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Serum alpha-fetoprotein in the three trimesters of pregnancy: effects of maternal characteristics and medical history

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KEYWORDS: alpha-fetoprotein; first-trimester screening; pre-eclampsia; preterm birth; 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 alpha-fetoprotein (AFP) in screening for pregnancy complications.*

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

Results *Serum AFP was measured in 17 071 cases in the first trimester, 8583 in the second trimester and 8607 in the third trimester. Significant independent contributions to serum AFP were provided by gestational age, maternal weight, racial origin, gestational age at delivery and birth-weight Z-score of the neonate of the previous pregnancy and interpregnancy interval. Cigarette smoking was found to significantly affect serum AFP in the first trimester only. The machine used to measure serum AFP was also found to have a significant effect. Random-effects multiple regression analysis was used to define the contribution of maternal variables that influence the measured level of serum AFP 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 AFP 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

Measurement of maternal serum alpha-fetoprotein (AFP) during the first and second trimesters of pregnancy has been shown to be useful in screening for aneuploidies, neural tube defects and adverse pregnancy outcome, including fetal death, pre-eclampsia (PE), fetal growth restriction and preterm birth[1–11].

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 recorded at different times during pregnancy. In normal pregnancy, serum AFP concentration is affected by gestational age and maternal characteristics, including maternal weight, racial origin and cigarette smoking[12–18]. Therefore, for the effective use of serum AFP 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 AFP levels, second, present a model for standardizing serum AFP 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

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normal outcome. Further details of the distribution of AFP MoM values in pregnancies with PE, SGA and fetal aneuploidies 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 2011 and January 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, 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[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 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 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 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 alpha-fetoprotein

Of the patients included in the study, maternal serum AFP was measured at each visit by automated biochemical analyzers within 10 min of blood sampling. In 17 638 cases the sample was analyzed using the DELFIA Xpress system (PerkinElmer Life and Analytical Sciences, Waltham, MA, USA) and in 16 623 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 of ≥ 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) developing 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[23].

Statistical analysis

The effect on serum AFP levels of the following variables from maternal characteristics and medical history 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, being parous, 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 $\log_{10}$ values of AFP 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 serum AFP levels and the effects of

Table 1 Maternal and pregnancy characteristics of women with singleton pregnancy attending for routine visits between January 2011 and January 2014, according to trimester of pregnancy

Characteristic	*11 + 0 to 13 + 6 weeks (n = 17 071)*	*19 + 0 to 24 + 6 weeks (n = 8583)*	*30 + 0 to 34 + 6 weeks (n = 8607)*
Maternal age (years)	31.1 (26.6–34.8)	31.1 (26.6–34.8)	31.2 (26.8–34.8)
Maternal weight (kg)	67.0 (59.0–78.0)	71.0 (63.3–81.9)	76.7 (68.7–87.0)
Maternal height (cm)	164.0 (160.0–168.7)	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	11 626 (68.1)	6207 (72.3)	6212 (72.2)
Afro-Caribbean	3404 (19.9)	1663 (19.4)	1717 (19.9)
South Asian	1018 (6.0)	354 (4.1)	318 (3.7)
East Asian	639 (3.7)	172 (2.0)	154 (1.8)
Mixed	384 (2.3)	187 (2.2)	206 (2.4)
Medical history			
Chronic hypertension	252 (1.5)	115 (1.3)	121 (1.4)
Diabetes mellitus	219 (1.3)	88 (1.0)	88 (1.0)
SLE/APS	31 (0.2)	14 (0.2)	13 (0.2)
Cigarette smoker	1711 (10.0)	854 (9.9)	840 (9.8)
Family history of PE	608 (3.6)	287 (3.3)	275 (3.2)
Obstetric history			
Nulliparous	7868 (46.1)	4090 (47.7)	4232 (49.2)
Parous with no previous PE	8593 (50.3)	4187 (48.8)	4049 (47.0)
Parous with previous PE	610 (3.6)	306 (3.5)	326 (3.8)
Interpregnancy interval (years)	3.1 (2.0–5.2)	2.9 (1.9–4.9)	3.1 (2.0–5.2)
GA at delivery of previous pregnancy (weeks)	40 (39–40)	40 (39–40)	40 (39–40)
Birth weight of previous pregnancy (g)	3420 (3085–3758)	3444 (3090–3775)	3440 (3110–3775)
Mode of conception			
Spontaneous	16 321 (95.6)	8290 (96.6)	8338 (96.9)
Ovulation induction	270 (1.6)	86 (1.0)	82 (1.0)
In-vitro fertilization	480 (2.8)	207 (2.4)	187 (2.1)
Pregnancy outcome			
PE	488 (2.9)	217 (2.5)	208 (2.4)
No PE	16 583 (97.1)	8366 (97.5)	8399 (97.6)

