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DO THE LEVELS OF cffDNA FRACTION CHANGE IN PREGNANCIES WITH PLACENTAL PROBLEMS?

SERBEST FETAL DNA FRAKSİYONU PLASENTAL PROBLEMLERE BAĞLI GEBELİK SONUÇLARINDA DEĞİŞİR Mİ?

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ABSTRACT

Objective: To investigate the value of the cell free fetal DNA (cff-DNA) for determining the important obstetric complications such as preeclampsia, intrauterine growth retardation (IUGR) and, preterm labor other than prenatal screening of fetal aneuploidies.

Methods: Our single center- retrospective study included 131 pregnant women in their 10-24th weeks of gestation, between the dates September 2019 and March 2020 who applied for cff-DNA analysis (HarmonyTM Prenatal Test; Ariosa Diagnostics Inc., San Jose, Calif., USA) with indications including advanced maternal age (≥40) and high risk for trisomy 13.18 and 21 according to the results of the first trimester prenatal screening or solely on their own desire.

Results: Oligohydraamnios was observed in 10 (8.1%) patients, gestational diabetes in 10 patients (8.1%), preeclampsia in 7 (8.6%) patients and ablatio plasenta in 2 (1.2%) patients in this study. Increasing levels of the extracellular fetal DNA fractions in 10-24th gestational weeks showed statistically significant correlation for predicting the risk for IUGR ($p<0.01$). There was not a statistically significant difference between the level of extracellular fetal DNA fractions and the other obstetric complications (preeclampsia, preterm labor, GDM, oligohydraamnios).

Conclusion: Although cff-DNA has many valuable implications as a novel biomarker for prenatal screening for special fetal aneuploidies, the association between the levels of cff-DNA and the risk of obstetric complications in clinical practice has not been clarified yet . Further studies should aim to investigate the cff-DNA levels in patients with pathological obstetric conditions in order to detect its potential predictive value and diagnostic implementation.

Keywords: cffDNA, pregnancy, placenta, pregnancy outcomes

ÖZ

Amaç: cff-DNA'nın non-invazif tanı uygulamaları dışında, bu biyobelirtecin preeklampsi, IUGR, preterm doğum gibi önemli obstetrik komplikasyonları belirlemede yerini araştırmak.

Yöntem: Çalışmamız Eylül 2019-Mart 2020 tarihleri arasında, kliniğimize başvuran 10-24. Gebelik haftaları arasında kendi isteği, ileri anne yaşı olan (≥40) ve ikili testte artmış trizomi 13, 18 ve 21 riski nedeniyle serbest fetal DNA analizi (HarmonyTM Prenatal Test; Ariosa Diagnostics Inc., San Jose, Calif., USA) yapılan 131 hastalarda tek merkezli retrospektif çalışma olarak planlandı.

Bulgular: Çalışmamızda hastaların 10'unda (%8,1) oligohidramnios, 10'unda (%8,1) gestasyonel diyabet, 7'sinde de (%8,6) preeklampsi gözlenmiştir. 2 hastada da (%1,2) dekolman plasenta izlenmiştir. 10-24. gebelik haftaları arası ölçülen hücre dışı fetal DNA fraksiyonlarının artmış düzeyleri IUGR ile sonuçlanan gebelikleri öngörmede anlamlı bulunmuştur ($p<0.01$). Diğer gebelik sonuçları (preeklampsi, GDM, preterm eylem, oligohidroamnios, dekolman plasenta) ve hücre dışı fetal DNA fraksiyon düzeyleri ile anlamlı ilişki bulunamamıştır.

Sonuç: cff-DNA'nın prenatal taramada bir dizi değerli uygulamaya sahiptir ancak gebelik komplikasyonlarını öngörmede cff-DNA'nın düzeyleri ile ilişkisi klinik uygulamada henüz yeri netleşmemiştir.Bu nedenle çalışmalar, cff-DNA'nın potansiyel öngörüsü ve tanısal uygulamalarını belirlemek için gebelikteki patolojik koşullar altındaki düzeylerinin belirlemesini amaçlamalıdır.

Anahtar Kelimeler: cffDNA, gebelik, plasenta, gebelik sonuçları

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Introduction

It is suggested that all pregnant women should be evaluated for fetal aneuplidies with prenatal screening and diagnostic tests at their first examination.[1] Prenatal screening tests are divided into traditional prenatal screening tests and non-traditional extracellular fetal DNA (cff-DNA, fetal DNA, NIPT). The use of cff-DNA in prenatal screening provided 62% less invasive procedures when compared with traditional tests.[2]

