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Evaluation of The Relationship between Cell-Free DNA Fetal Fraction of The Circulatory System and Fetal and Maternal Pregnancy Prognosis: A Prospective Study

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Abstract

Background: Non-invasive prenatal testing (NIPT), sometimes called noninvasive prenatal screening (NIPS), is a non-invasive prenatal genetic test using cell-free DNA in maternal blood. This method is used to diagnose fetal aneuploidy disorders such as Down syndrome (trisomy 21), Edwards syndrome (trisomy 18) and Patau syndrome (trisomy 13), which causes disability disorders or significant postpartum defects. The aim of this study was to investigate the relationship between high and low fetal fraction (FF) and prognosis of maternal pregnancy.

Materials and Methods: In this prospective study, after obtaining informed consent, 10 ml of blood was collected from 450 mothers with singleton pregnancies with gestational age above 11 weeks (11-16) at the request of NIPT for cell-free DNA BCT test. After obtaining the test results, maternal and embryonic results were evaluated based on the amount of non-cellular DNA FF. Data analysis was performed by using SPSS software version 21 and independent t test, chi-square statistical tests.

Results: Based on test results, 20.5% of women were nulli par. The mean FF index in the studied women was 8.3% with a standard deviation of 4.6. The minimum and maximum values were 0 and 27, respectively. The frequency of normal, low and high FFs was 73.2, 17.3 and 9.5%, respectively.

Conclusion: High FF has fewer risks to the mother and fetus than low FF. The use of FF level (high or low) can help us determining the prognosis of pregnancy and using it to better manage the pregnancy.

Keywords: Aneuploidy, Cell-free DNA, Fetal Fraction

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Introduction

Today, prenatal screening is the best strategy to reduce the birth of babies with a variety of inherited, congenital and genetic disorders in most parts of the world (1, 2). Neonatal health is one of the main goals of the health system that will eventually lead to a healthy society. Today, many genetic abnormalities can be diagnosed before birth. Among these, due to its relatively high prevalence, trisomy 21 is more important than others. There are three views on the origin of non-cellular DNA or free fetal DNA in the mother's blood: Placental tissue, fetal cells and direct transfer of fetal DNA to maternal blood (3). With the discovery of free-cellular DNA in the maternal circulation system (embryonic origin) by other scientists (4), Lo et al. (5), used this method as a non-invasive prenatal screening and evaluation method (it has attracted a lot of attention in genetic anomalies).

Cell free fetal DNA is released from trophoblastic cells during placental apoptosis appearing in the mother's blood from the fifth-week of pregnancy and its concentration is

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acceptable for the 9 to 10 pregnancy weeks. Therefore, it can be used in the first trimester of pregnancy for screening and evaluation before birth (6).

The main source of cell free DNA is placental tissue. Pregnancy problems associated with abnormal placentation are associated with abnormal increases in cell free DNA levels in the mother's blood. Pre-eclampsia is a complication of pregnancy (7).

One of the important disease in pregnancy is gestational diabetes (8). On the other hand, the prevalence and risk factors for intrauterine growth restriction (IUGR) of LBW and IUGR differ in various regions (9, 10). Factors that can affect fetal fraction (FF) in maternal plasma include gestational age, maternal weight, diabetes, smoking, *in vitro* fertilization (IVF), and placental biology. Therefore, the purpose of this study was to examine the relationship between low and high FF and predict pregnancy outcomes. In aneuploidy screening, i.e. the end of the first trimester to the beginning of the second trimester, the average FF in the mother's blood is almost 11 to 13% (11). The aim of this study was to use FF to predict the risk of preterm delivery and fetal adverse complications caused by problems such as pre-eclampsia or gestational diabetes and placental problems such as intrauterine growth restriction. So, the effect of low or high FF values in predicting pregnancy prognosis was investigated.

