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Maternal plasma cell-free DNA in the prediction of pre-eclampsia

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ABSTRACT

Objectives *To examine whether maternal plasma concentrations of total cell-free (cf)DNA and fetal fraction at 11–13 and 20–24 weeks' gestation in pregnancies that subsequently develop pre-eclampsia (PE) are different from those without this complication.*

Methods *Total cfDNA and fetal fraction were measured in 20 cases of early PE requiring delivery at < 34 weeks, in 20 cases of late PE with delivery at ≥ 34 weeks and in 200 normotensive controls, at 11–13 and 20–24 weeks' gestation. Total cfDNA and fetal fraction measured at 11–13 weeks were converted to multiples of the median (MoM), corrected for maternal characteristics and gestational age. The distributions of total cfDNA and fetal fraction at 20–24 weeks were expressed as MoM of values at 11–13 weeks. The Mann–Whitney* U*-test was used to determine the significance of differences in the median values in each outcome group relative to that in the controls.*

Results *In the early-PE group at 11–13 weeks, compared with controls, there was a significant increase in median total cfDNA (2104 genome equivalents (GE)/mL* vs *1590 GE/mL) and a decrease in median fetal fraction (6.8%* vs *8.7%). In the late-PE group at 20–24 weeks, compared with controls, there was a significant decrease in median fetal fraction (8.2%* vs *9.6%). These significant differences between groups were not observed when the values were converted to MoM.*

Conclusion *Measurements of total cfDNA and fetal fraction in maternal plasma at 11–13 and 20–24 weeks are not predictive of PE.*

INTRODUCTION

Pre-eclampsia (PE) complicates 2% of pregnancies and is one of the leading causes of maternal and perinatal morbidity and mortality in both developing and developed countries[1]. Development of the clinical signs of PE is thought to be the consequence of impaired trophoblastic invasion of the maternal spiral arteries, leading to placental hypoxia and the release of inflammatory cytokines, causing widespread vascular endothelial cell dysfunction[2,3].

Several studies have reported that in women with established PE, the plasma or serum concentrations of both total and fetal cell-free (cf)DNA are higher than in normotensive controls and the increase is particularly marked in those with severe PE[4–10]. These findings have been attributed to accelerated apoptosis of trophoblastic cells resulting from placental ischemia[4] and reduced clearance of the cfDNA from the maternal circulation in women with PE[11]. However, data are conflicting as to whether these altered levels precede the onset of the disease, and a recent systematic review was unable to draw definitive conclusions regarding the potential value of fetal cfDNA in the prediction of PE[12].

The aims of this study were to explore further whether maternal plasma concentrations of total cfDNA and fetal fraction at 11–13 and 20–24 weeks' gestation are increased in pregnancies that develop PE, and if these measurements are useful in the prediction of PE.

METHODS

Study population

This was a case–control study drawn from a prospective observational study of adverse pregnancy outcome in pregnant women attending their routine first- and second-trimester ultrasound scans at King's College Hospital, London, UK. The first-trimester visit, at 11–13 weeks' gestation, included recording of maternal characteristics and medical history and an ultrasound scan to, first, confirm gestational age from the measurement of the fetal crown–rump length (CRL)[13], second, diagnose any major fetal abnormalities and, third, measure fetal

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nuchal translucency thickness as part of combined screening for aneuploidies[14]. The second-trimester visit, at 20–24 weeks' gestation, included ultrasound examination for assessment of fetal anatomy, growth and wellbeing. In the two visits, plasma samples were collected and stored at −80 °C for subsequent biochemical analysis. Written informed consent was obtained from the women agreeing to participate in the study, which was approved by the National Health Service (NHS) Research Ethics Committee.

