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Serum free β-human chorionic gonadotropin in the three trimesters of pregnancy: effects of maternal characteristics and medical history

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KEYWORDS: first-trimester screening; human chorionic gonadotropin; pre-eclampsia; pyramid of pregnancy care; second-trimester screening; third-trimester screening; trisomy 18; trisomy 21

ABSTRACT

Objective *To define the contribution of maternal variables which influence the measured level of maternal serum free β-human chorionic gonadotropin (β-hCG) in screening for pregnancy complications.*

Methods *Maternal characteristics and medical history were recorded and serum free β-hCG 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 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 free β-hCG were determined from a linear mixed-effects multiple regression.*

Results *Serum free β-hCG was measured in 94 985 cases in the first trimester, 7879 in the second trimester and 8424 in the third trimester. Significant independent contributions to serum free β-hCG were provided by gestational age, maternal weight, age and racial origin, cigarette smoking, method of conception, diabetes mellitus and family history of pre-eclampsia (PE) in the mother of the patient. The effects of some variables were similar and those for others differed in each trimester. Random-effects multiple regression analysis was used to define the contribution of maternal variables that influence the measured level of serum free β-hCG 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 PE and in those without this pregnancy complication.*

Conclusions *A model was fitted to express measured serum free β-hCG 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

Maternal serum levels of free β-human chorionic gonadotropin (β-hCG) in the first trimester of pregnancy are increased in pregnancies with fetal trisomy 21 and decreased in fetal trisomies 18 and 13[1–3]. In pregnancies with established pre-eclampsia (PE) or PE in the third trimester before the clinical onset of the disease, serum free β-hCG is increased, but is decreased or unaltered at 11–13 weeks' gestation[4–8].

Our approach to risk assessment and screening for aneuploidies 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[8–10]. In normal pregnancy, serum free β-hCG concentration is affected by gestational age and maternal characteristics, including weight, racial origin, cigarette smoking, and method of conception[1–3]. Therefore, for the effective use of serum free β-hCG 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

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characteristics and medical history on serum free β-hCG levels, second, present a model for standardizing serum free β-hCG 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 free β-hCG MoM values in pregnancies with fetal trisomies and 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, 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. The second visit, at 19 + 0 to 24 + 6 weeks' gestation, and third visit, at 30 + 0 to 34 + 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 at 11–13 weeks or the fetal head circumference at 19–24 weeks[11,12].

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

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 = *94 985*)	*19 + 0 to 24 + 6 weeks* (n = *7879*)	*30 + 0 to 34 + 6 weeks* (n = *8424*)
Maternal age (years)	31.7 (27.4–35.4)	30.9 (26.4–34.7)	31.0 (26.6–34.7)
Maternal weight (kg)	66.0 (59.0–75.8)	71.2 (63.3–82.0)	77.0 (68.7–87.8)
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)	32.1 (32.0–32.5)
Racial origin			
Caucasian	69 161 (72.8)	5980 (75.9)	6291 (74.7)
Afro-Caribbean	15 754 (16.6)	1274 (16.2)	1481 (17.6)
South Asian	5047 (5.3)	332 (4.2)	314 (3.7)
East Asian	2576 (2.7)	141 (1.8)	152 (1.8)
Mixed	2447 (2.6)	152 (1.9)	186 (2.2)
Medical history			
Chronic hypertension	1188 (1.3)	108 (1.4)	122 (1.4)
Diabetes mellitus	766 (0.8)	84 (1.1)	82 (1.0)
SLE/APS	195 (0.2)	11 (0.1)	15 (0.2)
Cigarette smoker	8178 (8.6)	809 (10.3)	848 (10.1)
Family history of PE	3901 (4.1)	239 (3.1)	245 (3.0)
Obstetric history			
Nulliparous	46 697 (49.2)	3782 (48.0)	4141 (49.2)
Parous with no previous PE	45 274 (47.7)	3811 (48.4)	3973 (47.2)
Parous with previous PE	3014 (3.2)	286 (3.6)	310 (3.7)
Interpregnancy interval (years)	2.9 (1.9–4.9)	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)	3350 (3008–3700)	3399 (3030–3717)	3377 (3008–3700)
Mode of conception			
Spontaneous	91 398 (96.2)	7613 (96.6)	8146 (96.7)
Ovulation induction	1266 (1.3)	79 (1.0)	80 (0.9)
In-vitro fertilization	2321 (2.4)	187 (2.4)	198 (2.4)
Pregnancy outcome			
PE	2149 (2.3)	204 (2.6)	194 (2.3)
No PE	92 836 (97.7)	7675 (97.4)	8230 (97.7)

