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Serum soluble fms-like tyrosine kinase-1 in the three trimesters of pregnancy: effects of maternal characteristics and medical history

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KEYWORDS: first-trimester screening; pre-eclampsia; pyramid of pregnancy care; sFlt-1; second-trimester screening; soluble fms-like tyrosine kinase-1; third-trimester screening; trisomy 21

ABSTRACT

Objective *To define the contribution of maternal variables which influence the measured level of maternal serum soluble fms-like tyrosine kinase-1 (sFlt-1) in screening for pregnancy complications.*

Methods *Maternal characteristics and medical history were recorded and serum sFlt-1 was 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 pregnancies delivering phenotypically normal live births or stillbirths ≥ 24 weeks' gestation, variables from maternal demographic characteristics and medical history that are important in the prediction of sFlt-1 were determined from a linear mixed-effects multiple regression.*

Results *Serum sFlt-1 was measured in 7066 cases in the first trimester, 8078 in the second trimester and 10 464 in the third trimester. Significant independent contributions to serum sFlt-1 were provided by gestational age, maternal weight, racial origin, cigarette smoking, birth-weight Z-score of the neonate in the previous pregnancy and interpregnancy interval. Random-effects multiple regression analysis was used to define the contribution of maternal variables that influence the measured level of serum sFlt-1 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 pregnancy complication.*

Conclusions *A model was fitted to express measured serum sFlt-1 across the three trimesters of pregnancy as MoMs, after adjusting for variables from maternal characteristics and medical history that affect this measurement.* *Copyright © 2015 ISUOG. Published by John Wiley & Sons Ltd.*

INTRODUCTION

Soluble fms-like tyrosine kinase-1 (sFlt-1) is a circulating antiangiogenic protein implicated in the pathogenesis of pre-eclampsia (PE). The concentration of sFlt-1 is increased in the placenta and serum of women with PE and administering exogenous sFlt-1 to pregnant rats induces hypertension, proteinuria and glomerular endotheliosis[1]. There is also evidence that serum levels of sFlt-1 are higher in the weeks preceding clinical onset of PE and consequently may be a useful biochemical marker in screening for PE[2–6].

Our approach to risk assessment and screening for 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 recorded at different times during pregnancy[6–9]. In normal pregnancy, serum sFlt-1 concentration is affected by gestational age and maternal characteristics, including weight, racial origin and outcome of previous pregnancies[6]. Therefore, for the effective use of serum sFlt-1 measurements in risk assessment, these variables need to be taken into account which 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 characteristics and medical history on serum sFlt-1 levels, second, present a model for standardizing serum

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sFlt-1 measurements obtained in all three trimesters of pregnancy into 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 the pregnancies with a normal outcome. Further details of the distribution of sFlt-1 MoM values in pregnancies with PE 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 three routine hospital visits at King's College Hospital or 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. 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 19–24 weeks[10,11].

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 singleton pregnancy delivering a phenotypically normal live birth or stillbirth ≥ 24 weeks' gestation. Pregnancies with aneuploidies or major fetal abnormalities and those ending in termination, miscarriage or fetal death before 24 weeks were excluded.

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 at the first visit and weight at each visit.

Measurement of maternal serum soluble fms-like tyrosine kinase-1

Of the patients included in the study, maternal serum sFlt-1 was measured at each visit by an automated biochemical analyzer within 10 min of blood sampling (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[12]. 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 of ≥ 300 mg in 24 h or two readings of at least ++ on dipstick analysis of midstream or catheter urine specimens if no 24-hour 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 presence of hypertension at booking visit before 20 weeks' gestation in the absence of trophoblastic disease). The birth-weight *Z*-score for the neonate in the last pregnancy was derived from our reference range of birth weight for gestational age at delivery[13].

Statistical analysis

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

Multiple linear regression models were fitted to $\log_{10}$ values of sFlt-1 within each trimester. Continuous variables were coded initially into groups and represented as factors 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.

