000001843	0.0000000252	< 0.0001
Maternal weight				
Maternal weight (−69)†	−0.0020369614	−0.0021649241 to −0.0019089986	0.0000652871	< 0.0001
Maternal age				
Maternal age (−35)‡	0.0020439736	0.0016346976 to 0.0024532497	0.0002088143	< 0.0001
(Maternal age (−35))‡ × (Gestational age (−77))*	−0.0000124550	−0.0000173495 to −0.0000075605	0.0000024972	< 0.0001
Racial origin				
Afro-Caribbean	0.1894064984	0.1842504140 to 0.1945625828	0.0026306553	< 0.0001
East Asian	0.0385488933	0.0256023465 to 0.0514954401	0.0066053810	< 0.0001
South Asian	0.0724417799	0.0630847490 to 0.0817988108	0.0047739953	< 0.0001
Mixed	0.0724771966	0.060047314 to 0.084907078	0.0063417766	< 0.0001
Cigarette smoking	0.1493447902	0.1428045319 to 0.1558850485	0.0033368665	< 0.0001
Medical history				
Diabetes mellitus Type 1	−0.0634636332	−0.0941863797 to −0.0327408868	0.0156748706	< 0.0001
Diabetes mellitus Type 2 on insulin	−0.0580828726	−0.0975321241 to −0.0186336211	0.0201271691	0.00195
Obstetric history				
Parous	0.0221450947	0.0181331244 to 0.0261570649	0.0020469236	< 0.0001
Parous: gestational age of last birth (−40)§	0.0028963991	0.0013362846 to 0.0044565136	0.0007959768	0.00014
Parous: birth-weight *Z*-score	0.0038233889	0.0014407471 to 0.0062060307	0.0012156336	0.00083
Machine				
Roche	0.1864246691	0.1742907078 to 0.1985586303	0.0061907966	< 0.0001

Continuous variables were centered by subtracting the mean from each measured value: *−77 from gestastional age in days; †−69 from maternal weight in kg; ‡−35 from maternal age in years; §−40 from gestational age of last birth in weeks. SE, standard error.

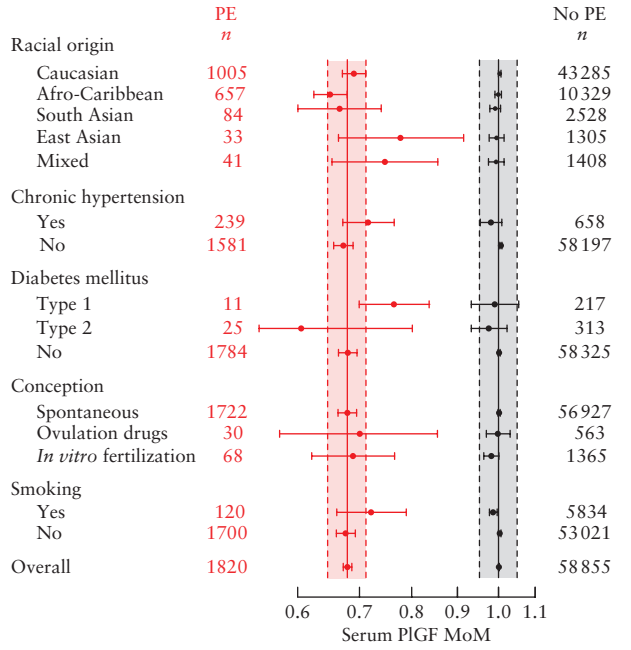

Figure 5 Median serum placental growth factor (PlGF) multiples of the median (MoM) (with 95% CI) derived from the model, according to racial origin, chronic hypertension, diabetes mellitus, method of conception and smoking in women who developed pre-eclampsia (PE) (red values) and in those unaffected by PE (black values). Median MoM of 1.0 (——) and median MoM ± 0.1 SD (----) of women unaffected by PE and median MoM of 0.6809 in women with PE (——) and median MoM ± 0.1 SD of unaffected pregnancies (----) are indicated (log MoM scale).

a maximum level at around 30 weeks and subsequently decreasing (Figure 1).