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

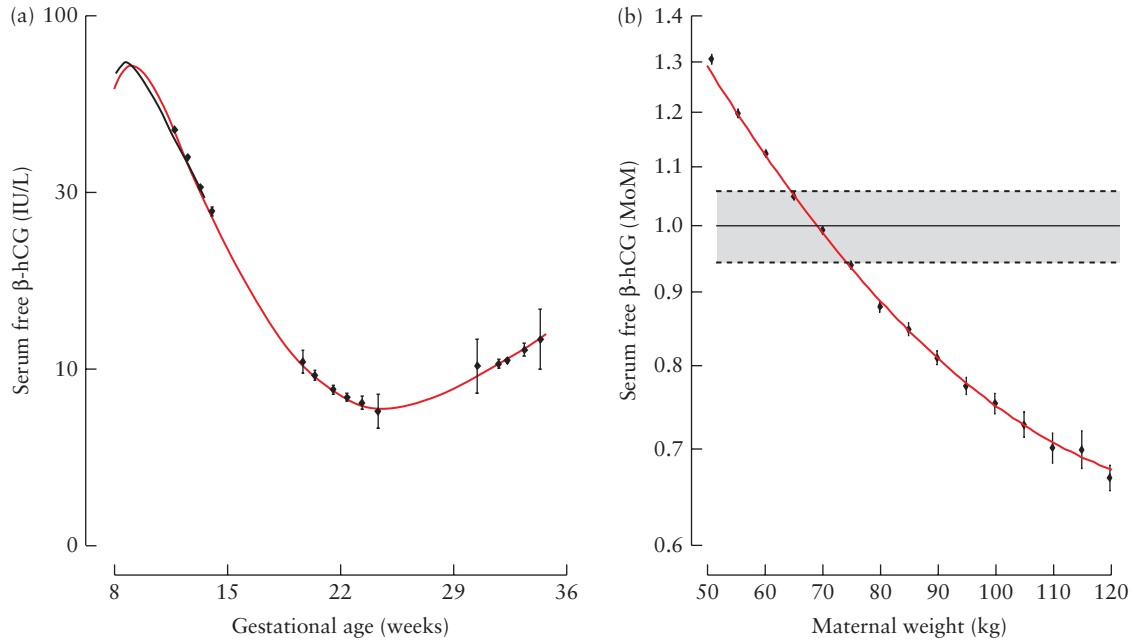

Figure 1 Relationship between median (95% CI) levels of serum free β-human chorionic gonadotropin (free β-hCG) and gestational age across the three trimesters of pregnancy (a) and maternal weight (b), plotted on the multiples of the median (MoM) scale after correcting for other factors. Black line in (a) represents the relationship between free β-hCG and gestational age in our previous model[2]. Fitted effects (——), median MoM of 1.0 (——), and median MoM ± 0.1 SD (- - - -) are indicated.

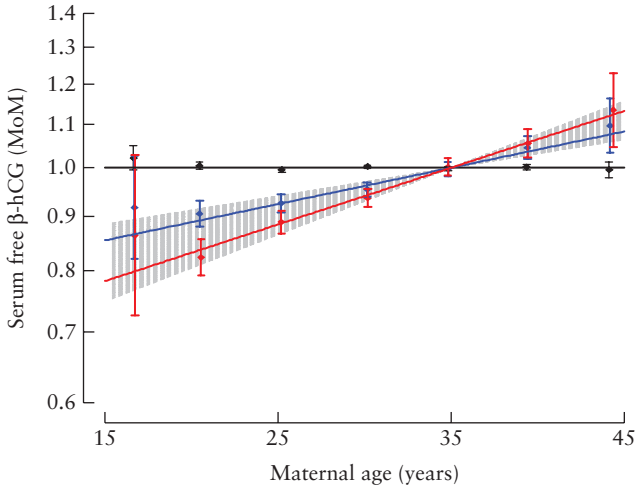

Figure 2 Effects of maternal age on median (95% CI) serum free β-human chorionic gonadotropin (free β-hCG) at 11 + 0 to 13 + 6 weeks' gestation (black), 19 + 0 to 24 + 6 weeks (blue) and 30 + 0 to 34 + 6 weeks (red), plotted on the multiples of the median (MoM) scale after correcting for other factors. Shaded areas show the range of possible relationships between maternal age and free β-hCG MoM, for gestational ages between 11 and 35 weeks.