Table 1 Maternal and pregnancy characteristics of 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 = *7066*)	*19 + 0 to 24 + 6 weeks* (n = *8078*)	*30 + 0 to 37 + 6 weeks* (n = *10 464*)
Maternal age (years)	31.0 (26.5–34.7)	31.0 (26.5–34.7)	31.1 (26.6–34.8)
Maternal weight (kg)	68.0 (59.7–79.0)	71.2 (63.5–82.1)	77.2 (69.0–88.2)
Maternal height (cm)	165.0 (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 (32.0–33.0)
Racial origin			
Caucasian	5247 (74.3)	6122 (75.8)	7768 (74.2)
Afro-Caribbean	1244 (17.6)	1314 (16.3)	1849 (17.7)
South Asian	292 (4.1)	339 (4.2)	388 (3.7)
East Asian	122 (1.7)	147 (1.8)	205 (2.0)
Mixed	161 (2.3)	156 (1.9)	254 (2.4)
Medical history			
Chronic hypertension	101 (1.4)	117 (1.4)	146 (1.4)
Diabetes mellitus	68 (1.0)	85 (1.1)	100 (1.0)
SLE/APS	9 (0.1)	11 (0.1)	19 (0.2)
Cigarette smoker	697 (9.9)	816 (10.1)	1027 (9.8)
Family history of PE	214 (3.0)	250 (3.1)	305 (2.9)
Obstetric history			
Nulliparous	3241 (45.9)	3894 (48.2)	5081 (48.6)
Parous with no previous PE	3546 (50.2)	3880 (48.0)	5024 (48.0)
Parous with previous PE	279 (3.9)	304 (3.8)	359 (3.4)
Interpregnancy interval (years)	3.0 (2.0–5.0)	3.1 (2.0–5.0)	3.2 (2.1–5.1)
GA at delivery of previous pregnancy (weeks)	40.0 (39.0–40.0)	40.0 (39.0–40.0)	40.0 (39.0–40.0)
Birth weight of previous pregnancy (g)	3444 (3100–3780)	3450 (3100–3780)	3450 (3126–3775)
Mode of conception			
Spontaneous	6818 (96.5)	7800 (96.6)	10 125 (96.8)
Ovulation induction	81 (1.1)	80 (1.0)	96 (1.0)
In-vitro fertilization	167 (2.4)	198 (2.4)	243 (2.3)
Pregnancy outcome			
PE	157 (2.2)	281 (3.5)	266 (2.5)
No PE	6909 (97.8)	7797 (96.5)	10 198 (97.5)

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

Graphical displays of the relationship between gestational age and sFlt-1 levels and the effects of variables from maternal characteristics including maternal weight, racial origin and previous pregnancy interval on sFlt-1 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 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[14].

RESULTS

Characteristics of the study population

The maternal characteristics and medical history of women that fulfilled the entry criteria are presented in Table 1. Serum sFlt-1 was measured in 7066 cases in the first trimester, in 8078 in the second trimester and in 10 464 in the third trimester. There were 2873 measurements taken in all three trimesters, 1177 in the first and second trimesters, 967 in the first and third trimesters, 1837 in the second and third trimesters, 2049 in the first trimester only, 2191 in the second trimester only and 4787 in the third trimester only.

Variables affecting serum sFlt-1

The variables with substantial effect on serum sFlt-1 were gestational age at assessment, maternal weight, racial origin, cigarette smoking, interpregnancy interval and birth-weight *Z*-score of the neonate in the previous pregnancy. Median levels of serum sFlt-1 showed an increasing curvilinear relationship with increasing gestational age; the increase was much steeper in the third trimester than in the first and second trimesters (Figure 1).

The effects of variables on serum sFlt-1 were similar in all three trimesters. Serum sFlt-1 levels decreased with increasing maternal weight, were higher in women of Afro-Caribbean racial origin than in Caucasian women and were lower in cigarette smokers than in non-smokers (Figure 2). In parous women, serum sFlt-1 levels were lower than in nulliparous women and increased with a greater interpregnancy interval and birth-weight *Z*-score of the neonate in the previous pregnancy (Figure 2).