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

maternal age, weight, height and other characteristics on AFP 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 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 data of 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 of the women that fulfilled the entry criteria are presented in Table 1. Serum AFP was measured in 17 071 cases in the first trimester, 8583 in the second trimester and 8607 in the third trimester. In the first phase of the study, the serum was measured only in the first-trimester visit but this was extended subsequently to the second- and then the third-trimester visits. There were 3081 measurements taken in all three trimesters, 1970 in the first and second trimesters, 1003 in the second and third trimesters, 2416 in the first and third trimesters, 9604 in the first trimester only, 2529 in the second trimester only and 2107 in the third trimester only.

Variables affecting serum AFP

The variables with substantial effect on serum AFP were the machine used for the measurement, gestational age at assessment, maternal weight, racial origin, cigarette smoking, and gestational age at delivery and birth-weight *Z*-score of the neonate in the previous pregnancy and interpregnancy interval. Median levels of serum AFP increased with increasing gestation in a curvilinear fashion (Figure 1) and decreased with greater maternal weight (Figure 2a). There was an Afro-Caribbean effect on serum AFP, which was dependent on gestational age (Figure 2b). Serum AFP was higher in cigarette smokers in the first trimester than in non-smokers, but not in the second and third trimesters (Figure 2c). In parous women, serum AFP was lower than in nulliparous women, decreased

with greater gestational age at delivery and birth-weight *Z*-score of the neonate in the last pregnancy and increased with a longer interpregnancy interval (Figure 3).

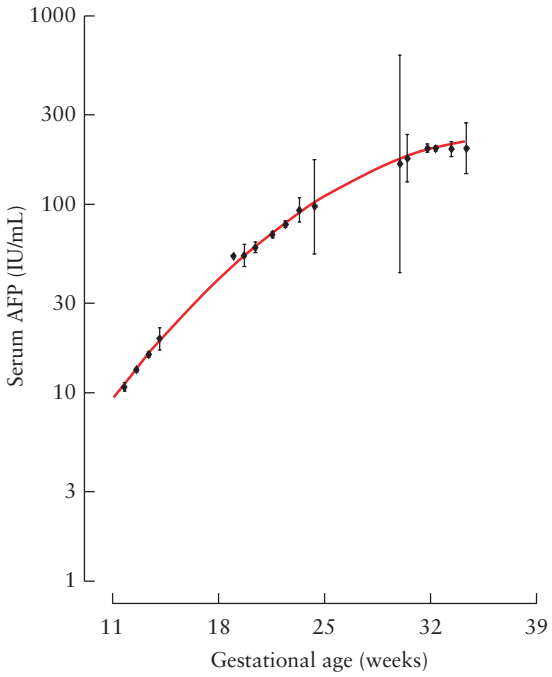

Figure 1 Relationship between median (95% CI) levels of serum alpha-fetoprotein (AFP) concentration and gestational age across three trimesters of pregnancy.

Final model on serum AFP

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. Smoking was found to have an effect on serum AFP levels in the first trimester. Effects of maternal weight, racial origin, parity, gestational age at delivery and birth-weight *Z*-score of the neonate in the last pregnancy and interpregnancy interval on the median level of serum AFP were considered constant across the three trimesters. The relationship between gestational age and median level of serum AFP was increasing and curvilinear.