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 pregnancies ≥ 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 free β-human chorionic gonadotropin

Of the patients included in the study, maternal serum free β-hCG was measured at each visit by automated biochemical analyzers within 10 min of blood sampling. Samples obtained in the first trimester were analyzed using the DELFIA Xpress system (PerkinElmer Life and Analytical Sciences, Waltham, MA, USA) and those in the second and third trimesters 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[13]. 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

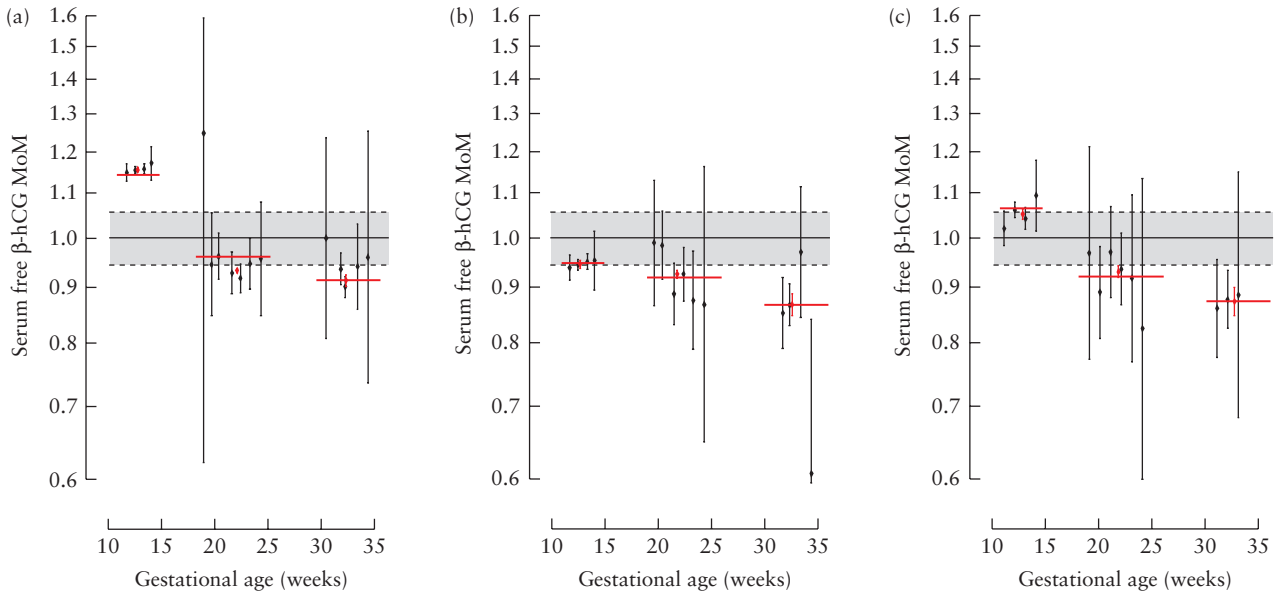

Figure 3 Effect of Afro-Caribbean (a), South Asian (b) and East Asian (c) racial origin on median (95% CI) serum free β-human chorionic gonadotropin, plotted on the multiples of the median (MoM) scale after correcting for other factors. Fitted effects (——), median MoM of 1.0 (——) and MoM ± 0.1SD (- - - -) are indicated. Black vertical lines represent values for individual gestational weeks and red vertical lines represent pooled estimates for each trimester.