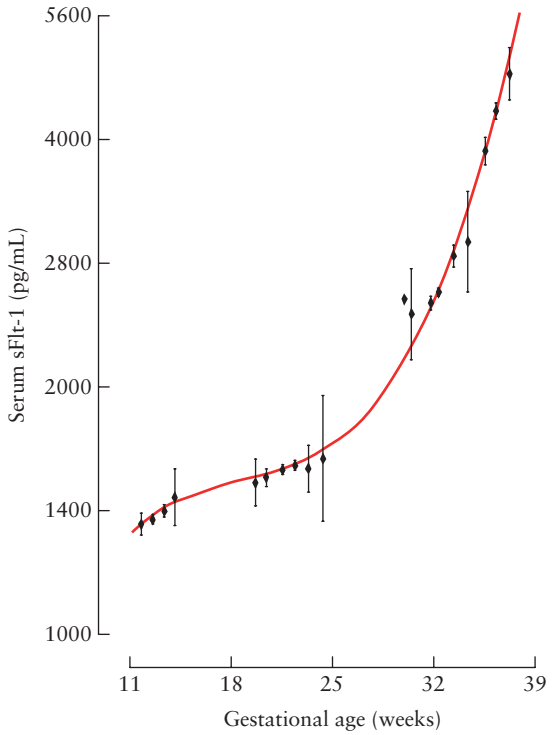

Figure 1 Relationship between median (95% CI) levels of serum soluble fms-like tyrosine kinase-1 (sFlt-1) and gestational age across the three trimesters of pregnancy.

Final model on serum sFlt-1

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, interpregnancy interval and birth-weight *Z*-score of the neonate in the last pregnancy on the median level of serum sFlt-1 were considered constant across the three trimesters. The relationship between gestational age and median level of serum sFlt-1 was curvilinear.

Figure 3 shows MoM diagnostics for racial origin, chronic hypertension, diabetes mellitus, method of conception and smoking in pregnancies unaffected by PE and those that developed PE. Figure 4 shows MoM diagnostics for maternal weight, birth-weight *Z*-score of the neonate in the last pregnancy and interpregnancy interval in women unaffected by PE and in those who developed PE. In unaffected women, 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 1.2657 (95% CI, 1.22429–1.30845) and the levels were consistently higher across the range of variables.

Distributional properties of serum sFlt-1 MoM values

Figure 5 shows a Gaussian distribution of serum sFlt-1 MoM values. The median and 5th, 10th, 90th and 95th percentiles were 1.0000 (95% CI, 0.99199–1.00710) and 0.48992 (95% CI, 0.48502–0.49713), 0.57415 (95% CI, 0.56757–0.57875), 1.78151 (95% CI, 1.76332–1.79773) and 2.12011 (95% CI, 2.09276–2.14919), respectively. Estimated SD and correlations with 95% CI are given in Tables 3 and 4, respectively. The correlations between $\log_{10}$ serum sFlt-1 MoM across trimesters were stronger between first and second trimesters and second and third trimesters than between first and third trimesters.