The inclusion criteria for the study were singleton pregnancy with plasma samples taken and stored at 11–13 and 20–24 weeks' gestation. The study population comprised 20 pregnant women who subsequently developed PE and delivered before 34 weeks (early PE), with plasma samples taken during the first trimester, and 20 pregnant women with PE delivering at or after 34 weeks (late PE), with plasma samples taken during the first and second trimesters of pregnancy. The control group consisted of 200 pregnancies matched with the cases for storage time; they did not develop any pregnancy complication and resulted in the live birth of phenotypically normal neonates. In the first trimester, all 200 controls were compared with 20 cases of early PE and 20 cases of late PE. In the second-trimester, 100 controls were compared with 20 cases of late PE.

Maternal demographic characteristics were recorded in a computer database. 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, as defined by the International Society for the Study of Hypertension in Pregnancy[15].

Laboratory analysis

Venous blood was collected in ethylenediamine tetraacetic acid (EDTA) BD Vacutainer™ tubes (Becton Dickinson UK Limited, Oxfordshire, UK) and, within 15 min of collection, was centrifuged at 2000 *g* for 10 min and again at 16 000 *g* for 10 min. Plasma samples were then stored at −80 °C until used for subsequent analyses. The samples were sent overnight, on dry ice, from London, UK, to the USA (Sequenom, Inc., San Jose, CA, USA). The laboratory personnel who processed the samples were blinded to all clinical sample information, including the diagnosis of PE. The purified cfDNA was evaluated using two independent methods: a targeted sequencing polymorphism-dependent method that allows assessment of genomic equivalents (GE)/mL; and a fetal fraction assessment derived from whole-genome random sequencing. This statistical approach exploits *a-priori* knowledge about the distribution of regional over-representations of sequencing counts that correlate well with the fetal cfDNA contribution. An analysis of a large set of genome-wide sequencing data revealed that specific regional over-representations of sequencing counts exist, which correlate well with the fetal cfDNA contribution. This effect is probably a result of differences between maternal and fetal cfDNA, such as size distributions or DNA-methylation patterns. The regions tend to be stable and the genome-wide pattern can be learned through a machine-learning approach. This trained model is validated in an independent test set using a secondary method for fetal fraction assessment. This model delivers an accurate measurement of fetal fraction (SeqFF). In pregnancies with delivery of male infants, fetal fraction was also determined by the chromosome Y dosage (ChrFFy)[16].

Statistical analysis

Comparisons between outcome groups were made using the Mann–Whitney *U*-test for continuous variables, and the chi-square test or Fisher's exact test for categorical variables. The distributions of total cfDNA and fetal fraction were made Gaussian after logarithmic transformation. In pregnancies with delivery of male infants, the significance of the association in fetal fraction determined by ChrFFy and SeqFF was examined. Regression analysis was used to determine the significance of the association between $\log_{10}$ values of total cfDNA with fetal fraction. Backward stepwise multiple regression analysis was used to determine which of the factors amongst the maternal characteristics and gestation were significant predictors of the $\log_{10}$ total cfDNA and $\log_{10}$ fetal fraction. The distributions of total cfDNA and fetal fraction at 11–13 weeks were converted to multiples of the median (MoM) in cases and controls, corrected for maternal characteristics and gestation. The distributions of total cfDNA and fetal fraction at 20–24 weeks were expressed as MoM of the expected median for that individual at 11–13 weeks in cases and controls. A Wilcoxon signed-rank test was used to compare total cfDNA and fetal fraction across the first- and second-trimester samples within each outcome group. A Mann–Whitney *U*-test was used to determine the significance of differences in the median values in each outcome group relative to that in the controls.

The statistical software package, SPSS 22.0 (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY, USA: IBM Corp.) was used for all data analyses.

RESULTS

The maternal characteristics of each outcome group are summarized in Table 1. In the early-PE and late-PE groups, compared with the normal group, there was a higher median maternal body mass index (BMI), a higher prevalence of personal history of PE and chronic hypertension. In addition, in the early-PE group, there was a higher prevalence of nulliparous women, a lower maternal height, a lower prevalence of Caucasian racial origin and fewer women with no personal history of PE. The median gestational age at delivery and neonatal birth weight were significantly lower in the early-PE group than in the normal group.

