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Retrospective results of our non-invasive prenatal test (NIPT) experience

Non-invaziv prenatal test (NIPT) deneyimimize ait retrospektif sonuçlar

Ufuk Atlıhan[1] Tevfik Berk Bildacı[2] Selçuk Erkılınç[2]
Onur Yavuz[3] Hüseyin Aytuğ Avşar[4] Can Ata[5]

[1] Private Karataş Hospital, Department of Gynecology and Obstetrics, Izmir, Türkiye

[2] Institute of Health Sciences, Department of Gynecology and Obstetrics, İzmir Democracy University, Izmir, Türkiye

[3] Institute of Health Sciences, Department of Gynecology and Obstetrics, Dokuz Eylul University, Izmir, Türkiye

[4] Torbalı State Hospital, Department of Gynecology and Obstetrics, Izmir, Türkiye

[5] Buca Seyfi Demirsoy Training and Research Hospital, Department of Gynecology and Obstetrics, Izmir, Türkiye

ABSTRACT

Aim: Non-invasive prenatal test (NIPT) has become widespread over the years with higher probabilities of detection and fewer false positives with regard to traditionally used screening techniques. We aimed to document the experience of introducing this kind of equipment into clinical practice, evaluate its impact on the detection of fetal-aneuploidies, analyze the demographic characteristics of females undergoing 1.trimester fetal-aneuploidy screening testing with those choosing the NIPT, and assess elements influencing cfDNAfetal fraction.

Materials and Methods: Our research was designed as anobservational, retrospective research of 406 pregnant females who underwent fetal-aneuploidy screening in the course of pregnancy, from January 2019 to April 2023. Some patients had the 1.trimester fetal-aneuploidy screening test between 11-13.weeks, while another group of patients chose to undergo the NIPT at their own request. Any abnormalities in trisomy 13,18,21 were reported in the NIPT results. Maternal age, parity, history of miscarriage, presence of hypertension, fetal anomaly detected on ultrasound were questioned.

Results: The average age of females who chose the 1.trimester fetal-aneuploidy screening test was 31.17±4.00, and that of those who chose NIPT was 32.84±5.09, and it was seen to be significantly higher in the NIPTgroup ($p<0.01$). The history of miscarriage in patients undergoing NIPT was significantlyhigher with regard to the other group ($p=0.027$). The presence of pregestational diabetes mellitus and hypertension in patients who underwent NIPT was found to be significantly higher than the other group ($p=0.016$, $p=0.024$, respectively). Age and body mass index (BMI) have a statistically significant negative connection versus cfDNA fetal fraction ($p<0.01$, $r=-0.506$) ($p<0.01$, $r=-0.509$).

Conclusion: Our study showed that the area of prenatal aneuploidy screening was greatly impacted by the introduction of NIPT, which replaced the 1.trimester screening test and decreased the number of intrusive testing. Our findings may be used as a reference for prenatal treatment and can offer clinics useful information when integrating NIPT into the prenatal screening flow.

Keywords: Non-invasive prenatal testing, fetal aneuploidi, fetal screening testing, fetal trisomy.

ÖZ

Amaç: *Non-invaziv prenatal test (NIPT), geleneksel olarak kullanılan tarama yöntemlerinden daha üstün saptama oranları ve düşük yanlış pozitiflik oranlarıyla yıllar içinde yaygınlaşmıştır.*

Corresponding author: Ufuk Atlıhan
Private Karataş Hospital, Department of Gynecology and Obstetrics, Izmir, Türkiye
E-mail: *cfl_ufuk@hotmail.com*
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Çalışmamızda, bu teknolojinin fetal anöploidilerin saptanmasına yönelik yaklaşımlarımıza etkisini raporlamak, 1. Trimester fetal anöploidi taraması yapılanların özelliklerini, NIPT testini seçenlerle karşılaştırmak ve serbest DNA fetal fraksiyonunu etkileyen faktörleri değerlendirmek amaçlanmıştır.