Figure 4 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 5 shows MoM diagnostics for gestational age at delivery and birth-weight *Z*-score of the neonate in the last pregnancy and interpregnancy interval, in women unaffected by PE and those that 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, median MoM values were consistently increased across most variables and the overall median MoM was 1.08826 (95% CI, 1.05371–1.12734).

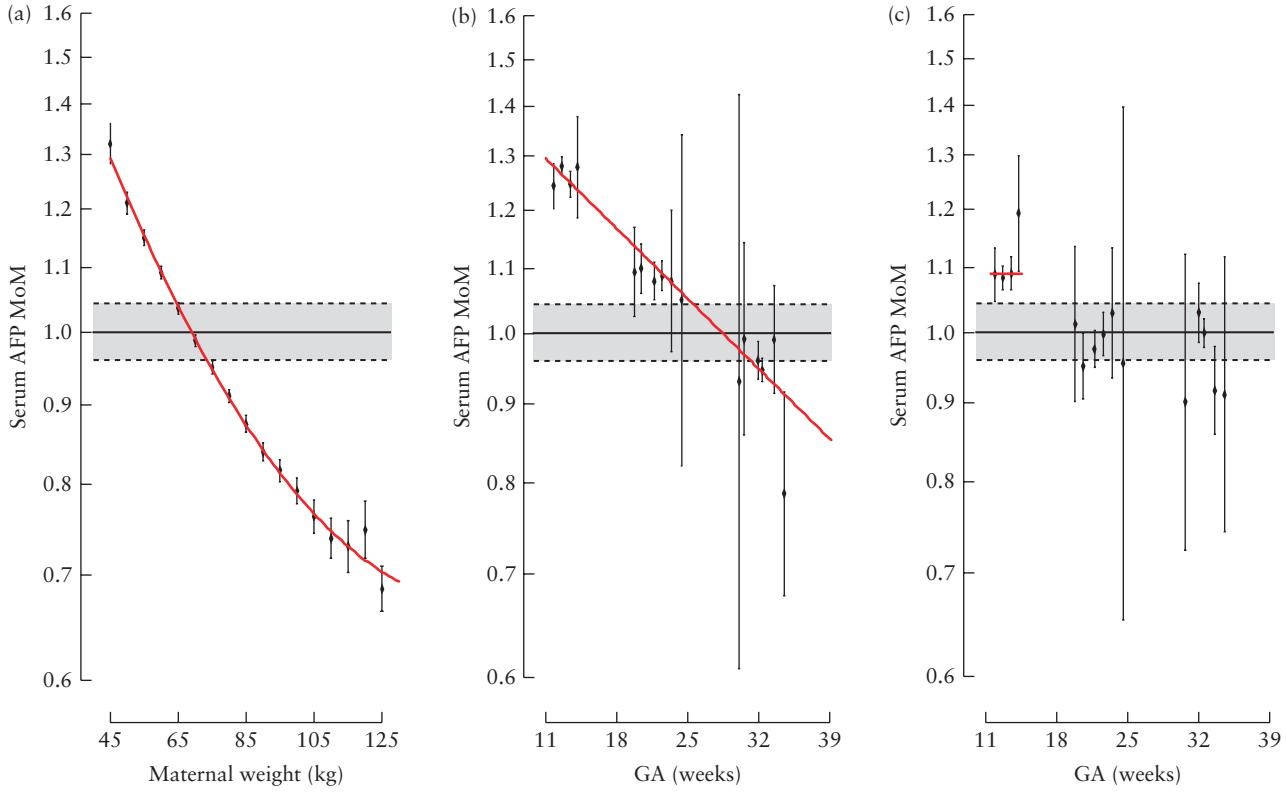

Figure 2 Effect of maternal weight (a), and Afro-Caribbean racial origin (b) and cigarette smoking (c) in relation to gestational age, on median (95% CI) serum alpha-fetoprotein (AFP), plotted on the multiples of the median (MoM) scale after correcting for other factors. Fitted effects (——), median MoM of 1.0 (——) and median MoM ± 0.1 SD (- - - -) are indicated. GA, gestational age.