Materials and Methods

In this prospective study, 450 pregnant women over 11 weeks were selected by requesting non-invasive prenatal testing (NIPT) after obtaining informed consent form. The mother's blood sample was then collected in special tubes (cell-free DNA BCT). After receiving the test results, maternal and fetal results were evaluated based on the amount of free cells FFs. After preparing the NIPT test result, the relationship between maternal and fetal prognosis and cell-free FF was investigated. Therefore, issues such as gestational hypertension, preterm delivery, IUGR, oligohydramnios, pre-eclampsia, postpartum hemorrhage and its relationship with diabetes, maternal progesterone use, maternal age, hematoma history and IVF are examined. After birth, its association with fetal weight, sex, birth age, intra uterine fetal death (IUFD), and abnormalities would be examined. Blood samples were taken from each patient for cell-free DNA testing. Blood collection protocols: i. Patients were selected based on the following login criteria, ii. Blood samples should be taken from 11-16 weeks of pregnancy, and iii. The mother must be older or equal to 18 years of age. Confirmed maternal blood samples were tested in the laboratory for NIPT and the result was announced (all mothers needed to be tested based on screening results, so the costs were paid by the patients). As a result, in all experiments, FF was determined and mentioned.

Ethical considerations

This project was carried out in compliance with all the principles of ethical regulations in the research approved by the Ministry of Health and after the approval of the Ethics Committee of Zabol University of Medical Sciences, with the code IR.ZBMU.REC.1398.169.

Data analysis

Data were described by mean ± SD and percent frequeny. Categorical factors were compared among different FFs using Chi square test. Crude and adjusted associations of FF on different outcomes of pregnancy was estimated using poisson regression models. the accuracy of FF on predicting different outcomes of pregnancy was assessed using sensitivity, specificity and likelihood ratio indices. Stata software version 14 was used for data analysis.

Results

Out of 450 pregnant women participating in the study, the information of 410 was complete and verifiable. The mean age of participants was 38.1 years with a standard deviation of 2.4. The youngest and oldest participants were 25 and 45 years old, respectively. 8.5% of women had a parity of more than 3. History of hypertension and gestational hypertension were present in 32.4 and 14.1% of women, respectively. 87.1% of women had no abortion history and in the rest, the history of various abortions was observed.

Anemia and hypothyroidism were present in 33.7 and 38.8% of women, respectively.

Premature delivery, abnormal bleeding, cesarean delivery and low birth weight were observed in 35.8, 21.2, 31.5 and 20.5% of participants, respectively.

The FF of mean index in the studied women was 8.3% with a standard deviation of 4.6. The minimum and maximum values were 3 and 27, respectively. The frequency of normal, low and high FFs was 73.2, 17.3 and 9.5%, respectively.

Table 1 shows that mothers with normal, lower and higher than normal FF, were not statistically and significantly different in terms of frequency of diabetes, hypertension, smoking, hypothyroidism, anemia, parity, IVF and age and weight mean.

Table 1: Comparison of women with different FFs in terms of demographic and clinical characteristics

Maternal Characteristics	Fetal fraction			P value
	Normal	Low	High	
Diabetes miletus	151 (50.33)	38 (53.52)	20 (51.28)	0.889
Hypertension	143 (47.67)	34 (47.89)	14 (35.9)	0.372
Cigarette	60 (20)	11 (15.49)	11 (28.21)	0.28
Hypothyroidism	113 (37.67)	35 (49.3)	11 (28.21)	0.071
Anemia	104 (34.67)	21 (29.58)	13 (33.33)	0.716
IUI	35 (11.67[a])	23 (32.39[b])	8 (20.51[ab*])	<0.0001
IVF	93 (31.1)	26 (36.62)	14 (35.9)	0.601
Weight (kg)	69.3 ± 21.7	60.5 ± 29.9	65.6 ± 26.6	0.137
Age (Y)	38 ± 2.3	38.3 ± 2.8	38 (2.2)	0.252

Data are presented as n (%) or mean ± SD. *; Each subscript letter denotes a subset of fetal fraction categories whose column proportions do not differ significantly from each other, FF; Fetal fraction, IUI; Intra uterine insemination, and IVF; *In vitro* fertilization.

Examination of pregnancy outcomes in mothers and infants showed that low FF had not a significant effect on the risk of preterm delivery (RR=1.11, P=0.643), high bleeding (RR=0.86, P=0.636), fetal abnormalities (RR=0.98, P=0.949). But the risk of low birth weight increased about three times (RR=2.99, P=0.006) which was statistically significant.

On the other hand, high FF increased the risk of the above consequences ,but this effect was not statistically significant (Tables 2-5).

