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Fetal fraction of cell-free DNA in maternal plasma in the prediction of spontaneous preterm delivery

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KEYWORDS: cell-free DNA; fetal fraction; first-trimester screening; preterm birth

ABSTRACT

Objectives *To investigate whether, in pregnancies complicated by spontaneous preterm delivery, fetal fraction of cell-free DNA (cfDNA) in maternal plasma at 11–13 weeks' gestation is altered and if this measurement could be useful in the prediction of preterm delivery.*

Methods *Fetal fraction of cfDNA was measured at 10 + 0 to 13 + 6 weeks' gestation in 3169 pregnancies, 3066 (96.7%) that delivered at ≥ 37 weeks and 103 (3.3%) with spontaneous delivery at < 37 weeks, including 21 that delivered at < 34 weeks and 82 that delivered at 34–37 weeks. The measured fetal fraction was converted to multiples of the median (MoM), corrected for maternal characteristics and gestational age, and the Mann–Whitney* U*-test was used to determine the significance of differences in the median values in the spontaneous preterm delivery groups from that in the term delivery group.*

Results *In the spontaneous preterm delivery groups (< 34 weeks' gestation, 34–37 weeks, < 37 weeks), compared to the term delivery group, there was no significant difference in the median fetal fraction MoM (1.004, 0.922 and 0.946, respectively,* vs *1.015).*

Conclusion *Measurement of fetal fraction in maternal plasma at 11–13 weeks' gestation is not predictive of spontaneous preterm delivery.* *Copyright © 2014 ISUOG. Published by John Wiley & Sons Ltd.*

INTRODUCTION

Preterm delivery is the leading cause of perinatal death and disability in children, and the vast majority of mortality and morbidity relates to early delivery before 34 weeks' gestation[1,2]. Spontaneous delivery before 34 weeks occurs in about 1% of singleton pregnancies. Studies have shown that the patient-specific risk for spontaneous early delivery can be determined at 11–13 weeks' gestation by combining maternal demographic characteristics and obstetric history, with an estimated detection rate of 28%, at a false-positive rate of 10%[3]. This performance of screening is not improved by biophysical or biochemical markers of placental perfusion or function, but the detection rates increased to 36% and 55% by the addition of maternal serum α-fetoprotein and cervical length measurement, respectively, at 11–13 weeks[3–5]. There is also some evidence that plasma cell-free DNA (cfDNA) is increased in pregnancies complicated by spontaneous preterm birth, with a suggested mechanism of early initiation of breakdown of the placental barrier in anticipation of labor[6–8]. However, there is contradictory evidence as to whether, in cases of spontaneous preterm birth, the increase in cfDNA precedes the clinical event[9–12].

The aims of this study were to explore whether, in pregnancies that are complicated by spontaneous preterm birth, fetal fraction of cfDNA in maternal plasma at 11–13 weeks' gestation is altered and if this measurement is useful in the prediction of this pregnancy complication.

METHODS

The data for this study were derived from clinical implementation of cfDNA testing in screening for trisomies 21, 18 and 13 at 10 + 0 to 13 + 6 weeks' gestation in women with singleton pregnancies, attending the Fetal Medicine Centre in London, UK, between October 2012 and November 2013.

We recorded maternal characteristics and medical history, provided pretest counseling and obtained written consent for cfDNA testing. Patients were asked to complete a questionnaire on maternal age, racial origin (Caucasian, Afro-Caribbean, South Asian, East Asian or mixed), method of conception (spontaneous or assisted conception requiring the use of ovulation drugs or

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in-vitro fertilization), cigarette smoking during pregnancy (yes or no) and parity (parous or nulliparous if no previous pregnancy at or after 24 weeks' gestation). The questionnaire was then reviewed by a doctor together with the patient. Maternal weight was measured and recorded. An ultrasound examination was carried out to determine if the pregnancy was singleton with a live fetus and to estimate gestational age from measurement of the fetal crown–rump length (CRL)[13]. 20 mL of maternal blood was obtained by venepuncture, using Streck cfDNA BCT™ tubes, and cfDNA testing was subsequently performed using the Harmony™ Prenatal Test (Ariosa Diagnostics, Inc., San Jose, CA, USA).

Data on pregnancy outcome were collected from obstetricians, general medical practitioners or the women themselves. The outcome measures were spontaneous delivery at < 34 weeks' gestation (early preterm), at 34 + 0 to 36 + 6 weeks (late preterm) and at < 37 weeks (total preterm). Spontaneous preterm birth included those with spontaneous onset of labor and those with preterm prelabor rupture of membranes (PPROM).

