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ORIGINAL ARTICLE

An analysis of factors affecting fetal fraction in cell-free fetal DNA test for aneuploidy screening

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ABSTRACT

Objective: To analyze factors affecting fetal fraction in cell-free DNA (cfDNA) test for aneuploidy screening.

Material and Methods: This is a retrospective cohort study conducted between 2018 and 2023. All pregnant women who had a cfDNA test for aneuploidy screening were included in the study. Maternal data, gestational age (GA) at the time of blood draw for the test, methodology of the chosen test, and results of cfDNA test (fetal fraction and risk status) were collected. Pregnancy outcome, GA at the time of delivery, presence of hypertensive disorders of pregnancy (HDP), and neonatal outcomes were obtained and analyzed.

Results: Data from a total of 447 women were analyzed. The median GA at the time of the cfDNA test was 12 (11–13) weeks. The median fetal fraction among women who have a healthy weight, who are overweight, and who are obese was 10.4% (7.7–13.1), 9% (7.1–12), and 7% (3.4–7.9), respectively (p=0.002). There were 12 (2.7%) cfDNA test results with low fetal fraction. All low fetal fraction results belonged to women who were obese (p<0.001). For each one-unit increase in BMI, there was a 0.4% drop in fetal fraction (95% CI 0.27–0.53). The decrease in fetal fraction remained significant when adjusted for maternal age and GA (-0.41, 95% CI 0.28–0.55). Maternal age, GA at the time of the test, and heparin and aspirin use were not associated with fetal fraction.

Conclusion: Maternal BMI has a negative effect on fetal fraction in cfDNA testing. Pre-test counseling should include factors influencing the fetal fraction—and therefore the accuracy—of cfDNA testing.

Keywords: Aneuploidy screening, cfDNA, fetal fraction, NIPT.

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INTRODUCTION

Recent advancements in the detection of cell-free DNA (cfDNA) in maternal serum have substantially changed our practice in prenatal screening options for fetal aneuploidies. The source of cfDNA in maternal circulation is thought to be the syncytiotrophoblasts in the intervillous space after apoptosis, hence the placenta.[1] The majority of these DNA fragments are of maternal origin. However, the sensitivity of the test is influenced by the quantity of fetal DNA in maternal circulation, commonly referred to as the fetal fraction.

Non-invasive prenatal testing (NIPT) results include the fetal fraction (as a percentage), and the generally accepted minimum threshold is 4%.[2]

Evidence shows that approximately 25% of "no-call" results due to low fetal fraction are associated with aneuploidies.[3] Nevertheless, it is important to acknowledge that there are additional factors, aside from aneuploidy, that might contribute to a low fetal fraction. These factors include maternal obesity, heparin use, and an earlier gestational age (GA).[4–6] The mode of conception is also a determining factor of fetal fraction, with pregnancies conceived by *in vitro* fertilization having lower fetal fractions.[7]

The objective of this study is to analyze and document the factors that influence the fetal fraction in an unselected cohort of pregnant women. This analysis aims to enhance our ability to provide guidance and improve our comprehension of cfDNA test outcomes.

MATERIAL AND METHODS

This study is designed as a retrospective cohort study conducted at Acıbadem Altunizade Hospital, Obstetrics and Gynecology Department. Pregnant women presented for antenatal care at outpatient clinics between 2018 and 2023 constituted the study population. Women who opted for cell-free fetal DNA test for aneuploidy screening and who have singleton pregnancies were included in the study. A keyword search of the electronic database identified cases using the keywords "cell-free fetal DNA" and "NIPT." Maternal and fetal characteristics, cell-free fetal DNA test results, and pregnancy and neonatal outcome data were extracted from electronic medical records. Research involving human subjects complied with all relevant national regulations and institutional policies and is in accordance with the tenets of the Helsinki Declaration (as revised in 2013), and has been approved by the authors' Institutional Review Board (Acıbadem Mehmet Ali Aydınlar University) (Decision Number: 2024-6/243).