Table 1 Maternal and obstetric characteristics of the study population

Characteristic	*Normal (n = 200)*	*Early PE (n = 20)*	*Late PE (n = 20)*
11–13 weeks' gestation ($n = 240$)			
Maternal weight (kg)	65.8 (58.8–77.7)	78.0 (60.3–94.8)	76.0 (63.7–91.2)
Maternal BMI (kg/m^2)	24.1 (21.5–27.9)	29.8 (24.4–35.2)*	28.6 (23.6–33.2)*
Gestational age at screening (weeks)	12.6 (12.1–13.0)	12.6 (12.3–12.8)	12.7 (12.3–13.3)
20–24 weeks' gestation ($n = 120$)			
Maternal weight (kg)	70.1 (62.7–82.1)	—	80.9 (66.9–99.0)*
Maternal BMI (kg/m^2)	25.7 (22.6–29.1)	—	31.5 (25.2–34.4)*
Gestational age at screening (weeks)	22.0 (21.7–22.2)	—	22.0 (21.7–22.0)
Maternal age (years)	31.6 (28.8–35.4)	29.3 (23.5–34.6)	32.0 (29.6–33.3)
Maternal height (cm)	166 (160–170)	160 (157–164)*	162 (160–166)
Racial origin			
Caucasian	127 (63.5)	5 (25.0)*	12 (60.0)
Afro-Caribbean	57 (28.5)	11 (55.0)	7 (35.0)
South Asian	6 (3.0)	3 (15.0)	0 (0.0)
East Asian	2 (1.0)	1 (5.0)	0 (0.0)
Mixed	8 (4.0)	0 (0.0)	1 (5.0)
Parity			
Nulliparous	81 (40.5)	14 (70.0)*	10 (50.0)
Parous			
No prior PE	116 (58.0)	3 (15.0)*	7 (35.0)
Prior PE	3 (1.5)	3 (15.0)*	3 (15.0)*
Family history of mother with PE	11 (5.5)	3 (15.0)	1 (5.0)
Cigarette smoker	12 (6.0)	1 (5.0)	2 (10.0)
Conception			
Spontaneous	194 (97.0)	18 (90.0)	19 (95.0)
Ovulation drugs	2 (1.0)	1 (5.0)	0 (0.0)
In-vitro fertilization	4 (2.0)	1 (5.0)	1 (5.0)
Chronic hypertension	1 (0.5)	4 (20.0)*	5 (25.0)*
Gestational age at delivery (weeks)	39.4 (38.9–40.3)	31.5 (28.2–33.3)*	39.0 (38.5–40.0)
Birth weight (g)	3402 (3117–3650)	1245 (712–1506)*	3398 (3074–3707)
Birth-weight percentile	52.6 (27.4–71.2)	1.7 (0.4–7.0)*	48.7 (30.8–86.7)

Data are given as median (interquartile range) or *n* (%). BMI, body mass index; PE, pre-eclampsia. Comparison between outcome groups was performed using Mann–Whitney *U*-test with post-hoc Bonferroni correction for continuous variables and chi-square test or Fisher's exact test for categorical variables. *Adjusted significance level < 0.025.

In pregnancies with delivery of male infants, there was a strong, significant correlation between $\log_{10}$ fetal fraction determined by SeqFF and $\log_{10}$ fetal fraction determined by ChrFFy ($r = 0.836$, $P < 0.0001$) (Figure 1). In all cases, there was a significant negative correlation between $\log_{10}$ total cfDNA and $\log_{10}$ fetal fraction determined by SeqFF ($r = -0.396$, $P < 0.0001$; Figure 2).

Normal pregnancy outcome

Multiple regression analysis demonstrated that for the prediction of $\log_{10}$ total cfDNA, a significant independent contribution was provided by Afro-Caribbean racial origin. Multiple regression analysis also demonstrated that for the prediction of $\log_{10}$ fetal fraction, significant independent contributions were provided by fetal CRL, maternal weight, maternal height, Afro-Caribbean racial origin and conception with *in-vitro* fertilization (IVF) (Table 2).