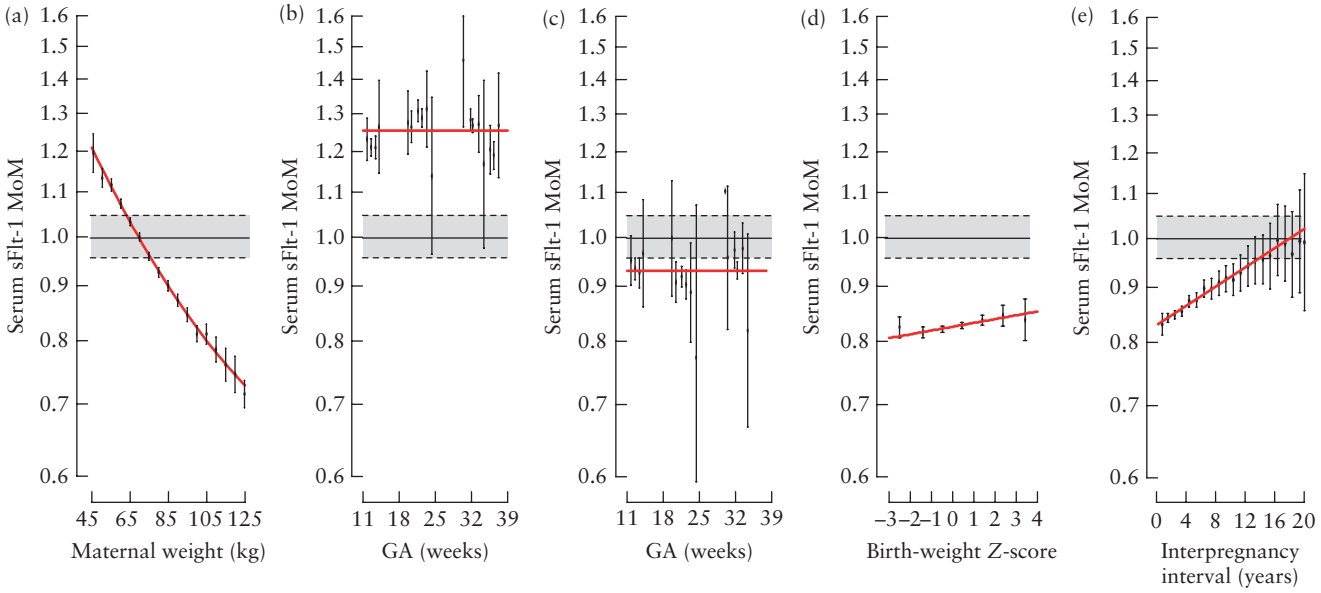

Figure 2 Effect of maternal weight (a), Afro-Caribbean racial origin (b), cigarette smoking (c), birth-weight *Z*-score of neonate in last pregnancy (d) and interpregnancy interval (e) on median (95% CI) serum soluble fms-like tyrosine kinase-1 (sFlt-1), plotted on the multiples of the median (MoM) scale after correcting for other factors. Fitted effects (——), median MoM of 1.0 (——), and median MoM ± 0.1 SD (----) are indicated.

Table 2 Linear mixed model with random effects for variables from maternal characteristics and history that contribute substantively to the measurement of serum soluble fms-like tyrosine kinase-1

Term	*Estimate*	*95% CI*	*SE*	P
Intercept	3.12286605747	3.10706227050 to 3.13866984444	0.00806315662	< 0.0001
Trimester-dependent effects				
Trimester 2				
Constant	−0.02990915977	−0.06941480349 to 0.00959648395	0.02015594067	0.0689
Trimester 3				
Constant	−0.14054865187	−0.21566932087 to −0.06542798288	0.03832687194	0.0001
Trimester-independent effects				
Gestational age				
Gestational age (−77)*	0.00198239941	0.00069749491 to 0.00326730391	0.00065556352	0.0012
(Gestational age (−77))^2*	−0.00002661606	−0.00004316600 to −0.00001006611	0.00000844385	0.0008
(Gestational age (−77))^3*	0.00000017880	0.00000012344 to 0.00000023416	0.00000002825	< 0.0001
Maternal weight				
Maternal weight (−69)†	−0.00319756531	−0.00348550543 to −0.00290962520	0.00014690822	< 0.0001
(Maternal weight (−69))^2†	0.00001166160	0.00000459081 to 0.00001873238	0.00000360755	0.0006
Racial origin				
Afro-Caribbean	0.09779318473	0.08988207324 to 0.10570429623	0.00403628137	< 0.0001
Cigarette smoking	−0.03385670162	−0.04369286391 to −0.02402053934	0.00501845014	< 0.0001
Obstetric history				
Parous	−0.08284560198	−0.09076402007 to −0.07492718390	0.00404000923	< 0.0001
Parous: birth-weight *Z*-score of last pregnancy	0.00352677147	−0.00013818491 to 0.00719172784	0.00186987570	< 0.0001
Parous: interpregnancy interval in years	0.00449701798	0.00319684919 to 0.00579718676	0.00066335142	0.0300

Continuous variables were centered by subtracting the mean from each measured value: *−77 from gestational age in days; †−69 from maternal weight in kg. SE, standard error.