Maternal data (age, parity, presence of any chronic illness, mode of conception, body mass index, heparin/aspirin use), GA at the time of blood draw for NIPT, methodology of the chosen NIPT brand (whole genome sequencing or targeted methodology), and results of NIPT (fetal fraction and risk status) were collected. Low fetal fraction was defined as fetal fraction below 4%. Gestational age was determined based on the last menstrual period (LMP) and dating by first-trimester crown-rump length (CRL) if there was a discrepancy or the date of LMP was unknown. Outcome of pregnancy (intrauterine fetal demise (IUFD), live birth), GA at the time of delivery, presence of hypertensive disorders of pregnancy (HDP), and neonatal outcomes (GA at delivery, birthweight) were obtained and analyzed. Preterm birth was defined as delivery before the 37th gestational week. Low birthweight was defined as birthweight below 2500 grams. Composite adverse pregnancy outcome was defined as the occurrence of either preterm birth, hypertensive disorders of pregnancy, intrauterine fetal demise, or low birthweight of the neonate.

Measures of association for categorical variables were analyzed with Chi-square and Fisher's Exact test. Linear regression analysis was used for determining effect size of the relationship between continuous outcomes. One-way analysis of variance (ANOVA) was used to assess differences in fetal fraction between groups based on age, BMI, and GA. All analyses were performed using STATA software, version 18.0 Basic Edition (Copyright 1985-2021 StataCorp LLC). A p-value of <0.05 was considered statistically significant.

RESULTS

The database search yielded 459 women who had a cfDNA test. After exclusion of 12 cases with multiple pregnancies, a total of 447 women were included in the study. The median GA at the time of blood draw for the cfDNA test was 12 (11–13) weeks. Demographic, clinical, and pregnancy characteristics of the cohort are outlined in Table 1.

The median fetal fraction among women who have a healthy weight, who are overweight, and who are obese was 10.4% (7.7–13.1), 9% (7.1–12), and 7% (3.4–7.9), respectively (p=0.002) (Fig. 1a). The median fetal fraction based on age categories was as follows: 8.9% (7.1–11) among women younger than 30, 9.8% (7.3–12.6) among those aged 30 to 35, 9.3% (7–12) among those aged 35 to 40, and 8.4% (7–11.1) among those 40 and older (p=0.28) (Fig. 1b). The median fetal fraction based on GA was as follows: 9.1% (7.1–11.8) between 10–12+6 weeks, 9.6% (7.5–12) between 13–16+6 weeks, 12.2% (7.2–14) between 17–21+6 weeks, and 12.7% (8.6–12.9) in 22 weeks and above (p=0.42) (Fig. 1c).

There were 12 (2.7%) cfDNA test results with low fetal fraction. All low fetal fraction results belonged to women who were obese (p<0.001). Three of the low fetal fraction results belonged to mothers 40 and older, three were aged between 35 to 40, and six were aged between 30 to 35 (p=0.14). Linear regression analysis showed that for each one-unit increase in BMI, there was a 0.4% drop in fetal fraction (95% CI 0.27–0.53). The decrease in fetal fraction remained significant when adjusted for maternal age and GA (-0.41, 95% CI 0.28–0.55).

There was only one high-risk result for trisomy 21, which was confirmed by amniocentesis, and the pregnancy was terminated at the 20th gestational week.

There was an adverse pregnancy outcome in 55 (12.6%) of the cohort. Seventeen (3.9%) of liveborn neonates were considered as low birthweight. Low fetal fraction was not associated with composite adverse pregnancy outcome (p=0.19).

Eleven women were on anticoagulants (low molecular weight heparin) due to high risk of thromboembolic events, and heparin use was not associated with low fetal fraction (p=0.56). Eleven women were on aspirin for preeclampsia prophylaxis, and aspirin use was also not associated with low fetal fraction (p=0.31).