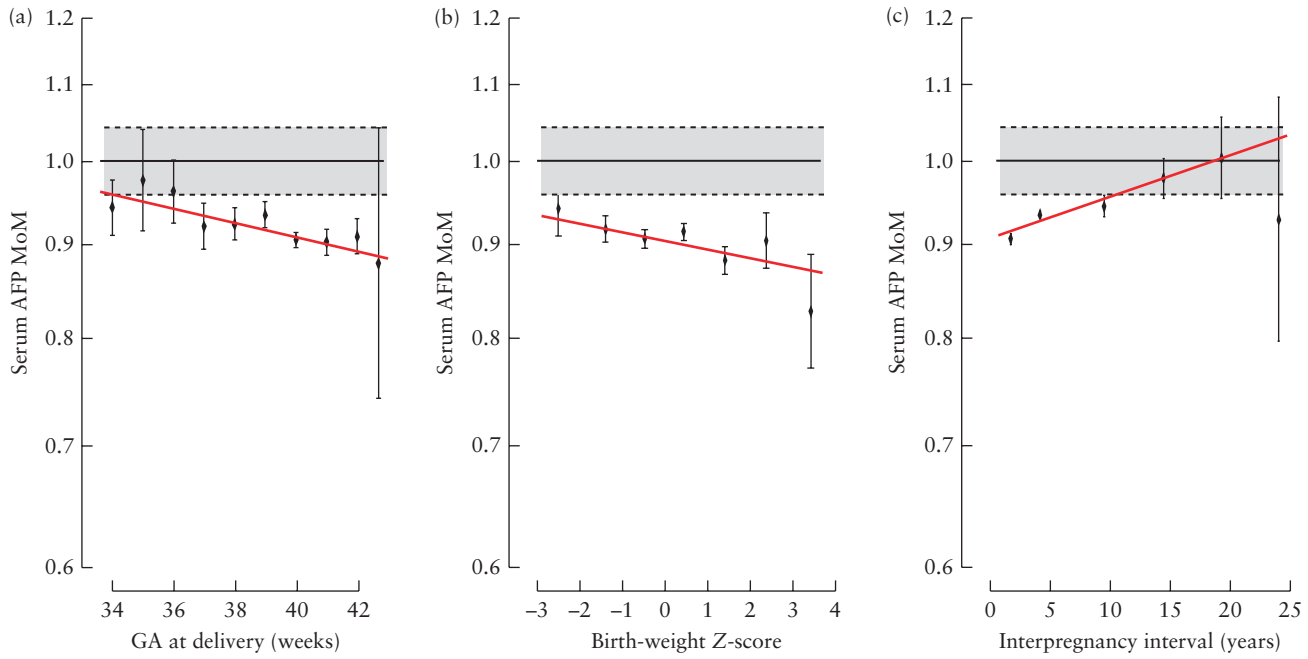

Figure 3 Effect of gestational age (GA) at delivery (a) and birth-weight *Z*-score of the neonate in the previous pregnancy (b) and interpregnancy interval (c) on median (95% CI) serum alpha-fetoprotein (AFP), plotted on the multiples of the median (MoM) scale after correcting for other factors. Fitted 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 alpha-fetoprotein