The relationship between maternal age and serum PlGF depended on gestational age (Figure 2). Allowing for other factors, there was a positive trend of serum PlGF with age in the first and second trimesters, and a negative trend in the third trimester. Therefore, maternal age and gestational age interactions were included in the model such that the relationship between serum PlGF and maternal age was in part defined by gestational age. The shaded regions in Figure 2 show the range of possible relationships between maternal age and serum PlGF for any given gestational age between 77 and 259 days (11–37 weeks).

The effects of variables on serum PlGF other than maternal age were similar across all three trimesters. PlGF decreased with increasing maternal weight (Figure 2), was higher in women of Afro-Caribbean, South Asian and East Asian racial origin than in Caucasian women, was increased in cigarette smokers and decreased in women with diabetes mellitus Type 1 and in those with Type 2 disease requiring treatment with insulin (Figure 3). In parous women, serum PlGF was higher than in nulliparous women and increased with gestational age at delivery and birth-weight *Z*-score of the neonate in the last pregnancy (Figure 4).

Final model on serum PlGF

A linear mixed model, with random effects to represent random effects between women, was fitted to the subset of variables that contributed substantively to the linear regression models (Table 2). Trimester effects were included with the first trimester being used as the reference. Effects of maternal weight, racial origin, smoking, diabetes mellitus and gestational age at delivery and birth-weight *Z*-score of the neonate in the last pregnancy on median level of serum PlGF were considered constant across the three trimesters. The relationship between gestational age and median level of serum PlGF was curvilinear with a maximum level at around 30 weeks' gestation.

Figure 5 shows MoM diagnostics for racial origin, chronic hypertension, diabetes mellitus, method of conception and smoking in women unaffected by PE and those that developed PE. Figure 6 shows MoM diagnostics for maternal weight and gestational age at delivery and birth-weight *Z*-score of the neonate in the last pregnancy in women unaffected by PE and those who developed PE. In unaffected pregnancies, the model provided an adequate fit with median MoM values falling well within 0.1 SDs of 1 MoM. In the PE group, the overall median MoM was 0.74762 (95% CI, 0.72811–0.77308) and the levels were consistently lower across the range of variables.

Distributional properties of serum PlGF MoM values

Figure 7 shows a Gaussian distribution of serum PlGF MoM values. The median and 5th, 10th, 90th and 95th percentiles were 1.0000 (95% CI, 0.99539–1.00441) and 0.45182 (95% CI, 0.44772–0.45832), 0.5608 (95% CI, 0.55753–0.56533), 1.80709 (95% CI, 1.79276–1.81879) and 2.22 (95% CI, 2.19421–2.24269), respectively. Estimated SD and correlations are given in Tables 3 and 4, respectively. The SD increased substantially over the three trimesters. The correlations between $\log_{10}$ serum PlGF MoM values across the three trimesters were slightly stronger between first and second trimesters and second and third trimesters than between first and third trimesters.

DISCUSSION

Main findings of the study

The findings of this study demonstrate that, in pregnancy, significant independent contributions to the measured maternal serum PlGF concentration are provided by maternal characteristics and variables from medical history. PlGF has a curvilinear relationship with gestational age, decreases with maternal weight, is increased in women of Afro-Caribbean, South Asian and East Asian racial origin and in cigarette smokers and decreased in women with diabetes mellitus Type 1 and in those with Type 2 disease requiring treatment with insulin. In the first and second trimesters, there is an increase in serum levels of PlGF with increased maternal age but, in the third trimester, the levels decrease with increased age. In parous women, PlGF is higher than in nulliparous women and the level is related to the outcome of the previous pregnancy, both in terms of gestational age at delivery and birth-weight *Z*-score of the neonate.

