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Association of Cell-Free Fetal DNA at 11-17 Weeks of Pregnancy and the Outcome of Pregnancy

Sedigheh Borna[1], Azam Tarafdari[1], Hedyeh Khenar[1], Amirhossein Zabolian[1], Ava Ajir[1], Shima Mohammadi[1], Ali Hosseini[1*]

Abstract

Objectives: This study aimed to evaluate the association between cell-free fetal DNA (cffDNA) levels in the first and second trimesters and adverse pregnancy outcomes.

Materials and Methods: This was a retrospective cohort study including all women who had cffDNA measurement as a primary or secondary screening test for chromosomal abnormalities in a screening laboratory in Iran.

Results: Totally, 481 out of 634 pregnant women (75.9%) did not develop any pregnancy complications. On the other hand, 55 pregnancies (8.7%) led to preterm delivery. Based on the results, 33 pregnant women (5.2%) gave birth to intrauterine growth restriction (IUGR) babies and 16 pregnant women (2.52%) developed preeclampsia (PE). Moreover, 15 women developed gestational diabetes. Further, 11 pregnancies resulted in abortion and 6 pregnancies led to fetal death. There was a positive association between the duration of pregnancies and the weight of the babies at birth ($r=0.362$, $P=0.000$). There was a statistically significant association between the increase in cffDNA level and trisomy ($P=0.000$) and the decrease in cffDNA level and the incidence of PE ($P=0.019$).

Conclusions: The reduction of plasma cffDNA level in pregnancies led to PE and the elevation of cffDNA results in fetal trisomy. However, further studies will be required to confirm these findings.

Keywords: Cell-free fetal DNA, Pregnancy outcomes, Non-invasive prenatal screening.

Introduction

In 1997, fetal DNA was found in the maternal circulation by the quantification of the male SRY gene in pregnant women (1). It resulted in the development of a new non-invasive prenatal test (NIPT), which was employed to identify abnormalities in the chromosome of fetuses using blood samples of the mother. Accordingly, concentration of cell-free fetal DNA (cffDNA) in the blood of the mother is determined to identify complications related to pregnancy, even in cases with the early loss of pregnancy (2, 3), preeclampsia (PE) (4), intrauterine growth restriction (IUGR) (5), and preterm infants (6).

As a novel field of research, studies on the association between cffDNA and pathogenesis of pregnancy-related outcomes are on the rise. Using blood samples of mothers, nearly 99% of trisomies during pregnancy can be identified, with a false-positive rate of below 1% (7%-9%) (7-9). Consequently, this methodology is much better than currently available methods that are currently used (10). Recently, it has been reported that the cffDNA level increases, as a marker for in-time diagnosis of disorders during pregnancy (e.g., PE, IUGR, preterm infant, placental Previa, etc).

However, conflicting evidence shows that cffDNA level may increase within the early stages of pathological changes and may later reduce with the progression of the disease. Therefore, levels of cffDNA increase preceding the onset of the clinical symptoms of pregnancy-related complications (11,12).

This study aimed to assess the relationship between maternal cffDNA during the first and second trimesters and some common pregnancy-related complications, such as PE, trisomy, gestational diabetes, abortion, fetal death, preterm delivery, chronic diabetes, and IUGR.

Material and Methods

Participants

This retrospective cohort observational study investigated adverse pregnancy outcomes in pregnant women who attended their routine first and second-trimester screening at a laboratory, Tehran, Iran (2017-2018). It incorporated the previous cffDNA test results of 634 pregnant women. The current study was approved by the National Health Service (NHS) Ethics Committee. Additionally, written informed consent was obtained from all participants.

The inclusion criteria were being pregnant and the availability of plasma samples taken at 11–17 weeks of gestation. The plasma samples were taken the year before this investigation, and the cffDNA test results were recorded accordingly. Data concerning outcomes

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[1]Department of Obstetrics and Gynecology, Imam Khomeini Hospital Complex, Tehran University of Medical Sciences, Tehran, Iran.
***Corresponding Author:** Ali Hosseini, Tel: (+98) 919 63 03 563, Email: alihosseini642@yahoo.com

Key Messages

- Total and fetal cell-free DNA in plasma or serum is higher in preeclamptic pregnancies than in normal pregnancies.
- The cffDNA could be employed as a non-invasive screening method to predict preeclampsia and trisomy.

of the pregnancy were obtained from these laboratory records of participants. We also collected demographic characteristics of women.