Term	*Estimate*	*95% CI*	*SE*	P
Intercept	0.95463921	0.95012120 to 0.95915722	0.00230511	< 0.0001
Trimester-dependent effects				
First trimester				
Smoking	0.03618913	0.02755020 to 0.04482807	0.00440762	< 0.0001
Second trimester				
Constant	0.00742188	0.00256029 to 0.01228347	0.00248040	0.00154
Third trimester				
Constant	0.00906895	0.00365116 to 0.01448674	0.00276418	0.00049
Trimester-independent effects				
Gestational age				
Gestational age (−77)*	0.01471000	0.01453705 to 0.01488295	0.00008824	< 0.0001
(Gestational age (−77))^2*	−0.00003909	−0.00004012 to −0.00003806	0.00000052	< 0.0001
Maternal weight				
Maternal weight (−69)†	−0.00398949	−0.00418527 to −0.00379371	0.00009989	< 0.0001
(Maternal weight (−69))^2†	0.00002220	0.00001702 to 0.00002738	0.00000264	< 0.0001
Racial origin				
Afro-Caribbean	0.11300040	0.10588194 to 0.12011886	0.00363187	< 0.0001
Afro-Caribbean: gestational age (−77)*	−0.00092456	−0.00100102 to −0.00084810	0.00003901	< 0.0001
Obstetric history				
Parous	−0.04135287	−0.04708246 to −0.03562329	0.00292326	< 0.0001
Parous: gestational age at delivery of previous pregnancy (−40)‡	−0.00390760	−0.00562421 to −0.00219098	0.00087582	< 0.0001
Parous: birth-weight *Z*-score	−0.00476072	−0.00737551 to −0.00214594	0.00133407	< 0.0001
Parous: interpregnancy interval in years	0.00226416	0.00135316 to 0.00317516	0.00046480	< 0.0001
Machine				
Roche	0.03948173	0.03467850 to 0.04428496	0.00245063	< 0.0001

Continuous data were centered by subtracting the mean from each measured value: *77 from gestational age in days; †69 from maternal weight in kg; ‡40 from gestational age at delivery of previous pregnancy in weeks. SE, standard error.