Laboratory analysis

Maternal venous blood samples were sent via courier to the USA for analysis using a chromosome-selective assay (Harmony Prenatal Test). The method relies on the assay of non-polymorphic and polymorphic loci, in which fetal alleles differ from maternal alleles, enabling simultaneous determination of chromosome proportion and fetal fraction[14]. Risk scores for trisomies 21, 18 and 13 and fetal fraction were provided in the test report[14–16].

Statistical analysis

Descriptive data are presented as median and interquartile range for continuous variables and as *n* (%) for categorical variables. Comparisons between outcome groups were performed using the Mann–Whitney *U*-test for continuous variables and the χ^2 test or Fisher's exact test for categorical variables.

The measured fetal fraction was $\log_{10}$ transformed to make the distribution Gaussian. Normality of distribution was assessed using probability plot. Regression analysis was used to examine the significance of association between $\log_{10}$ fetal fraction and gestational age at delivery. In the term delivery group, backward stepwise multiple regression was used to determine which of the factors among fetal CRL, maternal age, weight, racial origin, smoking status, parity and method of conception were significant predictors of $\log_{10}$ fetal fraction. The distributions of fetal fraction were converted to multiples of the median (MoM) in all cases, corrected for maternal characteristics and gestational age as determined. The Mann–Whitney *U*-test was used to determine the significance of differences in the median values between the outcome groups.

The statistical software package SPSS 22.0 (SPSS Inc., Chicago, IL, USA) was used for data analysis.

RESULTS

During the study period, cfDNA testing was performed in 3483 singleton pregnancies with a live fetus at 10 + 0 to 13 + 6 weeks' gestation. We excluded 314 (9.0%) cases owing to loss to follow-up or incomplete data on pregnancy outcome ($n = 128$), termination of pregnancy because of aneuploidies or other fetal abnormalities ($n = 57$), miscarriage ($n = 49$), cfDNA assay failure ($n = 12$) or iatrogenic delivery at < 37 weeks ($n = 68$). Thus the study population comprised 3169 pregnancies. In 3123 of these cases, the reported result from cfDNA analysis was low risk for the three trisomies and the fetal fraction was ≥ 4%. In 46 (1.5%) cases, the fetal fraction was < 4% and no risk was given for the three trisomies; these pregnancies resulted in the birth of phenotypically normal neonates and for the purpose of the analysis the fetal fraction was assumed to be 3%.

The study population comprised 3066 (96.7%) pregnancies that delivered at ≥ 37 weeks' gestation and 103 (3.3%) with spontaneous delivery at < 37 weeks. The maternal and pregnancy characteristics of each of the outcome groups are summarized in Table 1. In both early and late spontaneous preterm delivery groups (< 34 weeks and 34 + 0 to 36 + 6 weeks), compared to the term delivery group, the median gestational age at delivery and neonatal birth weight were significantly reduced.

The frequency distribution of $\log_{10}$ fetal fraction is shown in Figure 1. In the total study group, there was no significant correlation between $\log_{10}$ fetal fraction and gestational age at delivery ($r = 0.030$, $P = 0.095$; Figure 2). In the term delivery group, multiple regression analysis demonstrated that for the prediction of $\log_{10}$ fetal fraction, significant independent contributions were provided by fetal CRL, maternal weight, South Asian racial origin, parity and method of conception (Table 2). In the spontaneous preterm delivery groups (< 34 weeks, 34 + 0 to 37 weeks, < 37 weeks), compared to the term delivery group, there was no significant difference in the median fetal fraction (Table 3, Figure 3).

DISCUSSION

Main findings of the study

The findings of this study demonstrate that in pregnancies resulting in early and late spontaneous preterm delivery, the median fetal fraction of cfDNA in the maternal plasma at 11–13 weeks' gestation is not significantly different from that of those delivering at term.

In pregnancies that result in term delivery, the fetal fraction of cfDNA in maternal plasma increases with fetal CRL, decreases with maternal weight, is higher in parous than nulliparous women, lower in women of South Asian racial origin than in Caucasians and lower in women who had assisted, rather than spontaneous, conception. Consequently, the estimated fetal fraction was adjusted for these variables before comparing results between outcome groups.