The screening criteria were as follows: gestational diabetes, chronic diabetes, chronic hypertension, PE, preterm delivery, abortion, fetal trisomy, stillbirth, and IUGR. Moreover, confounding factors were considered, which include pregnancy methods, body mass index (BMI) of the mothers, smoking status, fetal gender, and previous pregnancies.

Statistical Analysis

Data were analyzed using SPSS version 26.0. The descriptive data were reported as mean, standard deviation, variance, and percentage. Quantitative comparison between the groups was performed using a *t* test. Mann-Whitney U test was used to investigate the differences concerning mean values of outcomes in each group.

Qualitative comparisons were performed using the chi-square test for categorical variables. Besides, Pearson's correlation coefficient was used to determine the relationship between cffDNA levels and pregnancy-related complications. A *P* value lower than 0.05 was considered statistically significant.

Discussion

The current research was a retrospective cohort study of cffDNA test in pregnant women who referred for pregnancy screenings (i.e., the first and second trimesters). We investigated the relationship between cffDNA level and some common pregnancy-related complications such as PE, fetal trisomy, gestational diabetes, chronic diabetes, abortion, stillbirth, preterm delivery, and IUGR.

According to the results, there was a negative association between BMI of the mothers and cffDNA levels, and a positive association between the increase in the weeks of pregnancy and the birthweight. However, there was no relationship between the duration of the pregnancies and the birthweight and between the change in cffDNA levels and the mother's chronic diseases, such as hypertension and gestational diabetes. Besides, no statistically significant associations were found between the baby's gender and the emergence of any pregnancy complications, and smoking and the changes in cffDNA levels. Additionally, the change in the cffDNA level was not related to the incidence of abortion. The number of deliveries and abortions was not related to the number of pregnancies. However, statistically significant associations were found between the increase in cffDNA concentration and incidence of trisomy and the decrease in cffDNA concentration and the incidence of PE. Additionally, there were no associations between changes in the concentration of cffDNA and the incidence of fetal death, preterm delivery, and IUGR.

Poon et al showed that the plasma levels of both maternal and fetal cffDNA during the 11 to 13 weeks of gestation were almost similar in mothers who suffered from PE, IUGR, or spontaneous preterm labor and those with normal pregnancy (13). Contrary to our results, there are studies which found that PE is associated with an increased level of cffDNA (4,14,15), which has been attributed to the release of necrotic or apoptotic syncytiotrophoblast fragments containing cffDNA into the maternal circulation (16). Several other studies reported that the level of cffDNA is often higher in PE pregnancies than in pregnancies with no complication (12,17,18). It is also established that the concentration of total and fetal cell-free DNA in plasma or serum is higher in PE pregnancies than in other pregnancies, particularly in pregnancies complicated by severe PE (19, 20). It is evident that in cases with early-onset PE, the increase in cffDNA level is associated with disease onset and can be diagnosed during the first trimester of pregnancy (5,21,22). In cases with early-onset PE, the concentration of cffDNA in the plasma of the mother starts to increase at

Table 1. Demographic Information of the Participants (n = 634)

	Total	Complication	*P* Value
Smoking	6 (0.9%)	1 (16.7%)	0.83
Chronic hypertension	8 (1.3%)	3 (37.5%)	0.33
Diabetic	6 (0.9%)	0 (0)	0.55
Gender			
Male	332 (52.4%)	32 (9.6%)	0.70
Female	302 (47.6%)	29 (9.6%)	
Methods of pregnancy			
Spontaneous	597 (94.2%)	56 (9.4%)	0.52
IVF	24 (3.8%)	4 (16.7%)	
Drug	13 (2.1%)	1 (7.7%)	

Table 2. Concentration of cffDNA (Mean ± SD) in Women with Pregnancy Outcomes (n=634)

Pregnancy Outcome	N	cffDNA
Normal	n=481	11.81 ± 3.97
Preeclampsia	n=16	9.44 ± 2.15*
Trisomy	n=11	17.36 ± 2.01**
Gestational diabetes	n=15	10.0 ± 3.20
Chronic diabetes	n=6	10.83 ± 2.78
Abortion	n=11	11.55 ± 4.39
Fetal death	n=6	13.67 ± 10.59
Preterm delivery	n=55	11.64 ± 5.21
IUGR	n=33	11.76 ± 3.70

* $P=0.03$ and ** $P=0.001$: significantly different from the group with normal delivery outcome.