Table 2: Crude and adjusted risk ratios of low and high FF in the occurrence of pregnancy and neonatal outcomes (term and preterm)

Outcomes	Fetal fraction	Term	Preterm	P value	Crude RR	P value	95% CI	Adjusted RR	P value	95% CI
PTL	Normal	198 (66)	102 (34)							
	Low	42 (59.15)	29 (40.85)	0.434	1.2	0.383	0.79-1.81	1.11		0.72- 1.7
	High	23 (58.97)	16 (41.03)		1.21	0.485	0.71- 2.04	1.19		0.7- 2.01

Data are presented as n (%). FF; Fetal fraction, PTL; Preterm labor, RR; Relative ratio, and CI; Confidence interval.

Table 3: Crude and adjusted risk ratios of low and high FF in the occurrence of pregnancy and neonatal outcomes (normal hemorrhage and PPH)

Outcomes	Fetal fraction	Normal hemorrhage	PPH	P value	Crude RR	P value	95% CI	Adjusted RR	P value	95% CI
PPH	Normal	235 (78.33)	65 21.67							
	Low	58 (81.69)	13 18.31	0.788	0.84	0.58	0.46- 1.53	0.86	0.636	0.47- 1.59
	High	30 (76.92)	9 23.08		1.06	0.859	0.53- 2.14	1.08	0.834	0.53-2.17

Data are presented as n (%). FF; Fetal fraction, PPH; Postpartum hemorrhage, RR; Relative ratio, and CI; Confidence interval.

Table 4: Crude and adjusted ratios of low and high FF in the occurrence of pregnancy and neonatal outcomes (normal baby and with anomalies)

Outcomes	Fetal fraction	Normal baby	With anomalies	P value	Crude RR	P value	95% CI	Adjusted RR	P value	95% CI
Anomalies	Normal	240 (80)	60 (20)							
	Low	56 (78.87)	15 (21.13)	0.895	1.06	0.849	0.60-1.86	0.98	0.949	0.54-1.77
	High	30 (76.92)	9 (23.08)		1.15	0.689	0.57-2.32	1.1	0.784	0.54-2.23

Data are presented as n (%). FF; Fetal fraction, RR; Relative ratio, and CI; Confidence interval.

Table 5: Crude and adjusted risk ratios of low and high FF in the occurrence of pregnancy and neonatal outcomes (normal wight and LBW)

Outcomes	Fetal fraction	Normal wight	LBW	P value	Crude RR	P value	95% CI	Adjusted RR	P value	95% CI
LBW	Normal	282 (94.31)	17(5.69[a])							
	Low	59 (83.1)	12 (16.9[b])	0.007	2.97	0.004	1.42-6.22	2.99	0.006	1.38-6.49
	High	36 (92.31)	3 (7.69[ab])		1.35	0.629	0.40-4.62	1.29	0.682	0.38-4.45

Data are presented asn (%). FF; Fetal fraction, RR; Relative ratio, CI; Confidence interval, and LBW; Low birth weight.

Examination of FF accuracy indicators in predicting pregnancy outcomes showed that low FF sensitivity varied from 14.9 to 37.5%. The specificity of low FF in predicting pregnancy outcomes also ranged from 82 to 84.3%. The most sensitivity in predicting fetal anomalies and most specificity in predicting LBW is observed. That is, predicted LBW with less false positives. The highest degree of magnification (LR) was also related to LBW prediction. This means that the test can identify low birth weight more accurate than the other maternal/neonatal outcomes (Table 6).

Table 6: High and low FF accuracy indices in predicting pregnancy outcomes

Fetal fraction	Outcome	Sensitivity	Specificity	PPV	NPV	LR
Low FF	PTL	19.7	80.3	40.8	65	1.23
	PPH	14.9	82	18.3	78.2	0.83
	Anomaly	17.9	82.8	21.1	79.6	1.04
	LBW	37.5	84.3	16.9	94.1	2.39
High FF	PTL	10.9	91.2	41	64.7	1.24
	PPH	10.3	90.7	23.1	79	1.11
	Anomaly	10.7	90.8	23.1	79.8	1.16
	LBW	9.4	90.4	7.7	92.2	0.98

FF; Fetal fraction, PTL; Preterm labor, PPH; Postpartum hemorrhage, LBW; Low birth weight, PPV; Positive predictive value, NPV; Negative predictive value, and LR; Likelihood ratio.

On the other hand, the sensitivity of high FF in predicting pregnancy outcomes varied from 9.4 to 10.9% and its specificity varied from 90.4 to 91.2%. The most important feature for the accuracy of this test was the PTL prediction. That is, there were fewer false positives in predicting preterm delivery. The highest degree of magnification was related to PTL prediction. Therefore, in general, FF (low FF and high FF) had low sensitivity and positive predictive value and also high Specificity in predicting pregnancy outcomes. It seems that low FF was more valuable in predicting LBW and high FF in predicting preterm delivery (Tables 2, 3).