In the controls, there was a significant increase of 10% in both the median fetal fraction and its MoM values across the first and second trimesters ($P < 0.0001$; Table 3). The median total cfDNA and its MoM values were not significantly different across the first and second trimesters.

Pre-eclampsia

In the early-PE group, compared with the controls, there was a significant increase in the median total cfDNA and a significant decrease in the median fetal fraction at 11–13 weeks but these significant alterations were not observed in their MoM values (Table 3).

In the late-PE group, there was no significant change in the median total cfDNA and fetal fraction and in their MoM values between the first and second trimesters. Compared with the controls, the median fetal fraction was significantly reduced at 20–24 weeks but the MoM values were not significantly different between the late-PE and control groups.

DISCUSSION

Main findings of the study

This study has demonstrated that, at 11–13 weeks' gestation in pregnancies that subsequently develop early PE, the median maternal plasma concentration of total cfDNA is increased and fetal fraction is reduced. In pregnancies that develop late PE the median fetal fraction

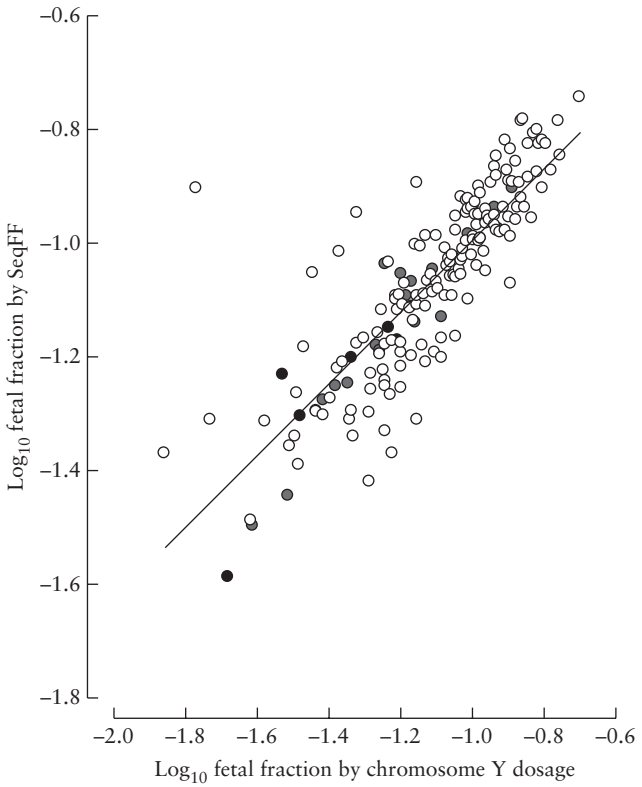

Figure 1 Relationship between $\log_{10}$ values of fetal fraction measured by SeqFF and by chromosome Y dosage in pregnancies delivering male neonates (○, controls; ●, early pre-eclampsia; ●, late pre-eclampsia).