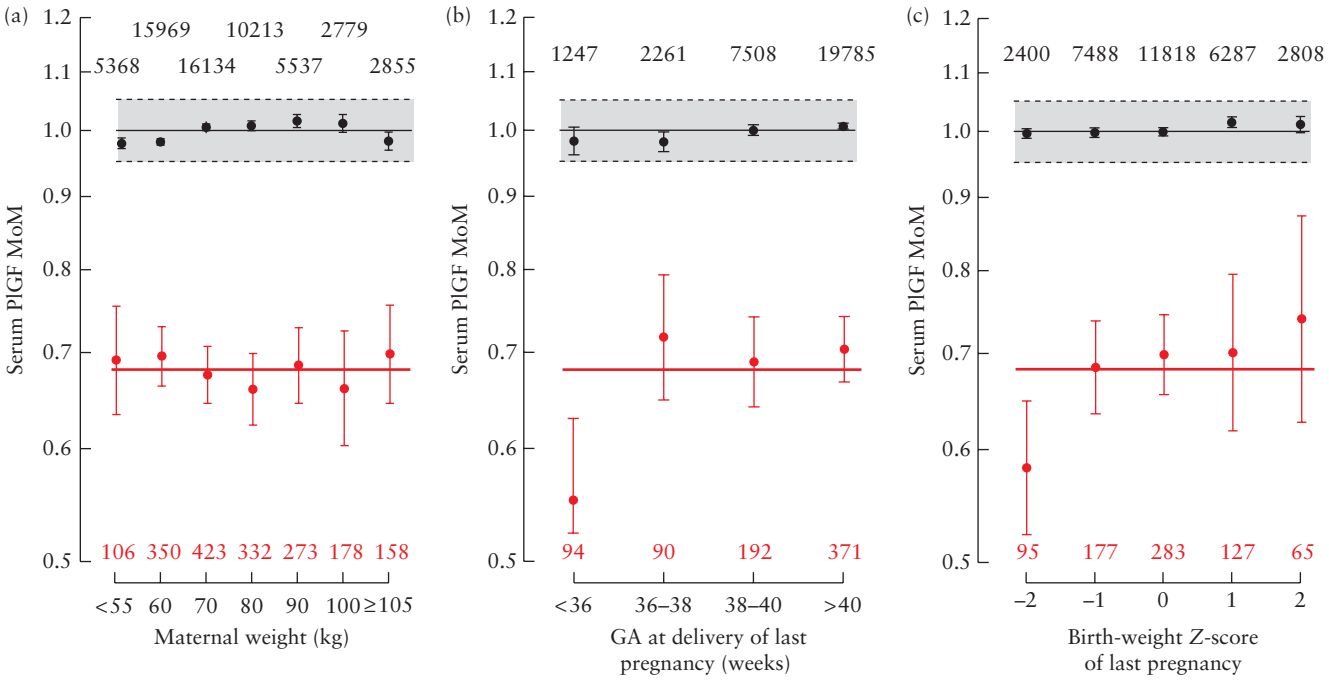

Figure 6 Median serum placental growth factor (PlGF) multiples of the median (MoM) (with 95% CI) derived from the model according to maternal weight (a) and gestational age at delivery (b) and birth-weight *Z*-score (c) of neonate in last pregnancy in women who developed pre-eclampsia (PE) (red values) and in those unaffected by PE (black values). Median MoM of 1.0 (——) and median MoM ± 0.1 SD (----) are indicated.