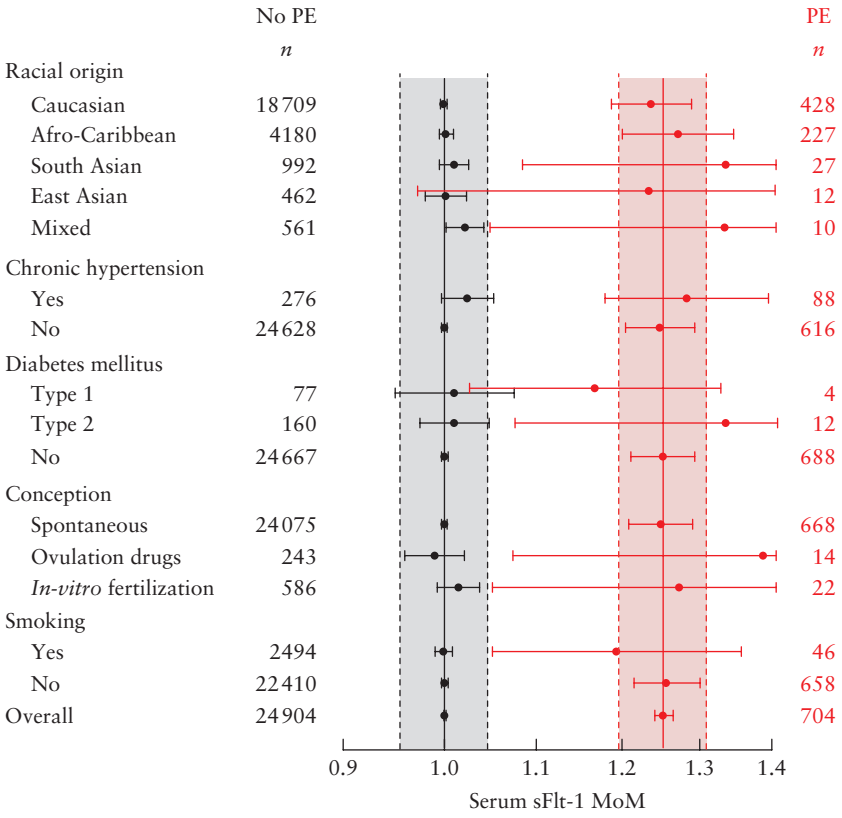

Figure 3 Median serum soluble fms-like tyrosine kinase-1 (sFlt-1) multiples of the median (MoM) (with 95% CI) derived from the model according to racial origin, chronic hypertension, diabetes mellitus, method of conception and smoking in women who developed pre-eclampsia (PE) and in those unaffected by PE. Median MoM of 1.0 (——) and median MoM ± 0.1 SD (----) of women unaffected by PE and median MoM of 1.2657 in women with PE (——) and median MoM ± 0.1 SD of women unaffected by PE (----) are indicated (log MoM scale).

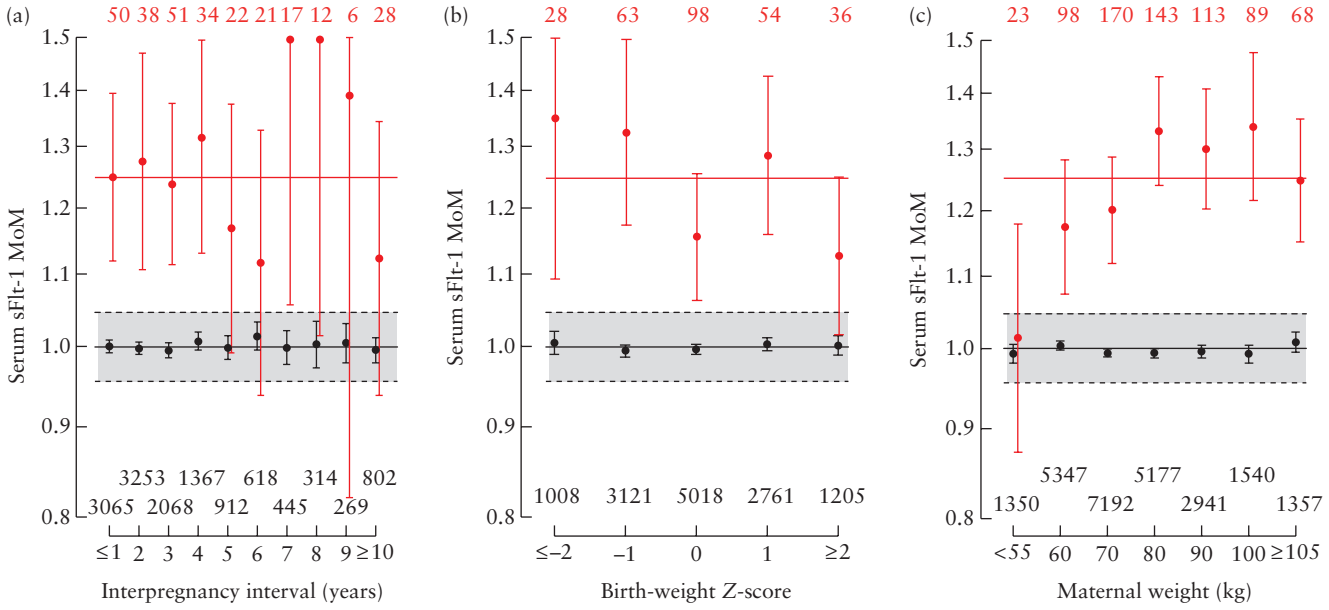

Figure 4 Median serum soluble fms-like tyrosine kinase-1 (sFlt-1) multiples of the median (MoM) (with 95% CI) derived from the model, according to interpregnancy interval (a), birth-weight *Z*-score of neonate in last pregnancy (b) and maternal weight (c) in women who developed pre-eclampsia (PE) (red 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 1.2657 in women with PE (——) are indicated (log MoM scale).