Extracellular fetal DNA (cff-DNA) screening for aneuploidies analyse extracellular DNA fragments in maternal circulation as early as from 9-10th gestational week and can be performed until term pregnancy. Cff-DNA components are released from plasental trophoblastic cells into maternal circulation during programmed cell death. Fetal components known as fetal fraction composes approximately 3-13 % of the whole extracellular fetal DNA in maternal circulation.[3,4] The quantity of extracellular DNA in fetal components increases during pregnancy. The quantity of fetal fraction is affected by many factors such as maternal age, maternal body mass index (BMI), maternal drug exposure, maternal race, aneuploidy, fetal or materal mosaicism, single or multiple pregnancy.[5-8] Cff-DNA as a common fetal aneuploidy screening test is the most specific and sensitive screening test up to date. However, it has potentially false positive and negative results. Therefore, positive results should be confirmed with invasive diagnostic tests such as karyotyping. Cff-DNA is an only laboratory screening test which defines sex determination and sex chromosome aneuploidy except patients with organ transplantation. It is not recommended in patients with organ transplantation with an opposite gender donor since it may lead to erroneous results.

The usage of cff-DNA reports more than %99 detection rate for fetal trisomy 21, more than %98 detection rate for fetal trisomy 18, more than % 99 detection rate for trisomy 13 with a combined false positive rate of %0.13.[9] Cff-DNA screening in the patients with vanishing twin is not adviced since it may yield results with high risk of aneuploidy.[10] Also, some reports also recommended to investigate rare aneuploidies such as trisomy 16, trisomy 22, microdeletion syndromes in addition to these common aneuploidies.[11] Increasing cff-DNA quantity in maternal circulation during pathologic obstetric conditions such as early pregnancy loss, intrauterin growth retardation and preterm delivery was reported in the literature.[12-15] Today, the benefit of its clinical use, limitations and advantages of the cff-DNA and, to which population the test should be applied is still a controversial issue. In our study, we aimed to investigate the role of cff-DNA fraction in predicting obstetric diseases and complications.

Methods

Our single center retrospective study included 131 pregnant women in their 10-24th weeks of gestation, between the dates September 2019 and March 2020 who applied for cff-DNA analysis (HarmonyTM Prenatal Test; Ariosa Diagnostics Inc., San Jose, Calif., USA) with indications including advanced maternal age (≥40) and high risk for trisomy 13, 18 and 21 according to the results of the first trimester prenatal screening or solely on their own desire.

Physicians who are following patients provided consultancy and blood samples were taken after obtaining informed consent. Seven patients which were detected as trisomy excluded from the study because of termination and 124 patients were followed until the end of their pregnancies. All findings and, examination results were recorded. The demographic data of the patients including maternal age, maternal weight and height, medical and disease history, and cff-DNA fraction at non-invasive prenatal test (NIPT) week, indications for NIPT were obtained. Birth weight, birth week as gestastional outcomes and preterm delivery, intrauterine growth retardation, preeclempsia, gestastional diabetes mellitus and oligohydraamnios and ablatio placenta as obstetric complications were analyzed. Preterm delivery was regard as delivery before 37th gestational week and intrauterine growth retardation was accepted below 5th percentil. Cff- DNA fraction was compared between the patiens with and without poor gestastional results.

The statistical analysis was performed using Student's t test and Pearson x^2 test IBM SPSS 20.0 (SPSS Inc., Chicago, IL, USA). Categorical variables were presented as absolute values and percentages. Differences between groups were compared by using Student's t test. Student's t test or Pearson x^2 test were performed respectively groups which showed normal and not normal distribution. All tests were accepted as two-tailed and the significance value was accepted as 5% ($p<0.05$).

Results

The indications for performing NIPT included 25 patients (20.16%) older than 40 years of age, 93 patients (75%) had high risk of first trimester prenatal screening test, 6 patients (4.83%) applied with their own desire. Table 1 shows the data of the demographic parameters.

Table 1. Demographic parameters

Maternal Age, mean±SD (min - max)	35±4,8 (22-44)
BMI (kg/m²), mean±SD (min - max)	24.6±4 (18,1-41.8)
Gestational age at NIPT, mean±SD (min - max)	14.6±3 (10-24.6)
Fraction of cffDNA, mean±SD (min – max)	10.6±3.7 (4.5-24.6)
NIPT cause, n (%)	
≥ 40 year	25 (20.16%)
High risk of prenatal screening	93 (75%)
Voluntary demand	6 (4.8%)

Pregnancy outcomes data are shown in table 2. When we consider the gestational outcomes, the mean birth week was 37.5±2.1 (25-40), birth weight was 2.128±647 (1.200-2.940). Preterm labor was observed in 21 patients (16.9%) and intrauterine growth retardation was detected in 4 patients (3.2%). Also, 10 patients (8.1%) had oligohydroamnios, 10 patients (8.1%) had gestational diabetes mellitus, 7 patients (8.6%) had preeclampsia and 2 patients had ablatio placenta (1.2%).