11 to 13 weeks of gestation, which in turn causes decreased fetal fraction. Cases with late-onset PE have reduced fetal size at 20 to 24 weeks of gestation. Characteristics of the mother influence the concentration of cffDNA and fetal size. However the multiples of the median are same in PE and normotensive controls. As a result, it can be argued that cffDNA and fetal fraction at 11–13 and 20–24 weeks of gestation are not predictors of PE, according to a study conducted by Rolnik (23). In a review article including 3 prospective cohorts, and 10 case-control studies, 11 (out of 13) studies reported higher fetal cffDNA levels in pregnancies complicated by PE. Four studies which evaluated pregnancies complicated by severe or early-onset PE reported increased levels of fetal cffDNA before the diagnosis of PE. Nevertheless, it was mentioned that they could not adequately control confounding factors such as BMI, smoking habits, and race. Additionally, the authors reported no difference in the frequency and severity of PE (18). Consistent with our study, a correlation was found between lower cffDNA levels at 25 weeks and developing PE. Based on what mentioned before, cffDNA level lower than the 5th percentile and higher than the 90th percentile at the 25th week of gestation are at increased risk of PE. Moreover, the increased level of PAPP-A was not associated with the increased detection rate of PE. When dividing the levels of cffDNA into 20 equal percentile groups, they reported that PE was associated with both high cffDNA levels and low cffDNA levels. Therefore, low levels of cffDNA are associated with PE (24). It has been suggested that by using characteristics of mothers, arterial pressure (mean), uterine artery pulsatility index, serum levels of PAPP-A, and placental growth factor at 11 to 13 weeks of gestation, an effective program for PE screening can be implemented (25).

Although we could not find a relationship between cffDNA and PE, it is evident that in PE pregnancies the plasma levels of cffDNA are increased, which indicates the early breakdown of the placental barrier (26). A review article in 2018 showed a significant relationship between increased levels of cffDNA in the second and third trimesters of gestation and preterm delivery. Nevertheless, similar to our study, in 3 out of 10 studies including 159 preterm deliveries from a total of 5729 pregnancies, there were no significant correlations (27). In 2016, a retrospective cohort study was conducted on single pregnancies who had cffDNA testing at 10–20 weeks of gestation and increased risk for aneuploidy. Out of 1349 pregnancies that were eligible for inclusion, 119 (8.8%) were preterm before 37 weeks of pregnancy, and 49 cases (3.6%) had delivery before 34 weeks of pregnancy. In addition, at 1 to 20 weeks of gestation, the elevated fetal fraction was associated with an increased risk of preterm birth, which was statistically significant (28). In line with our study, Sekizawa et al reported that levels of fetal DNA in IUGR pregnancies were not significantly different from those in controls, although their study was limited to 9 cases of IUGR and 20 controls (29). Later, a study conducted in 2006 reported enhanced cffDNA levels in fetuses with fetal growth restriction (30).

Parity, BMI, ethnicity, family history of hypertension, and other factors affect the detection rate of cffDNA and can consistently modify the development of cffDNA. Poon et al (13) found an association between cffDNA and race, smoking habits, and BMI. In the end, the detection rate was greatly influenced by gestational age (31). Vora et al investigated 16 and 14 obese and lean women, respectively, and found an association between BMI and level of cffDNA. This study had also 10 controls. These findings indicate increased risk of stromal vascular apoptosis and adipocyte necrosis in women with obesity (32).

Smoking during pregnancy can increase the levels of cell-free DNA (33). Based on our investigation, the history of smoking during pregnancy was not associated with the level of cffDNA during the first trimester of pregnancy.

The present study had some potential limitations. For instance, we lost 95 samples due to a lack of information. Additionally, reasons for changes in cffDNA levels in mothers with diabetes mellitus and gestational diabetes are still vague and require more specific prospective studies. Therefore, it is suggested that further prospective studies be conducted to clarify cffDNA concentration changes in PE based on gestational age in high-risk PE mothers.

Conclusions

cffDNA testing could be employed as a non-invasive screening method to predict common pregnancy complications such as PE and trisomy. A beneficial consequence of such measurement is the early diagnosis of PE. Hence, women who are at risk of these complications should be screened during pregnancy and receive appropriate interventions.

It is recommended that the association between family marriages and cffDNA levels be investigated in future investigations. Further studies are needed to establish the real usefulness of cffDNA quantification in PE, either as a diagnostic tool or prognostic marker.

Authors' Contribution

SB and AT: Project development, manuscript writing; HK, AZ and AA: Data collection; SM: Data analysis; AH: Project development, data analysis, manuscript writing.

Conflict of Interests

The authors declare that they have no conflict of interest.

Ethical Issues

This study was approved by the Ethics Committee of Tehran University of Medical Sciences as a residency thesis (97-05-10949) and was therefore performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and all subsequent revisions.

Informed Consent

The participant's written informed consent form was collected before the study.

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