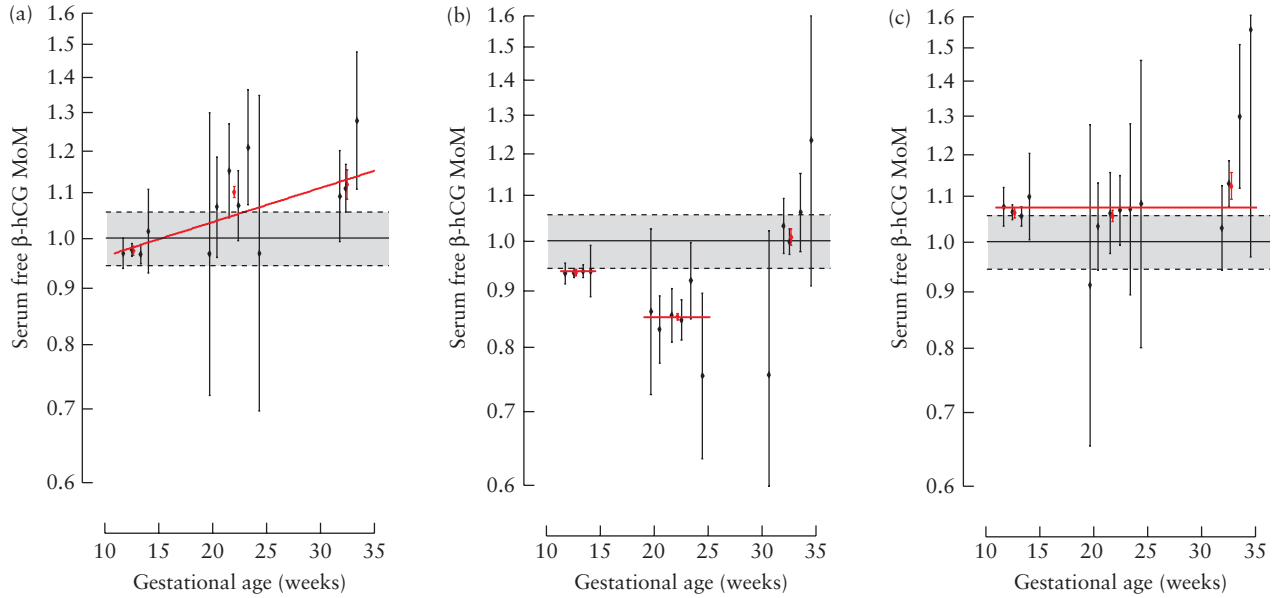

Figure 4 Effect of family history of pre-eclampsia (a), smoking (b) and assisted conception (c) on median (95% CI) serum free β-human chorionic gonadotropin (β-hCG), plotted on the multiples of the median (MoM) scale after correcting for other factors. Fitted effects (——), median MoM of 1.0 (——) and MoM ± 0.1SD (- - - -) are indicated. Black vertical lines represent values for individual gestational weeks and red vertical lines represent pooled estimates for each trimester.

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 for the neonate in the last pregnancy was derived from our reference range of birth weight for gestational age at delivery[14].

Statistical analysis

The effect on serum free β-hCG 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 last pregnancy and interpregnancy interval, method of conception, smoking during pregnancy and gestational age at assessment.

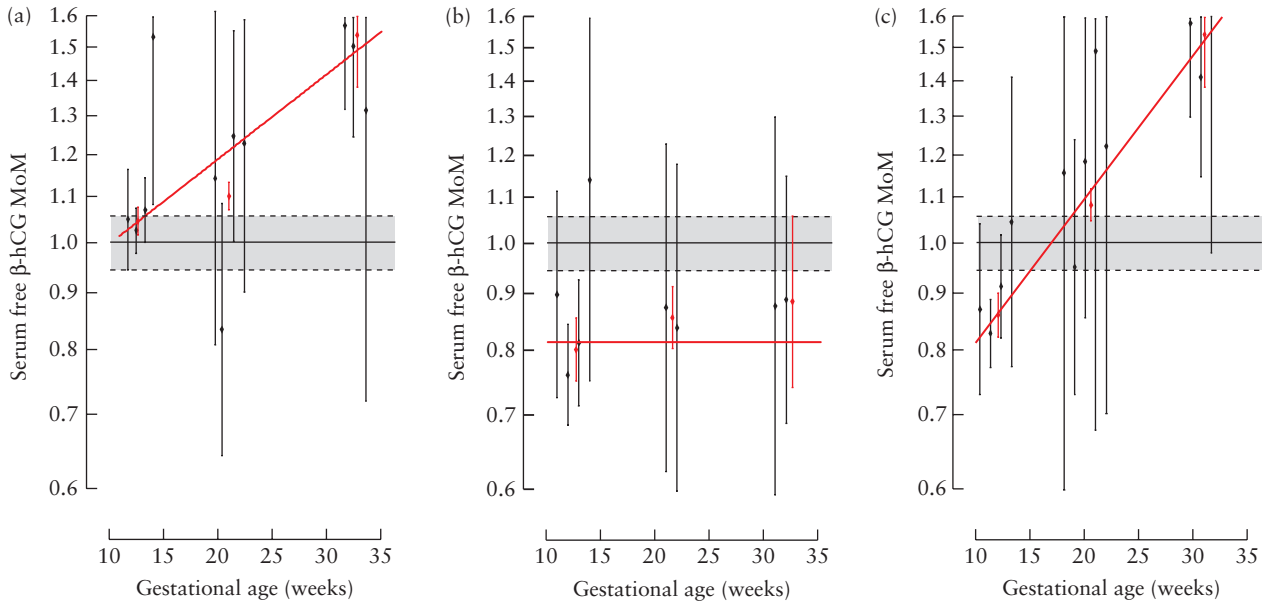

Figure 5 Effect of maternal diabetes mellitus Type 1 (a) and Type 2, treated with diet or metformin (b) or insulin (c), on median (95% CI) serum free β-human chorionic gonadotropin (β-hCG), plotted on the multiples of the median (MoM) scale after correcting for other factors. Fitted effects (——), median MoM of 1.0 (——) and MoM ± 0.1SD (- - - -) are indicated. Black vertical lines represent values for individual gestational weeks and red vertical lines represent pooled estimates for each trimester.