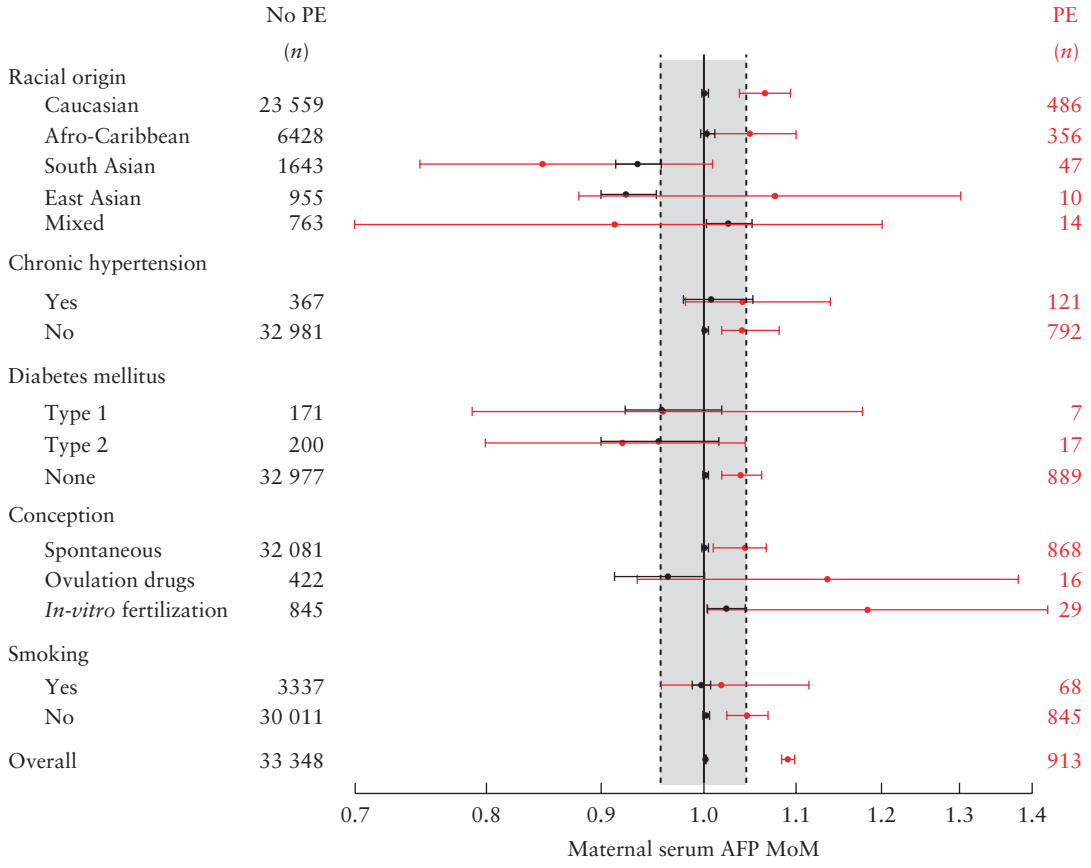

Figure 4 Median serum alpha-fetoprotein (AFP) multiples of the median (MoM) (with 95% CI) derived from the model according to racial origin, chronic hypertension, diabetes mellitus, method of conception and smoking in women who developed pre-eclampsia (PE) (red values) and in those unaffected by PE (black values). Median MoM of 1.0 (——) and median MoM ± 0.1 SD (- - - -) of women unaffected by PE are indicated.

Distributional properties of serum AFP MoM values

Figure 6 shows a Gaussian distribution of serum AFP MoM values. The median and 5th, 10th, 90th and 95th percentiles were 1.0000 (95% CI, 0.99492–1.00595) and 0.5247 (95% CI, 0.52004–0.52924), 0.60308 (95% CI, 0.59834–0.60721), 1.67624 (95% CI, 1.66447–1.6904) and 1.95664 (95% CI, 1.93647–1.97833), respectively. Estimated SD and correlations with 95% CI are given in Tables 3 and 4, respectively. The SD were similar across trimesters. The correlations between $\log_{10}$ serum AFP MoM across trimesters were stronger between first 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 AFP concentration are provided by maternal characteristics and variables from medical history. Serum AFP increases with increasing gestational age and decreases with greater maternal weight. There is an Afro-Caribbean effect on serum AFP, which is dependent on gestational age, whereby relative serum AFP levels are high at early gestations and low at late gestations. In parous women, serum AFP is lower than in nulliparous women and the level is related to the outcome of the previous pregnancy in terms of gestational age at delivery, birth-weight *Z*-score of the neonate and interpregnancy interval.

Random-effects multiple regression analysis was used to define the contribution of maternal variables that influence the measured serum AFP 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 AFP by automated machines that provide reproducible results within 40 min of sampling so that complete assessment and counseling can