Table 1: Maternal characteristics and pregnancy outcomes of the study cohort

Maternal age (years)	34±4
Body mass index (kg/cm^2)	22.2 (20.4–24.3)
Gravidity	1 (1–2)
Parity	0 (0–1)
Mode of conception	
Spontaneous	422 (94.4)
IVF	25 (5.6)
Gestational age (weeks)	
10+0 to 12+6	315 (70.5)
13+0 to 16+6	104 (23.3)
17+0 to 21+6	18 (4)
22 and above	10 (2.2)
Methodology of the chosen test	
Whole genome sequencing	163 (36.5)
Targeted	284 (63.5)
Fetal fraction	9.4 (7.1–12)
Outcome of pregnancy	
Live birth	437 (97.7)
Spontaneous abortion	7 (1.6)
Intrauterine fetal demise	2 (0.5)
Termination of pregnancy	1 (0.2)
Adverse pregnancy outcome	
Preterm birth	41 (9.2)
Hypertensive disorders of pregnancy	11 (2.6)
GA at the time of delivery (weeks)	38 (38–39)
Birthweight (gram)	3275 (3050–3555)

IVF: *In vitro* fertilization; GA: Gestational age. Data presented as mean± standard deviation, median (interquantile range) and n (percentage).

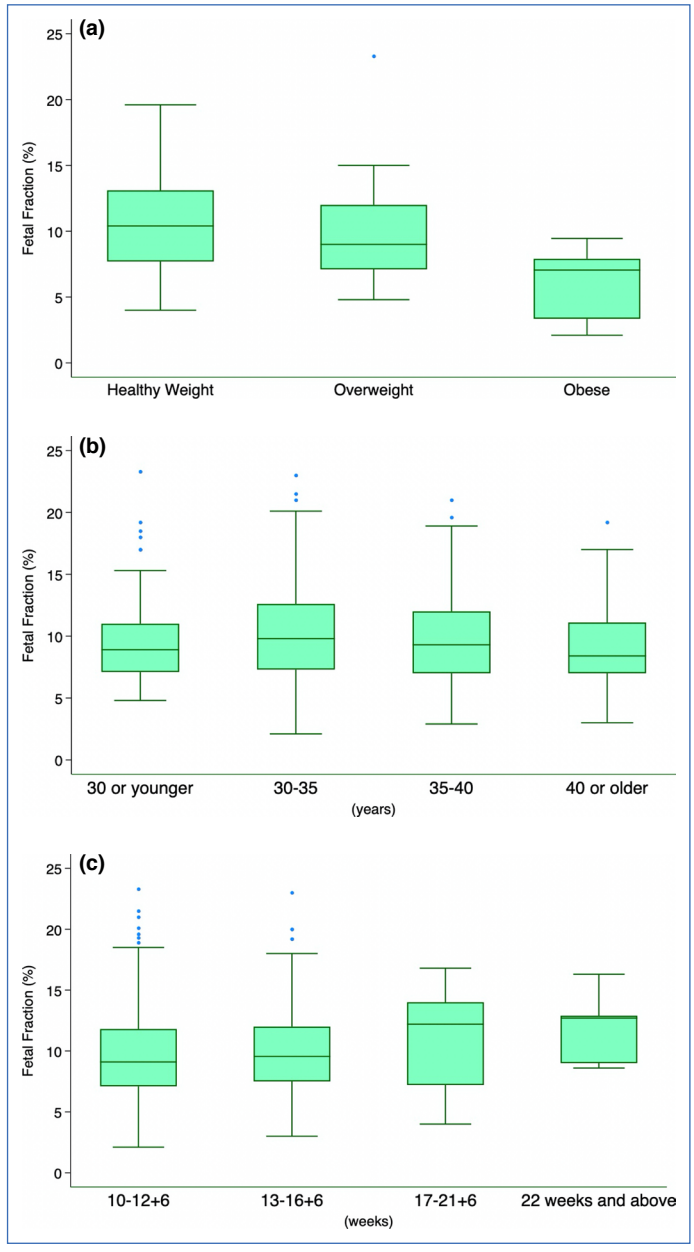

Figure 1: (a) Box-plot of fetal fraction based on maternal body mass index. **(b)** Box-plot of fetal fraction based on maternal age. **(c)** Box-plot of fetal fraction based on gestational age.

The upper, middle and lower bars in the boxes represent the 25th, 50th, and 75th percentiles, respectively.

DISCUSSION

Our results have shown that as BMI increases, fetal fraction in cfDNA testing—as a percentage—decreases. Fetal fraction tends to increase with gestational age, but this finding has not reached statistical significance.