The relationship modelled with gestational age in the first trimester from our earlier work[2] has shown to provide a good fit across a range of settings and analyzers and provides a model for standardization of free β-hCG levels in the first trimester, from 8 weeks' gestation. This is important in some settings for aneuploidy screening. The current data set is restricted to pregnancies of a gestational age of 11 weeks or more. We therefore applied a penalized regression so that the relationship with gestational age between 8 and 11 weeks was consistent with our previously published model[2]. This enabled us to produce a model that captures the relationship between free β-hCG and gestational age across all three trimesters, from as early as 8 weeks' gestation.

Multiple linear regression models were fitted to $\log_{10}$ values of free β-hCG 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.

Graphical displays of the relationship between gestational age and free β-hCG levels and the effects of maternal age, weight, height and other characteristics on free β-hCG 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 between women random effects. 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[15].

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 free β-hCG was measured in 94 985 cases in the first trimester, in 7879 in the second trimester and in 8424 in the third trimester. In the first phase of the study, the serum free β-hCG was measured only in the first-trimester visit but this was subsequently extended to the second- and third-trimester visits. There were 4164 measurements taken in all three trimesters, 2739 in the first and second trimesters, 455 in the second and third trimesters, 3028 in the first and third trimesters, 85 054 in the first trimester only, 521 in the second trimester only and 777 in the third trimester only.

Variables affecting serum free β-hCG

The variables with substantial effect on serum free β-hCG were gestational age at assessment, maternal age, weight and racial origin, cigarette smoking, diabetes mellitus, method of conception and family history of PE in the mother of the patient. There were no substantive differences between pregnancies conceived after ovulation induction and those of *in-vitro* fertilization and therefore the data from the two groups were pooled into one

Table 2 Linear mixed model with random effects for variables from maternal characteristics and history that contribute substantively to the measurement of serum free β-human chorionic gonadotropin

Term	*Estimate*	*95% CI*	*SE*	P
Intercept	−3.2017961815	−3.2045 to −3.1991	0.0013923761	< 0.0001
Trimester-dependent effects				
First trimester				
Racial origin				
Afro-Caribbean	0.0581833585	0.053417 to 0.062949	0.0024316957	< 0.0001
East Asian	0.0271660661	0.016417 to 0.037915	0.0054844043	< 0.0001
South Asian	−0.0235599528	−0.031384 to −0.015736	0.0039919673	< 0.0001
Smoker	−0.0265506276	−0.032675 to −0.020426	0.0031247774	< 0.0001
Second trimester				
Constant	−0.1163860016	−0.12378 to −0.10899	0.0037747795	< 0.0001
Maternal age (−35)*	0.0013592235	−0.00052856 to 0.003247	0.0009631557	0.0791
(Maternal age (−35))* × (GA (−77))†	0.0000268367	0.000011468 to 0.000042205	0.0000078412	< 0.0001
Racial origin				
Afro-Caribbean	−0.0173179678	−0.031926 to −0.0027095	0.0074532837	0.0101
East Asian	−0.0353279016	−0.075302 to 0.004646	0.0203948688	0.0416
South Asian	−0.0367260663	−0.063384 to −0.010068	0.0136010151	0.0035
Mixed	−0.0708951890	−0.099054 to −0.042736	0.0143669023	< 0.0001
Smoker	−0.0679371188	−0.08556 to −0.050314	0.0089912978	< 0.0001
Third trimester				
Constant	−0.1689767209	−0.1761 to −0.16185	0.0036364278	< 0.0001
Maternal age (−35)*	0.0013592235	−0.00052856 to 0.003247	0.0009631557	0.0791
(Maternal age (−35))* × (GA (−77))†	0.0000268367	0.000011468 to 0.000042205	0.0000078412	< 0.0001
Racial origin				
Afro-Caribbean	−0.0389548822	−0.052693 to −0.025217	0.0070090152	< 0.0001
East Asian	−0.0582539872	−0.097058 to −0.01945	0.0197980277	0.0016
South Asian	−0.0612492598	−0.088545 to −0.033954	0.0139261770	< 0.0001
Mixed	−0.0708951890	−0.099054 to −0.042736	0.0143669023	< 0.0001
Trimester-independent effects				
Gestational age				
GA (−77)†	−0.0502070100	−0.052325 to −0.048089	0.0010806350	< 0.0001
$(GA (-77))^2$†	0.0002936003	0.00020407 to 0.00038313	0.0000456804	< 0.0001
$(GA (-77))^3$†	0.0000006074	0.0000019835 to 0.0000010165	0.0000002087	0.002
Log_{10} (GA (−40))‡	3.1516350000	*a*	*a*	*a*
Maternal weight				
Maternal weight (−69)§	−0.0046737097	−0.0048221 to −0.0045253	0.0000757006	< 0.0001
$(Maternal weight (-69))^2$§	0.0000282358	0.000023822 to 0.00003265	0.0000022520	< 0.0001
Medical history				
Type 1 DM	0.0070047125	−0.022773 to 0.036782	0.0151927177	0.3224
Type 1 DM × (GA (−77))†	0.0011029181	0.00046655 to 0.0017393	0.0003246796	0.0003
Type 2 DM on diet or metformin	−0.0882893188	−0.1453 to −0.03128	0.0290863479	0.0012
Type 2 DM on insulin	−0.0869218689	−0.12682 to −0.047025	0.0203557636	< 0.0001
Type 2 DM on insulin × (GA (−77))†	0.0018108941	0.0010456 to 0.0025762	0.0003904539	< 0.0001
Family history of PE	−0.0116959941	−0.021166 to −0.0022264	0.0048314323	0.0077
Family history of PE × (GA (−77))†	0.0004449220	0.00023237 to 0.00065748	0.0001084461	< 0.0001
Assisted conception	0.0295488782	0.020704 to 0.038394	0.0045127608	< 0.0001