Table 1 Characteristics of the study population of 3169 women with a singleton pregnancy, according to term, early-preterm or late-preterm delivery

	GA at delivery (weeks)		
Characteristic	*≥ 37 (term)* (n = *3066*)	*< 34 (preterm)* (n = *21*)	*34 + 0 to 36 + 6 (late preterm)* (n = *82*)
Maternal age (years)	36.8 (33.8–39.6)	38.8 (35.6–41.1)	35.9 (33.1–39.3)
Maternal weight (kg)	63.0 (57.5–71.0)	62.7 (54.2–67.0)	60.5 (54.9–69.9)
Crown–rump length (mm)	64.7 (60.4–69.4)	65.0 (58.9–68.4)	65.2 (60.1–71.1)
Racial origin			
Caucasian	2673 (87.2)	18 (85.7)	70 (85.4)
Afro-Caribbean	63 (2.1)	—	1 (1.2)
South Asian	174 (5.7)	3 (14.3)	7 (8.5)
East Asian	103 (3.4)	—	3 (3.7)
Mixed	53 (1.7)	—	1 (1.2)
Parity			
Nulliparous	1384 (45.1)	11 (52.4)	43 (52.4)
Parous	1682 (54.9)	10 (47.6)	39 (47.6)
Cigarette smoker	23 (0.8)	—	1 (1.2)
Conception			
Spontaneous	2620 (85.5)	18 (85.7)	72 (87.8)
Assisted reproduction	446 (14.5)	3 (14.3)	10 (12.2)
GA at delivery (weeks)	39.6 (38.9–40.3)	31.6 (27.9–33.2)*	35.7 (35.3–36.3)*
Birth weight (g)	3405 (3133–3710)	1839 (1016–2003)*	2670 (2300–2935)*
Birth-weight percentile	53.3 (28.8–78.3)	44.3 (36.3–66.0)	53.5 (20.6–76.8)
Birth-weight percentile < 5^{th}	97 (3.2)	—	7 (8.5)

Data are given as median (interquartile range) or *n* (%). *Statistically significant difference on comparison between each preterm-delivery group with term-delivery group (Mann–Whitney *U*-test with *post hoc* Bonferroni correction and χ^2-test or Fisher's exact test for categorical variables, $P < 0.025$). GA, gestational age.

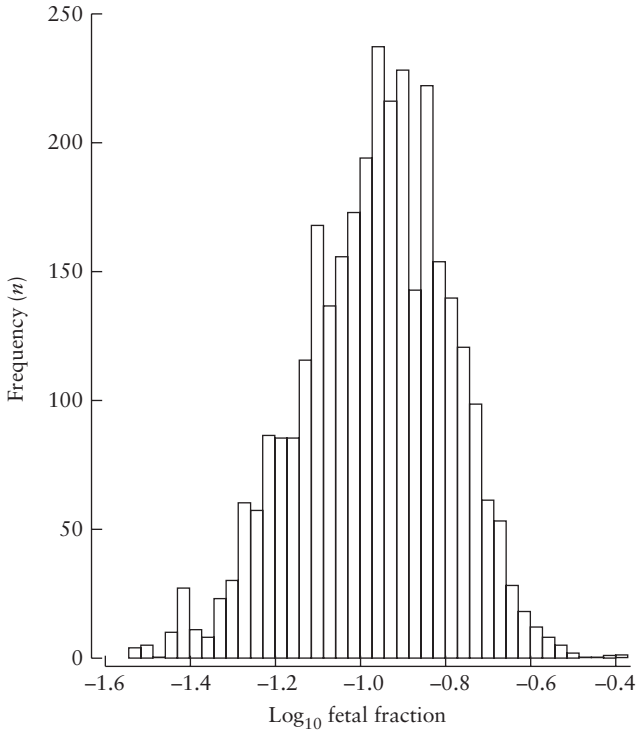

Figure 1 Frequency distribution of $\log_{10}$-transformed fetal fraction in maternal plasma cell-free DNA in the total study group of 3169 singleton pregnancies.