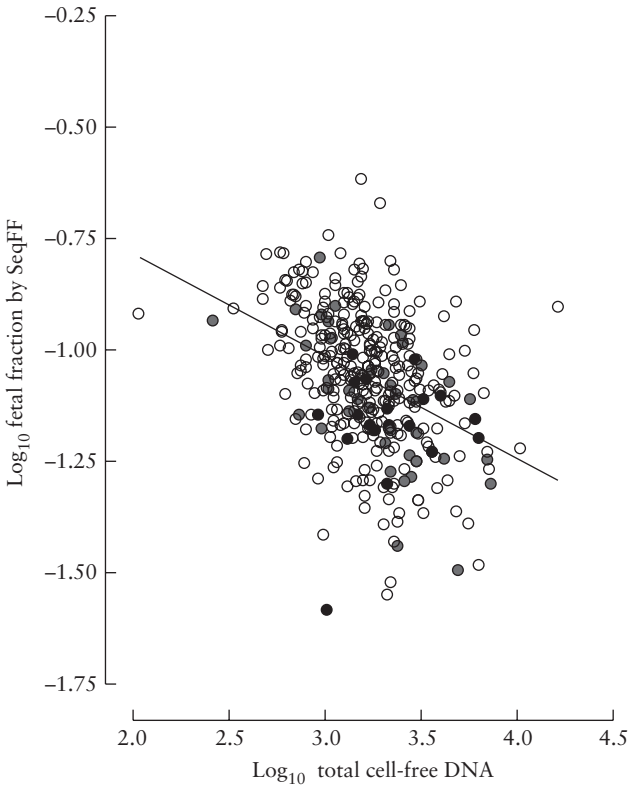

Figure 2 Relationship between $\log_{10}$ values of fetal fraction measured by SeqFF and total cell-free DNA (○, controls; ●, early pre-eclampsia; ●, late pre-eclampsia).

at 20–24 weeks is reduced. However, both total cfDNA and fetal fraction are affected by maternal characteristics and when these associations are taken into account the MoM in PE are not significantly different from those in normotensive controls.

The study has also shown that the values of fetal fraction obtained through independent methods correlate strongly with each other. Values obtained by chromosome Y dosage have traditionally been the methods most commonly used for fetal fraction measurement. Measurement of fetal fraction through SeqFF can enable a more widespread adaptation of this measurement in genome-wide cfDNA testing approaches because it is applicable to all pregnancies, irrespective of the fetal sex, and eliminates the need for an additional assay format.

In normal pregnancies, total cfDNA is higher in women of Afro-Caribbean racial origin than in Caucasians and the fetal fraction increases with fetal CRL and maternal height, decreases with maternal weight, is higher in women of Afro-Caribbean racial origin than in Caucasians and is lower in women who conceived with IVF.

Limitations of the study

PE is a heterogeneous disorder in terms of maternal phenotype, pathophysiology and severity. Consequently, the total number of normal and pathological pregnancies examined in this study may be inadequate for concluding that the maternal and fetal cfDNA levels are not altered in all types of PE. Nevertheless, the findings suggest that measurement of maternal plasma total cfDNA or fetal fraction is unlikely to be useful in screening for PE either at 11–13 or at 20–24 weeks' gestation.

Comparison with findings from previous studies

A recent systematic review investigated the usefulness of cfDNA quantification in the prediction of PE[12]. The review included three prospective cohort studies and 10 case–control studies with a total of 440 cases of PE and 2576 controls. The authors reported that 11 of the 13 studies found significantly higher concentrations of fetal cfDNA in women who developed PE. Four studies that evaluated cases of severe or early PE found significantly elevated fetal cfDNA concentrations before disease onset. Nevertheless, the authors alluded to the fact that most of the included studies did not adequately control for possible confounding factors, such as BMI, smoking status and racial origin, and that the definitions of PE and its severity varied. Owing to the significant heterogeneity between the published studies, a clinically meaningful meta-analysis could not be performed and therefore no precise conclusions could be drawn[12].

The majority of the published studies used a chromosome Y gene marker to quantify fetal cfDNA in pregnancies with male fetuses. Evaluating 44 PE cases and 176 controls, Sifakis *et al.* assessed the DYS14 locus in chromosome Y using the polymerase chain reaction to determine fetal cfDNA and reported that increased

Table 2 Fitted regression model for $\log_{10}$ total cell-free DNA and $\log_{10}$ fetal fraction by SeqFF at 11–13 weeks

Independent variable	*Regression coefficient (95% CI)*	*SE*	P
$\log_{10}$ total cell-free DNA			
Intercept	3.15813 (3.11670 to 3.19956)	0.021010	< 0.0001
Afro-Caribbean racial origin	0.19047 (0.11286 to 0.26708)	0.039356	< 0.0001
$\log_{10}$ fetal fraction by SeqFF			
Intercept	−1.59735 (−2.11240 to −1.082298)	0.26115	0.0001
Fetal crown–rump length (mm)	0.0034851 (0.0010566 to 0.0059135)	0.0012313	0.005
Maternal weight (kg)	−0.0035392 (−0.0048478 to −0.0022306)	0.00066350	< 0.0001
Maternal height (cm)	0.0034944 (0.00044763 to 0.0065411)	0.0015448	0.025
Afro-Caribbean racial origin	−0.055280 (−0.099198 to −0.011363)	0.022267	0.014
In-vitro fertilization	−0.13937 (−0.27639 to −0.0023592)	0.069471	0.046

SE, standard error.