Continuous variables were centered by subtracting the mean from each measured value: *−35 from maternal age in years; †−77 from gestational age in days; ‡−40 from gestational age in days; §−69 from maternal weight in kg. DM, diabetes mellitus; GA, gestational age; PE, pre-eclampsia; SE, standard error. *a*, this parameter is fixed on the basis of previous findings[2].

of assisted conception. Median levels of serum free β-hCG showed a curvilinear relationship with increasing gestational age, with a decrease in the first and second trimester, reaching a minimum level at around 24 weeks and then increasing in the third trimester (Figure 1a). Levels decreased with increasing maternal weight (Figure 1b) and increased with a greater maternal age in the second and third trimesters; in the first-trimester there was no substantive trend (Figure 2). Serum free β-hCG in women of Afro-Caribbean and East Asian racial origin was increased in the first trimester and decreased in the second and third trimesters, whereas, in South Asian women, levels were decreased in all three trimesters (Figure 3). In women with a family history of PE, the levels of serum free β-hCG increased with increasing gestational age, were lower in the first and second trimesters of smokers compared to non-smokers and were increased in all three trimesters of pregnancies conceived by assisted conception (Figure 4). Serum free β-hCG levels in women with diabetes mellitus Type 1 increased with increasing gestational age, were decreased in all three trimesters of women with Type 2 disease treated with diet alone or metformin, and increased with increasing gestational age in women with Type 2 disease treated with insulin, increasing from below the median level in the first trimester to greater than the median level in the second and third trimesters (Figure 5).