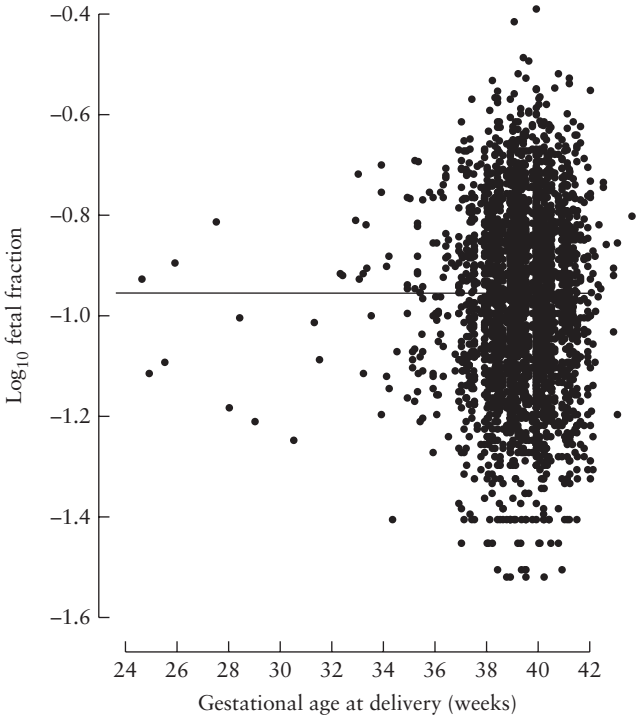

Figure 2 Association between $\log_{10}$-transformed fetal fraction of cell-free DNA in maternal plasma and gestational age at delivery in 3169 singleton pregnancies. Pearson correlation coefficient, $r = 0.030$; $P = 0.095$.

Limitations of the study

This was a cross-sectional study at 10 + 0 to 13 + 6 weeks' gestation and the conclusions, in relation to the inability of fetal fraction to distinguish between pregnancies that subsequently deliver preterm and those delivering at term, are confined to this early gestational age. Longitudinal studies are needed to examine whether a change in fetal fraction precedes the onset of labor and the interval between the two events.

Table 2 Fitted regression model for $\log_{10}$ fetal fraction at 10–13 weeks' gestation in 3169 women with a singleton pregnancy

Independent variable	*Regression coefficient (95% CI)*	*Standard error*	P
Intercept	−0.68531 (−0.74661 to −0.62491)	0.031263	< 0.0001
Fetal crown–rump length (mm)	0.0010460 (0.00022669 to 0.0018653)	0.00041786	0.012
Maternal weight (kg)	−0.0055255 (−0.0060105 to −0.0050405)	0.00024735	< 0.0001
South Asian racial origin	−0.033763 (−0.058070 to −0.0094556)	0.012397	0.007
Parous	0.045157 (0.033727 to 0.056587)	0.058293	< 0.0001
Assisted conception	−0.061662 (−0.077795 to −0.045529)	0.0082280	< 0.0001

Table 3 Fetal fraction of cell-free DNA in maternal plasma according to different pregnancy outcome groups in 3169 women with a singleton pregnancy

	Fetal fraction	
Outcome group	*Percent*	*MoM*
Term: delivery ≥ 37 weeks ($n = 3066$)	11.1 (8.3–14.3)	1.015 (0.787–1.284)
Early preterm: spontaneous delivery < 34 weeks ($n = 21$)	11.7 (7.8–12.5)	1.004 (0.705–1.184)
Late preterm: spontaneous delivery 34 + 0 to 36 + 6 weeks ($n = 82$)	10.8 (7.7–13.8)	0.922 (0.768–1.218)
*Preterm: spontaneous delivery < 37 weeks ($n = 103$)	10.8 (7.7–13.6)	0.946 (0.753–1.207)

Data are given as median (interquartile range). *Comprises both early-preterm and late-preterm deliveries. Early-preterm and late-preterm groups were each compared to term group (*P*-value cut-off 0.025) and preterm group was compared to term group (*P*-value cut-off 0.05) using Mann–Whitney *U*-test and with *post hoc* Bonferroni correction, with no significant difference found in any comparison. MoM, multiples of the median.