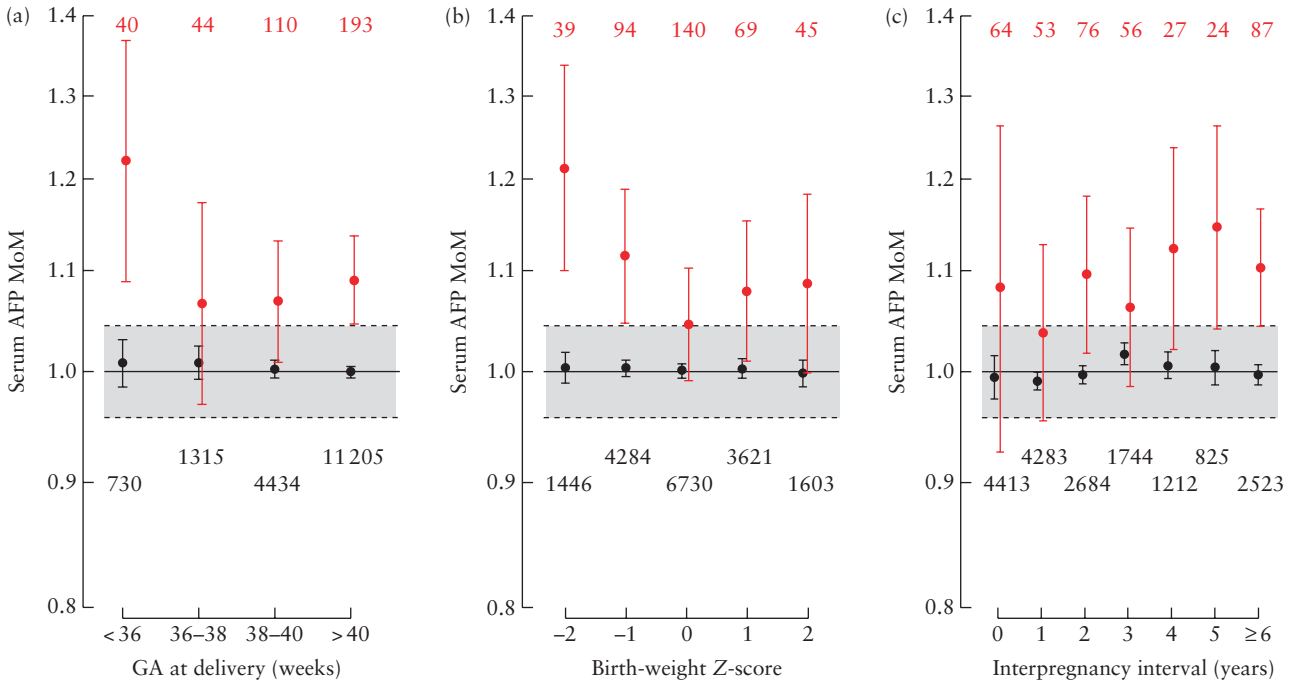

Figure 5 Median serum alpha-fetoprotein (AFP) multiples of the median (MoM) (with 95% CI) derived from the model according to gestational age (GA) at delivery (a) and birth-weight *Z*-score of the neonate (b) in the last pregnancy and interpregnancy interval (c) in women who developed pre-eclampsia (PE) (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 are indicated.

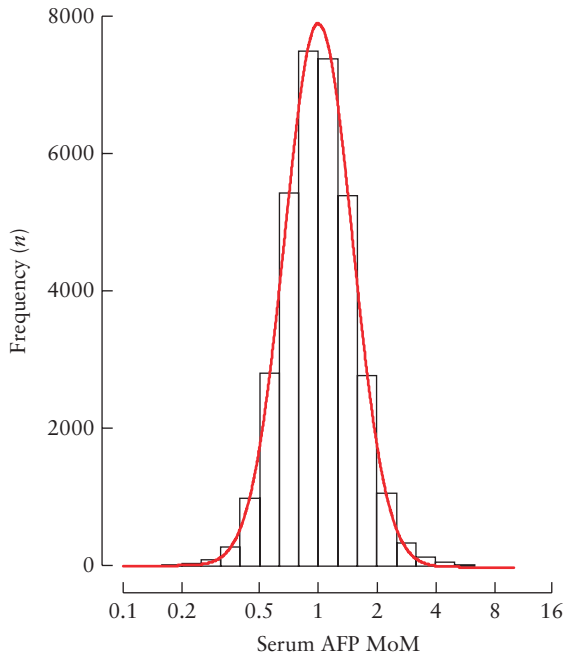

Figure 6 Gaussian distribution of serum alpha-fetoprotein (AFP) multiples of the median (MoM) values.

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 AFP 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.

Table 3 Standard deviations for $\log_{10}$ serum alpha-fetoprotein multiples of the median values for each trimester of pregnancy

Trimester	*SD Estimate (95% CI)*
First	0.19104 (0.19028–0.1918)
Second	0.15101 (0.15041–0.15161)
Third	0.17680 (0.17610–0.17751)

Table 4 Correlation of $\log_{10}$ serum alpha-fetoprotein multiples of the median values in each trimester of pregnancy

Trimester	*Second*	*Third*
First	0.36609 (0.33464–0.39673)	0.14189 (0.10658–0.17683)
Second	1	0.60852 (0.58545–0.63061)
Third	—	1

Values in parentheses are 95% CI.

Comparison with findings of previous studies

Previous studies, mainly undertaken in the second trimester, have also reported that serum AFP concentration is affected by gestational age and maternal characteristics, including maternal weight, racial origin and cigarette smoking[12–18]. 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. Additionally, we included variables such as the outcome of the previous pregnancy, because standardizing the measured values of biomarkers for any variables included in the prior model is essential for the application of Bayes' theorem in combined screening for pregnancy complications by maternal characteristics and biomarkers. The distribution of serum AFP should be specified conditionally on any terms included in the prior model.

Implications for clinical practice

Measurement of serum AFP may be useful in screening for aneuploidies, neural tube defects and adverse pregnancy outcome. Effective use of serum AFP 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.

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