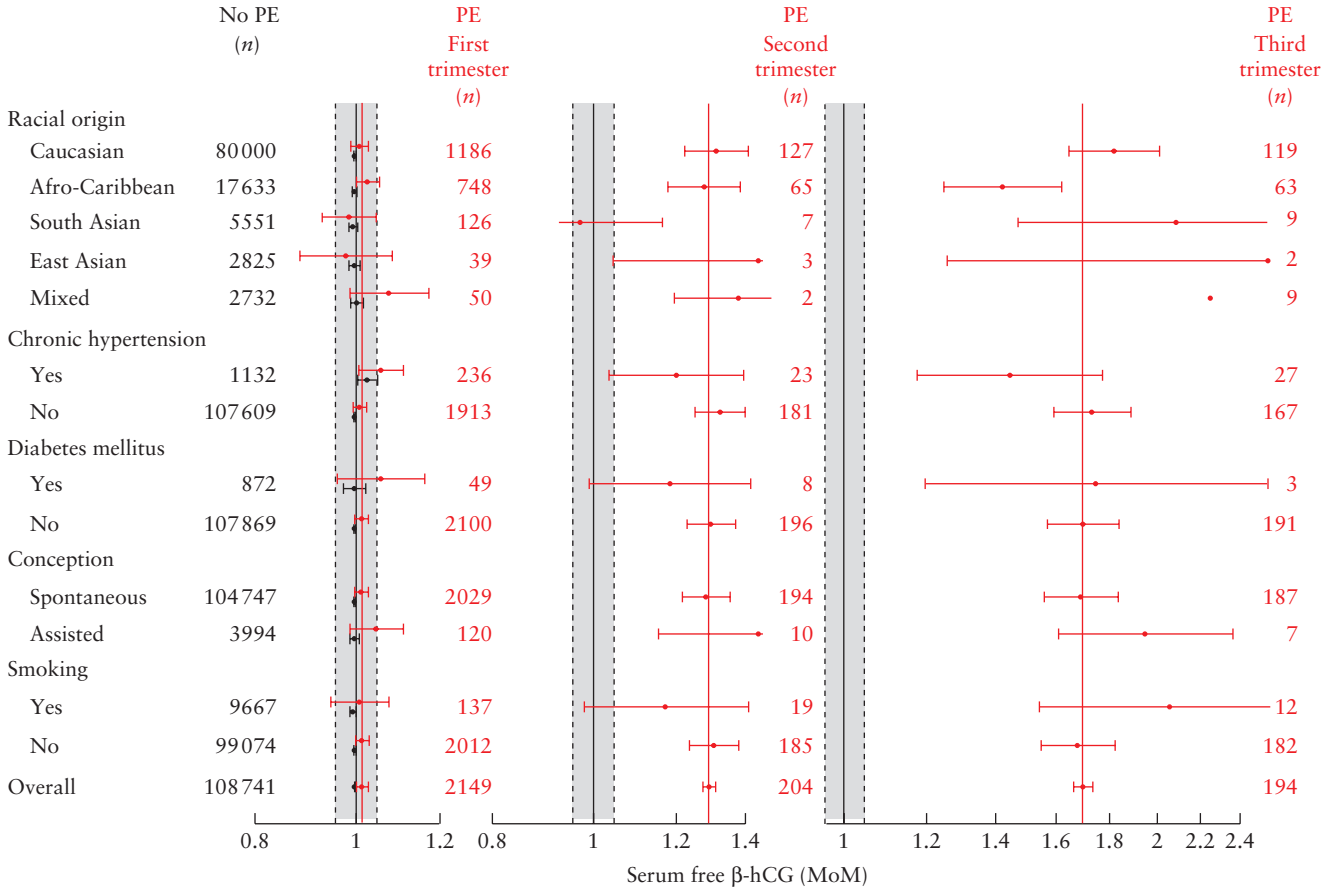

Figure 6 Median serum free β-human chorionic gonadotropin (β-hCG) multiples of the median (MoM) (with 95% CI) derived from the model according to racial origin, chronic hypertension, diabetes mellitus, method of conception and smoking in women who developed pre-eclampsia (PE) (red lines and numbers), according to trimester, and in those unaffected by PE (black lines and numbers). Data for PE are presented separately for each trimester. Median MoM of 1.0 (——) and median MoM ± 0.1 SD (- - - -) of women unaffected by PE and median MoM (——) of women with PE for each trimester are indicated.

Final model on serum free β-hCG

A linear mixed model, with random effects to represent between-women random effects, 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, method of conception, diabetes mellitus and family history of PE on median level of serum free β-hCG were considered constant across the three trimesters. In contrast, the effects of maternal age and racial origin were trimester dependent. The relationship between gestational age and median level of serum free β-hCG was curvilinear with a minimum at around 24 weeks.

The regression coefficient of −0.11639 for the effect of the second trimester and −0.16898 for the effect of the third trimester means that levels of free β-hCG, after adjusting for all the variables in the model, are decreased in these trimesters by about 24% and 32%, respectively. Such difference could be the consequence of the machine or reagents used for β-hCG measurements, which were different in the first than in the second and third trimesters and/or other trimester-related effects.

Figure 6 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. 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 1.0136 (95% CI, 0.9896–1.0381) in the first trimester, 1.2878 (95% CI, 1.2527–1.3239) in the second trimester and 1.6802 (95% CI, 1.6207–1.7419) in the third trimester; in the last two trimesters, β-hCG levels were consistently increased across the range of variables.

Distributional properties of serum free β-hCG MoM values

Figure 7 shows a Gaussian distribution of serum free β-hCG MoM values. The median and 5th, 10th, 90th and 95th percentiles were 1.0000 (95% CI, 0.99549–1.00506) and 0.37846 (95% CI, 0.37578–0.38152), 0.47267 (95% CI, 0.46985–0.47541), 2.25437 (95% CI, 2.23952–2.26987) and 2.89177 (95% CI, 2.86747–2.91458), respectively. Estimated SD and correlations with 95% CI are given in Tables 3 and 4, respectively. The SD