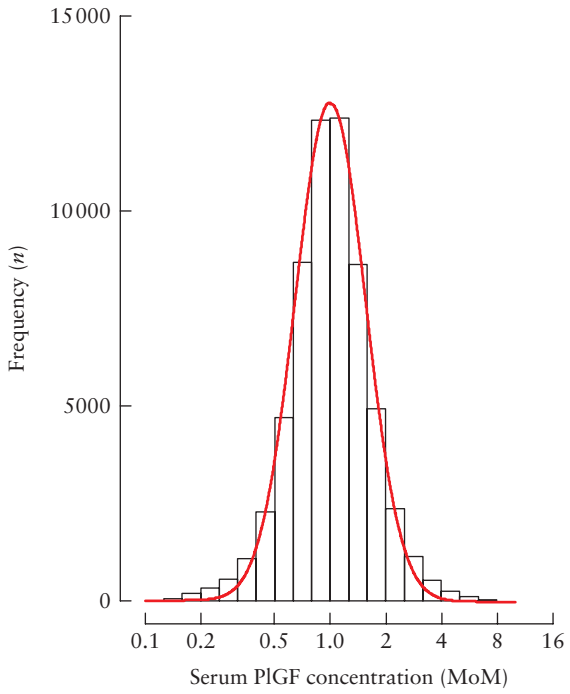

Figure 7 Gaussian distribution of serum placental growth factor (PlGF) multiples of the median values.

Random-effects multiple regression analysis was used to define the contribution of maternal variables that influence the measured serum PlGF concentration and express the values as MoMs. The model was shown to provide an adequate fit of MoM values for all covariates, both in pregnancies that developed PE and in those without this pregnancy complication.

Table 3 Standard deviations for $\log_{10}$ serum placental growth factor multiples of the median values for each trimester

Trimester	*Estimate (95% CI)*
First	0.17186 (0.17117–0.17255)
Second	0.20424 (0.20342–0.20505)
Third	0.32978 (0.32847–0.33110)

Table 4 Correlations of $\log_{10}$ serum placental growth factor multiple of the median (MoM) values in each trimester of pregnancy

Trimester	*Second*	*Third*
First	0.45747 (0.43175–0.48245)	0.38862 (0.36106–0.41549)
Second	1	0.58739 (0.566–0.608)
Third		1

Values in parentheses are 95% CI.

Strengths and limitations of the study

The strengths of this study are first, prospective examination of a large population of pregnant women attending for routine care in three well-defined gestational-age ranges which are widely used for first-trimester screening for chromosomal defects and second- and third-trimester assessment of fetal anatomy, growth and wellbeing, second, measurement of serum PlGF by automated machines that provide reproducible results within 40 min of sampling so that complete assessment and counseling can potentially be undertaken in the same hospital visit and

third, application of multiple regression analysis to define the contribution and interrelations of maternal variables that influence the measured serum PlGF across the three trimesters of pregnancy.

An alternative to the use of data from three gestational-age ranges would have been a cross-sectional study with inclusion of each gestational week from the beginning to the end of pregnancy. However, we adopted the pragmatic approach of collecting data from the gestational-age ranges used in routine clinical practice.

Comparison with findings of previous studies

In previous smaller studies examining the potential value of serum PlGF in screening for trisomies in the first trimester and for PE or delivery of SGA neonates in the first, second or third trimesters, we have used multiple regression analysis to express the measured serum concentration as MoMs after adjusting for variables that affect this measurement in pregnancies unaffected by aneuploidies, PE, SGA or GH[4–10]. In this expanded series of pregnancies in all three trimesters, we developed a model that incorporates variables with common effects across the trimesters and those with trimester-specific effects. The factors affecting serum PlGF in this study, such as maternal weight, racial origin and smoking, were also identified in our previous studies. However, in this study we have also investigated the effects and found significant contributions from diabetes mellitus Types I and 2 and the outcome of the previous pregnancy.

Implications for clinical practice

Effective use of serum PlGF in risk assessment and screening necessitates that variables from maternal characteristics and medical history which affect this measurement in normal pregnancies are taken into account. Standardizing the measured values of biomarkers for any variables included in the prior model is also essential in the application of Bayes' theorem in combined screening for pregnancy complications by maternal characteristics and biomarkers; the distribution of serum PlGF should be specified conditionally on any terms included in the prior model.

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