Table 2: Pregnancy outcomes

Gestational week at delivery, mean±SD (min – max)	37.5±2.1 (25-40)
Birth weight, mean±SD (min – max)	2.128±647 (1.200-2.940)
Preterm delivery, n (%)	21 (16.9%)
IUGR, n (%)	4 (3.2%)
Preeclampsi, n (%)	7 (8.6%)
Gestational Diabetes, n (%)	10 (8.1%)
Oligohidramnios, n (%)	10 (8.1%)
Placental abruption, n (%)	2 (1.2%)

Table 3 shows the relation between cff-DNA fractions and gestational outcomes. Increasing levels of cff-DNA fraction in between 10th and 24th gestastional week were determined significant for predicting IUGR ($p<0.01$). No significant correlation was observed between other gestational outcomes (preeclampsia, GDM, preterm delivery, oligohydraamnios, ablatio plasenta) and cff-DNA fraction levels.

Table 3: Comparison of pregnancy complications and cff DNA fraction values

	Positive	**Negative**	**P**
Preeclampsia	9.6±3.3	10.6±3.7	0.49
IUGR	7.1±1.5	10.7±3.7	0.01*
Total GHD	9.1±3	10.7±3.7	0.14
GDM	9.5±6.6	10.6±3.5	0.33
Oligohidramnios	9.7±3.5	10.6±3.7	0.43
Preterm delivery	10.4±2.3	10.6±3.9	0.82

GDM: gestational diabetes, GHD gestational hypertensive diseases. *The significance level was regarded as $p<0.05$.

Discussion

cff-DNA is a promising new, rapidly applicable biomarker that can be used to determine prenatal diagnosis and pregnancy complications. Although it is thought that increasing levels of cff-DNA can be a marker for predicting obstetric complications such as preeclampsia, preterm delivery, IUGR, conflicting evidence has showed that cff-DNA may increase in the early stage of disease and decrease in the advanced stage. Extracellular fetal DNA and their association with gestational outcomes is an interesting subject that attracts many researchers' attention.

Pregnancy with placental pathologies such as preeclampsia, IUGR had an increasing cff-DNA quantity in maternal circulation.[13,16-18] One study in 2003 by Sekizawa et al. was focused on extracellular cff-DNA in maternal circulation, in pregnancies complicated with IUGR. Although they examined 9 IUGR and 20 control cases, extracellular fetal DNA was found to be similar in pregnacies complicated with IUGR and, the control group.[19] Al Nakib et al. detected that cff-DNA levels in pregnancies complicated with fetal growth retardation was higher than normal in pregnancies with partial placental insufficency.[20] In our study, we found that the cff-DNA levels were significantly high in pregnancies complicated by IUGR.

Levine et al. suggested that apoptosis of trophoblasts, requiring for placental differentation occurs secondary to hypoxia in the first trimester and cff-DNA remains stable in preeclempsia in early pregnancy.[21] Thurik showed that there was no relation between cff-DNA and preeclempsia in the first trimester similar to the Poon's study.[22,23] However, Sifakis et al. and Illanes et al. showed that cff-DNA was increased in only early onset preeclampsia in first trimester.[24,25] Correlation between preeclempsia and cff-DNA was not statistically significant in our study.

Leung et al. and Farina et all. determined that pregnancies resulting in spontaneus preterm delivery had higher cff-DNA levels.[26,27] However, these studies evaluated measurements taken in 2nd and 3rd trimester of pregnancies. Illanes et al. performed a study comparing cervical length measurements with cff-DNA levels in order to evaluate predictive value of cff-DNA in 22-24*th* gestational week. There was no association between labor week and cff-DNA levels and this combination showed no predictive value.[25] Quezeda et al. reported that there was no correlation between cff-DNA levels in 10-19*th* gestastational week and spontaneus preterm delivery before 34*th* week and, 34-37*th* week.[28] Likewise, our study also showed no relation between preterm delivery and cff-DNA levels.

Bauer et al. reported that there was an association between increasing cff-DNA levels and GDM development in study group which consisted of average 15 week of pregnant women.[29] However, according to gestational week, only one study by Thurik et al. which calculated cff-DNA levels as MoM value detected that first trimester cff-DNA levels in pregnant women with GDM was lower than control group.[22] cff-DNA levels did not show any statistically significant correlation with GDM, oligohydroamnios, or ablatio placenta in our study.

As a conclusion, Studies, performed by now have shown that cff-DNA might be a valuable tool to evaluate serious gestational complications earlier and as a non-invasive prenatal screening test. While Cff-DNA is used as prenatal screening test, it is not performed to predict pregnacy-related diseases clinically. Therefore, studies should focus on investigating precise physiologic pathways which defines the amount of the cff-DNA release in order to determine the potential predictive and diagnostic implementations of cff-DNA.

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Compliance with Ethical Standards

This study was approved by Kocaeli university non-interventional clinical research ethics committee (GOKAEK 2022/03.04, Protocol no:2022/05).

Conflict of Interest

The authors declare no conflicts of interest.

Author Contribution

Authors contributed equally to this work.

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