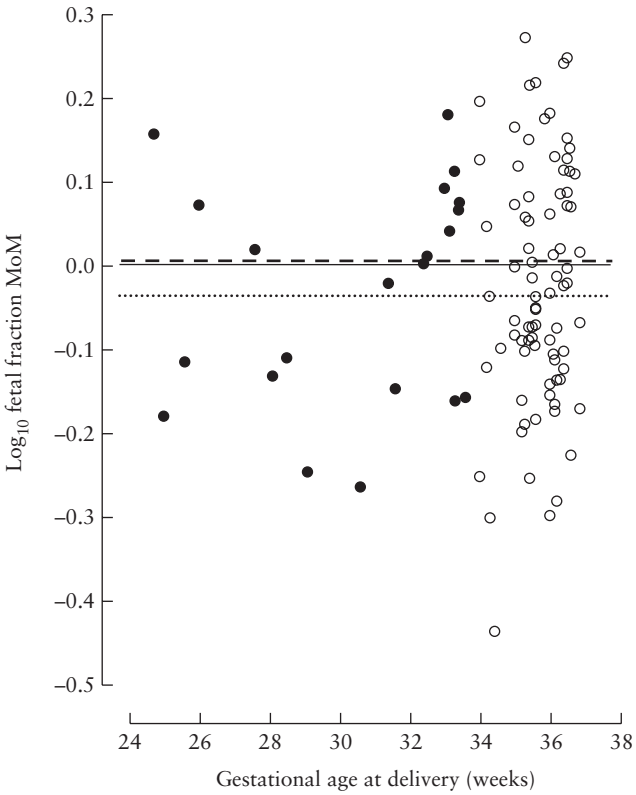

Figure 3 Association between $\log_{10}$-transformed fetal fraction multiples of the median (MoM) and gestational age at delivery in cases of spontaneous delivery at < 34 weeks' gestation (●; ——, median) and spontaneous delivery at 34–37 weeks (○; ....., median). – –, Median $\log_{10}$MoM of term delivery.

Comparison with findings from previous studies

Two previous studies examined women presenting with contractions and/or PPROM, and reported that, in those with spontaneous preterm delivery, maternal plasma fetal cfDNA was increased[6,7]. Leung *et al.*[6], using real-time quantitative polymerase chain reaction (PCR) for the detection of the *SRY* gene, demonstrated that the median fetal cfDNA was higher in 13 pregnancies with male fetuses, presenting with threatened preterm labor and subsequent delivery at 26–34 weeks, when compared to 17 controls that delivered at term. Farina *et al.*[7] assessed the *DYS1* locus on the Y chromosome by real-time PCR to determine fetal cfDNA in 29 women with preterm labor delivering at < 36 weeks, 21 with PPROM delivering at < 36 weeks and 21 with preterm labor delivering at ≥ 36 weeks. They reported that cumulative rates of delivery at < 30 weeks and delivery at < 36 weeks were significantly higher for women with fetal cfDNA ≥ 1.82 MoM than for those with fetal cfDNA concentrations below this cut-off (early preterm delivery: 45% *vs* 14%; preterm delivery: 73% *vs* 66%). The observed increase in fetal cfDNA levels appears to be part of the process that initiates the onset of labor and subsequent delivery.

Some contradictory evidence suggests that, in cases of spontaneous preterm delivery, the increase in cfDNA precedes the clinical event. A cohort study of 876 women undergoing routine fetal rhesus D (RhD) genotyping, at 23–28 weeks' gestation, reported that if the fetal cfDNA level was above the 95th percentile there was a 6- and 16-fold increase in risk for spontaneous delivery at < 37 weeks ($n = 19$) and at < 34 weeks ($n = 8$), respectively[9]. In contrast, Stein *et al.*[12] reported that, in a cohort study of 611 women undergoing routine fetal RhD genotyping at 24–25 weeks' gestation, the levels of fetal cfDNA were not altered in pregnancies complicated by preterm delivery. A study examining 34 women with a short cervix and 22 women with normal cervical length at 22–24 weeks' gestation assessed the *DYS14* locus on the Y chromosome by real-time PCR to determine fetal cfDNA levels, and reported no significant difference in the level of fetal cfDNA between those that delivered before 37 weeks and those delivering at term[11]. More

recently, a study used chromosome-selective sequencing of non-polymorphic and polymorphic loci, in which fetal alleles differ from maternal alleles, to determine the cfDNA counts of fetal and maternal origin in maternal plasma at 11–13 weeks' gestation[17]. Both fetal and maternal cfDNA counts were affected by maternal characteristics, but the corrected values in 20 cases of spontaneous preterm delivery were not significantly different from those of 1805 unaffected pregnancies.

Implications for clinical practice

First-trimester screening for spontaneous preterm delivery can be achieved using a combination of factors, including maternal characteristics and obstetric history, cervical length and serum α-fetoprotein levels, at 11–13 weeks' gestation[3–5]. The benefit of such early identification of pregnancies at high risk for spontaneous preterm birth is the potential to reduce the prevalence of this complication through early surgical and pharmacological therapies[18]. 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[19]. A beneficial consequence of such measurement of the fetal fraction could have been the improved performance of early screening for spontaneous preterm delivery. However, as demonstrated by our study, the use of cfDNA testing is unlikely to be useful for the prediction of this pregnancy complication.

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