Table 3 Comparison of mean of $\log_{10}$ multiples of the median (MoM) total cell-free DNA (cfDNA) and fetal fraction (SeqFF) of outcome groups

	Total cfDNA		*Fetal fraction (SeqFF)*	
Outcome group	*GE/mL*	*MoM*	*%*	*MoM*
Controls				
First trimester ($n = 200$)	1590 (1111–2312)	0.963 (0.693–1.348)	8.74 (6.73–11.03)	1.037 (0.804–1.261)
Second trimester ($n = 100$)	1746 (1162–2311)	1.073 (0.692–1.438)	9.65 (7.60–11.98)	1.146 (0.932–1.408)
Comparison across trimesters (P)	0.611	0.672	< 0.0001*	< 0.0001*
Early PE				
First trimester ($n = 20$)	2104 (1454–3547)†	1.199 (0.927–1.631)	6.85 (6.29–7.81)†	0.993 (0.879–1.098)
Late PE				
First trimester ($n = 20$)	2178 (1123–2847)	1.138 (0.687–1.910)	7.69 (6.49–9.83)	0.969 (0.771–1.222)
Second trimester ($n = 20$)	2140 (1067–2934)	1.158 (0.728–1.734)	8.20 (5.70–10.68)‡	0.935 (0.803–1.314)
Comparison across trimesters (P)	0.911	0.881	0.232	0.247

Data are given as median (interquartile range). Comparisons within outcome group and across trimesters were made using the Wilcoxon signed-rank test: *significant at $P < 0.05$. Comparison with controls in the first trimester was carried out using the Mann–Whitney U-test with post-hoc Bonferroni correction: †significant at adjusted $P < 0.025$. Comparison with controls in the second trimester was carried out using the Mann–Whitney U-test: ‡significant at $P < 0.05$. GE, genomic equivalent; PE, pre-eclampsia.

concentrations preceded the clinical onset at 11–13 weeks in women who developed early PE, but there was no difference when total PE and late PE groups were compared with controls[17]. Leung *et al.*, using an assay for the detection of the *SRY* gene, demonstrated that the median fetal cfDNA at 11–22 weeks was higher in 18 women who developed PE when compared with 33 normal controls[18]. However, two subsequent studies that quantified the *SRY* and *RHD* genes, respectively, in a total of 60 cases of PE and 639 controls in the second trimester of pregnancy, found no significant differences between the two groups in maternal plasma fetal or total cfDNA levels[19,20].

A recent study has used chromosome-selective sequencing of non-polymorphic and polymorphic loci, in which fetal alleles differ from maternal alleles, to determine cfDNA counts of fetal and maternal origin in maternal plasma at 11–13 weeks' gestation[21]. Both fetal and maternal cfDNA counts were affected by maternal characteristics, but the corrected values in 46 cases that developed PE were not significantly different from 1805 normal pregnancies that did not develop PE.

Implications for clinical practice

Effective screening for PE can be provided by a combination of maternal characteristics, mean arterial pressure, uterine artery pulsatility index and serum pregnancy-associated plasma protein-A and placental growth factor at 11–13 weeks' gestation[22]. The benefit of such early identification of high-risk pregnancies for PE is the potential to reduce the prevalence of the disease through the prophylactic use of low-dose aspirin[23,24]. The reported high performance of cfDNA analysis of maternal blood in screening for fetal trisomies will inevitably lead to widespread uptake of this technique, and an integral part of such aneuploidy screening is measurement of the fetal fraction[25]. A beneficial consequence of such measurement of the fetal fraction would have been improved performance of early screening for PE. However, as demonstrated by our study, this is unlikely to be the case.

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Disclosure

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