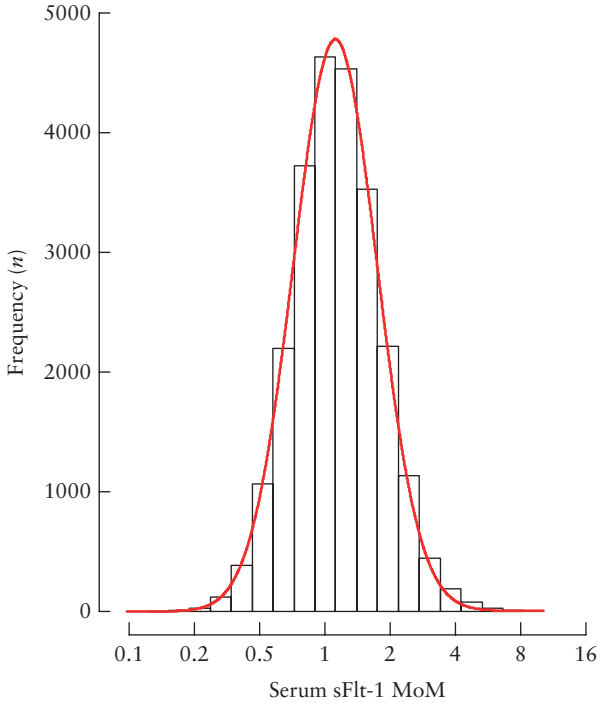

Figure 5 Gaussian distribution of serum soluble fms-like tyrosine kinase-1 (sFlt-1) multiples of the median values.

Table 3 Standard deviations (SD) for $\log_{10}$ serum soluble fms-like tyrosine kinase-1 (sFlt-1) multiples of the median (MoM) values for each trimester

Trimester	*SD Estimate (95% CI)*
First	0.16989 (0.16919–0.17058)
Second	0.21433 (0.21346–0.21521)
Third	0.19107 (0.19029–0.19186)

Table 4 Correlation of $\log_{10}$ serum soluble fms-like tyrosine kinase-1 (sFlt-1) multiples of the median (MoM) values in each trimester of pregnancy

Trimester	*Second*	*Third*
First	0.67985 (0.65901–0.69965)	0.37218 (0.33918–0.40427)
Second	1	0.68232 (0.6616–0.702)
Third		1

Values in parentheses are 95% CI.

DISCUSSION

Main findings of the study

The findings of this study demonstrate that, in pregnancy, significant independent contributions to the measured maternal serum sFlt-1 concentration are provided by maternal characteristics and variables from medical history. Serum sFlt-1 has a curvilinear relationship with gestational age, decreases with increasing maternal weight, is increased in women of Afro-Caribbean racial origin and decreased in cigarette smokers and in parous women, but increases with a greater birth-weight *Z*-score of the neonate in the last pregnancy and interpregnancy interval.

Random-effects multiple regression analysis was used to define the contribution of maternal variables that influence the measured serum sFlt-1 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.

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 sFlt-1 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 sFlt-1 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

Most previous studies investigated sFlt-1 in the late second or third trimesters of pregnancy for the prediction of PE and they did not adjust the measured values according to maternal characteristics and medical history[2–5]. The findings of this study, that serum sFlt-1 decreases with increased maternal weight, is higher in women of Afro-Caribbean racial origin than in Caucasian women and is lower in parous than in nulliparous women, were also observed in our previous third-trimester study[6]. Additionally, in this extended study, we found that sFlt-1 is decreased in cigarette smokers, is affected by outcome of the previous pregnancy in parous women and the effects of variables on serum sFlt-1 are similar in all three trimesters.

Implications for clinical practice

Effective use of serum sFlt-1 in risk assessment and screening necessitates that variables from maternal characteristics and medical history which affect this measurement in normal pregnancy 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 sFlt-1 should be specified conditionally on any terms included in the prior model.

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