The observed impact of maternal weight on cfDNA testing in this study aligns with findings from prior studies.[4,5] Ashoor et al.[4] reported that for each unit of increase in maternal weight (in kilograms), there was a 0.2% decrease in fetal fraction. Zhou et al.[8] reported a moderate negative correlation (correlation coefficient -0.4) between maternal BMI and fetal fraction in a large cohort of approximately 23,000. Another analysis of results from a large cohort of 140,000 women showed that every 5 kg/cm² increase in maternal BMI resulted in a 1.2% decrease in fetal fraction.[9] We have demonstrated a 0.4% decrease in fetal fraction for each 1 kg/cm² increase. However, the relationship between maternal BMI and fetal fraction should be interpreted cautiously, as BMI generally increases as gestational age increases. Nevertheless, our regression coefficient (-0.4) remained the same after adjusting for confounders, maternal age, and gestational age.

Although there was an increasing trend in fetal fraction as gestational age increased in our cohort, this finding was not significant. Multiple studies have reported a positive association of gestational age with fetal fraction, with an approximately 0.1% rise in fetal fraction for each week of gestation until 21 weeks, when the rate climbed to almost 1% per week.[5,8] However, a recent study did not report any relationship

between gestational age and non-reportable cfDNA test results due to low fetal fraction.[10] Both the aforementioned study and our study have lower median gestational ages at the time of blood draw for the cfDNA test (Nitsche et al.[10] 16 weeks, our study 12 weeks), which might be the reason for the lack of significant association between increasing gestational age and fetal fraction in these cohorts.

Two previous studies stated that there was a positive association between maternal heparin use and non-reportable cfDNA test results.[6,11] One study asserted that the decreased fetal fraction observed in cases when heparin is used is actually attributable to the underlying autoimmune disorders.[12] The majority of their cohort consisted of women taking heparin because of an autoimmune disease. Yet, this result is far from being generalizable—low molecular weight heparin has been prescribed by obstetricians around the globe for various reasons. Our cohort had eleven women on low molecular weight heparin, a small number to draw conclusions. Not unexpectedly, we could not establish a relationship between heparin use and low fetal fraction in the cfDNA test. We also do not have records of the indication for heparin use, which we believe should be investigated in larger studies to understand its influence on fetal fraction.

There is limited research available about the impact of low-dose aspirin use on cfDNA test results. Nitsche et al.[10] found that low-dose aspirin use was associated with a nearly 3-fold increase in non-reportable cfDNA test results. This interesting finding warrants further validation in future research, as low-dose aspirin is frequently used during pregnancy for the prevention of preeclampsia. We had eleven women on low-dose aspirin and could not find a significant relationship between aspirin use and low fetal fraction, probably due to the small sample size.

The patients in our study population are generally at low risk, as the women who seek routine antenatal care at our center usually have private medical insurance in addition to the national social security system. This allows them to make a more independent decision to have cfDNA testing for aneuploidy screening, without being burdened by financial concerns—as opposed to subjects of studies conducted in government-funded hospitals, where cfDNA may be used as "reflex testing" for high-risk serum screening results or ultrasound findings. This might account for the low rate of adverse pregnancy outcomes and high-risk test results.

CONCLUSION

In conclusion, we have shown that maternal BMI at the time of cfDNA testing has a negative effect on fetal fraction, potentially compromising the accuracy of the test. Providers should incorporate considerations of the factors that influence fetal fraction—and consequently, the outcome of the cfDNA test—into their pre-test counseling.

Statement

Ethics Committee Approval: The Acıbadem Mehmet Ali Aydınlar University Medical Research Ethics Committee granted approval for this study (date: 18.04.2024, number: 2024-6/243).

Author Contributions: Concept – IA, SY; Design – IA; Supervision – IA; Resource – SY; Materials – SY; Data Collection and/or Processing – SY; Analysis and/or Interpretation – IA; Literature Search – IA; Writing – IA, SY; Critical Reviews – IA, SY.

Conflict of Interest: The authors have no conflict of interest to declare.

Informed Consent: Written informed consent was obtained from patients who participated in this study.

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