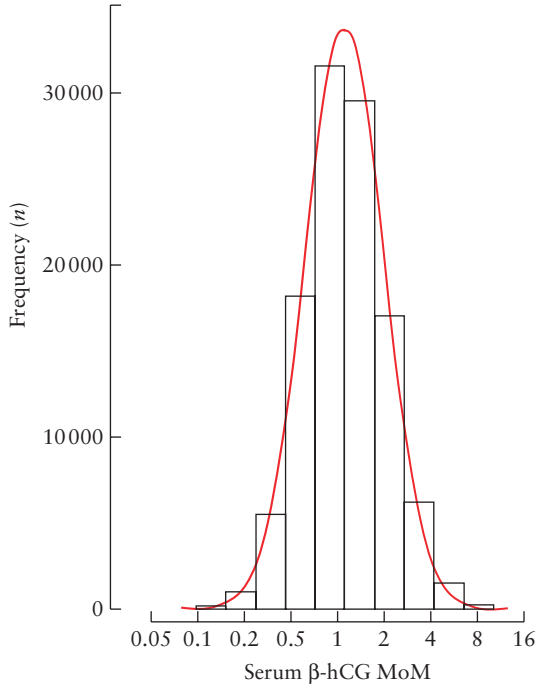

Figure 7 Gaussian distribution of serum free β-human chorionic gonadotropin (β-hCG) multiples of the median values.

Table 3 Standard deviations (SD) for $\log_{10}$ serum free β-human chorionic gonadotropin multiples of the median values for each trimester of pregnancy

Trimester	*SD Estimate (95% CI)*
First	0.25208 (0.25107–0.25311)
Second	0.27613 (0.27502–0.27725)
Third	0.35633 (0.35489–0.35777)

Table 4 Correlation of $\log_{10}$ serum free β-human chorionic gonadotropin multiples of the median values in each trimester of pregnancy

Trimester	*Second*	*Third*
First	0.59685 (0.57662–0.61634)	0.42347 (0.39782–0.44845)
Second	1	0.80582 (0.79473–0.81637)
Third	—	1

Values in parentheses are 95% CI.

was increased with a later trimester and the correlations between $\log_{10}$ serum free β-hCG MoM across trimesters were slightly stronger between first and second 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 free β-hCG concentration are provided by maternal characteristics and variables from medical history. Serum free β-hCG has a curvilinear relationship with gestational age, decreases with increasing maternal weight, increases with increasing maternal age in the second and third trimesters, is increased in the first trimester and decreased in the second and third trimester in women of Afro-Caribbean and East Asian racial origin, is decreased in all three trimesters of women of South Asian racial origin, is decreased in cigarette smokers and is increased in women with assisted conception and in the second and third trimesters of women with a family history of PE. Serum free β-hCG is increased in women with diabetes mellitus Type 1, is decreased in Type 2 disease treated with diet alone or metformin, and increases with gestational age from below the median in the first trimester to above the median in the second and third trimesters in Type 2 disease treated with insulin.

Random effects multiple regression analysis was used to define the contribution of maternal variables that influence the measured serum free β-hCG 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. In pregnancies affected by PE, serum free β-hCG MoM levels were higher in the second and even more so in the third trimester than in unaffected pregnancies.

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 free β-hCG 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 free β-hCG across the three trimesters of pregnancy. The single relationship that we have fitted may not be appropriate in all settings and, in some circumstances, separate relationships should be used in the different trimesters.

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

Previous studies, mainly in the first trimester, have also reported that serum free β-hCG concentration is affected

by gestational age and maternal characteristics, including maternal weight, racial origin, cigarette smoking and method of conception[1,2]. Three studies examining 35, 489 and 178 women with diabetes mellitus in the first trimester reported that the median free β-hCG MoM was 0.74, 1.0 and 1.0, respectively, but, in all three studies, the data from Type 1 and Type 2 diabetes were presented together[16–18]. Another study on 194 cases with Type 1 diabetes mellitus and 122 with Type 2 disease reported that the median free β-hCG MoM was 1.0 and 0.86, respectively[19].

In this 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. In the context of diabetes mellitus, we found that the levels are dependent on the type of the disease and the method of treatment applied.

Implications for clinical practice

Measurement of serum free β-hCG may be useful in screening for aneuploidies, neural tube defects and adverse pregnancy outcome. Effective use of serum free β-hCG 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. In the clinical implementation of the presented model it is important that adjustments are made to the various coefficients for the machines or reagents used and other possible local effects.

To emphasize the need for standardizing measured levels into MoM values, consider a spontaneous pregnancy at 11 weeks' gestation in a Caucasian, nulliparous, non-smoking, non-diabetic woman, with no family history of PE and of age 35 years. If the serum free β-hCG is 55 IU/L and the maternal weight is 140 kg, the measurement is translated into a MoM value of 1.546, which is on the 76th percentile for normal pregnancies. For a woman with a weight of 69 kg, the same serum free β-hCG corresponds to a MoM value of 0.999 which is on the 50th percentile for normal pregnancies. Consequently, for the same level of serum free β-hCG, the risk for PE may be increased if the maternal weight is low or decreased if the weight is high.

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