Discussion

Assessing fetal health during pregnancy and preventing the birth with congenital and genetic diseases is one of the most important challenges of the medical system. NIPT using fetal free cell DNA in maternal serum is a valuable non-invasive method that allows patients to be aware of prenatal fetal health without the risk of abortion associated with invasive amniocentesis and CVS procedures. NIPT can be performed any time after 10 weeks, therefore, there is enough time to make better decisions about the fate of the affected fetus (12). NIPT is not currently recommended for all pregnant women due to its very high cost, but with high sensitivity and accuracy value, it can be a suitable alternative for more invasive methods such as amniocentesis and CVS, which have complications such as spontaneous abortion, amniotic fluid leakage, etc., for high-risk pregnant women (13).

This test was performed at approximately 11 weeks of gestation in pregnant women. In a retrospective descriptive cross sectional study, Porreco et al. (14) and Grati et al. (15) reported the mean gestational age at non-invasive tests to examine trisomy was 12 to 13 week. Although the mean gestational age among the patients in our study was about one month, it was very close to Porreco and Grati's findings. One of these reasons is the uniformity of routine pregnancy care timeline in our country.

In the study of Bektashian et al. (16), the best time to perform screening tests is the first trimester between 10-14 weeks and the average age of the fetus based on scientific sources which is 11-13 weeks and 6 days.

Ashoor et al. (17) for evaluating FF in the first trimester of pregnancy, showed that the FF decreased with increasing maternal weight, so that the median of the FF was 10%, and this fraction is changed to 11.7% in 60 kg reduced by 3.9% in 160 kg with maternal weight. This was more evident in African women than in white women.

In 2019, Gerson et al. (18) performed an NIPT test on 639 mothers, in this study, the FF of 8.4 was considered as the minimum cut off. In mothers with low FFs, an association was observed between the incidence of placental insufficiency , high blood pressure, IUGR, and oligohydramnios.

Rolnik et al. (19) showed that there is a significant association between FF result and first trimester markers for adverse pregnancy outcome. They also showed that low FF is associated with an increase in a high-risk pregnancy which is consistent with the results of our study.

In Krishna et al. (20) with a retrospective study in 148 patients with control group (witness) and normal FF compared with lower than normal FF groups concluded that decreased FF could be associated with a poor prognosis of pregnancy consistent with the results of the present study.

Sifakis et al. (21) examined the complications of pregnancy with FF and the possible association of low FF with IUGR, and fully demonstrated that elevated FF levels could be a predictor for early detection of pregnancy-related disorders, such as preeclampsia. IUGR, preterm delivery, placental disorders and hyperemesis gravid arum, however, conflicting evidence suggests that FF levels may increase in the early stages of pathological changes and later decrease as the disease progresses.

Many comorbidities and high-risk exposures have increased in sick women today, including high blood pressure, diabetes, and obesity, high-risk exposures including alcohol consumption and exposure to viral infections. These factors play an important role in proving prenatal screening and diagnostic tests (22).

Also, among the variables related to pregnancy problems in univariate analysis, history of blood pressure, preeclampsia, bleeding and lack of care during pregnancy were significantly associated with IUGR, but in multivariate analysis only blood pressure and lack of care were important and known as the risk factor for IUGR. In one study, Mansour et al. (23) explained that high blood pressure and preeclampsia as risk factors for low birth weight, which is the result of the present study.

High FF have fewer risks to the mother and fetus than low one, which are less significant than low FF.

Conclusion

The present study concludes that the sensitivity and

specificity of low FF in predicting pregnancy outcomes are different: it is most sensitive in predicting fetal anomalies and most characteristic in predicting low birth weight. Also women with IUI history having low FF has no effect on preterm delivery and amount of bleeding. The sensitivity and specificity of high FF in predicting pregnancy outcomes are also different the most characteristic is related to its accuracy in predicting preterm labor and the highest degree of magnification is related to PTL.

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Authors' Contributions

Kh.R.K., M.M., L.R.K., M.E., S.A., F.F., M.A.; Contributed to conception and design, to all experimental work, data and statistical analysis, and interpretation of data. M.M.; Were responsible for overall supervision. All authors read and approved the final manuscript.

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