Gereç ve Yöntem: *Çalışmamız, Ocak 2019-Nisan 2023 arasında prenatal dönemde fetal anöploidi taraması yapılan 406 gebeye ilişkin gözlemsel, retrospektif bir çalışma olarak tasarlandı. Hastaların bir bölümü 11-13.hafta arasında 1. trimester fetal anöploidi tarama testi yaptırmış olup, bir grup hasta ise kendi isteğiyle NIPT yaptırmıştır. NIPT sonuçlarında trizomi 13,18 ve 21 kromozomlarındaki olası bir anomali rapor edildi. Anne yaşı, gebelik sayısı, abortus öyküsü, hipertansiyon varlığı, ultrasonda saptanmış fetal anomali varlığı gibi demografik veriler çalışmada sorgulandı.*

Bulgular: *1. trimester fetal anöploidi taraması testini seçen kadınların ortalama yaşı 31,17±4,00, NIPT'i seçenlerin ise 32,84±5,09 olarak saptanmış olup, NIPT grubunda anlamlı yüksek saptanmıştır (p<0.01). NIPT yapılan hastalarda abortus öyküsü, diğer gruba göre anlamlı şeklide yüksek olduğu tespit edilmiştir (p=0.027). NIPT yapılan hastalarda pregestasyonal diabetes mellitus ve hipertansiyon varlığı, diğer gruba göre anlamlı yüksek saptanmıştır (p=0.016, p=0.024 sırasıyla). Yaş ve vücut kitle indeksi ile hücre dışı serbest DNA fetal fraksiyonunu arasında sırasıyla negatif yönlü bir ilişki saptanmıştır (p<0.01, r=-0,506) (p<0.01, r=-0,509).*

Sonuç: *NIPT uygulamasının, 1. trimester fetal anöploidi taraması testlerinin yerini alarak ve invaziv testleri azaltarak doğum öncesi anöploidi taraması alanını önemli ölçüde etkilediğini göstermiştir. Çalışmamız, NIPT'nin prenatal tarama akışına entegrasyonu sürecinde kliniklere pratik bilgiler sunabilir ve doğum öncesi bakımda referans bilgiler verebilir.*

Anahtar Sözcükler: *Non-invaziv prenatal tanı testi, fetal anöploidi, fetal tarama testi, fetal trizomi.*

INTRODUCTION

Prenatal diagnosis enables the molecular and biochemical detection of hereditary diseases, allowing for their identification through advanced methods (1,2). Simultaneously, it provides the opportunity for prenatal treatment, if possible, and the implementation of necessary postnatal measures. It also facilitates the option of terminating the pregnancy within the legal timeframe when deemed necessary (1, 2). The increasing prevalence of delayed marriages and childbirth in societies worldwide, parallel to cultural development, has led to a rise in pregnancies at advanced maternal ages, indicating a growing tendency (3, 4). The significant increase in pregnancies at advanced ages has raised concerns regarding the heightened risk of fetal aneuploidy such as trisomy 13,18 and 21 (5, 6). The methods used for screening fetal chromosomal abnormalities can be divided into two categories. Invasive methods encompass direct intervention techniques performed on the fetus and its appendages. These techniques include fetal biopsies, amniocentesis, chorionic villus sampling, and cordocentesis (7, 8). Non-invasive tests, on the other hand, include fetal ultrasonography (Nuchal Translucency measurement during the 11-13th week screening) and biochemical tests analyzed from maternal blood (first trimester screening-test (FTST), second-trimester screening test (STST) (9, 10). These tests are still widely used as standard procedures (9,10). However, since the commercial release of the non-invasive prenatal test (NIPT) on the basis of cell-free-DNA (cfDNA) sequencing and its rapid global proliferation, private clinics have started providing this high-performing device to expectant mothers. The NIPT test has shown a detection rate of over 99% for fetal aneuploidies with an approximately 0.1% false positive rate and a 0.2% false negative rate (11-14). In comparison, the FTST has a 5% false positive probability and a detection probability of 95% (15, 16). However, recent discussions on the effectiveness of the test have arisen, suggesting that the false negative rates of the FTST and NIPT are comparable. This is because approximately 4% of patients undergoing NIPT have a low fetal fraction, which increases the false negative result risk (17). In our study, we retrospectively evaluated our experiences with patients who underwent NIPT at our hospital and aimed to report its impact on the detection of fetal aneuploidies in accordance with our current approaches. We also aimed to make an evaluation regarding clinically significant factors that influence the cfDNA-fetal fraction in NIPT.

MATERIALS and METHODS

The present study was designed as a retrospective observational study. The Helsinki-Declaration's Principles were followed in the composition of this research. Informed consent documents were received from all patients. The ethics committee approval numbered 2023/200 was obtained from the ethics committee. This is a retrospective observational study covering a total of 406 pregnant females who underwent fetal aneuploidy screening during January 2019 to April 2023 in a tertiary hospital. All patients in our study received information about the availability and limitations of FTST and NIPT, as well as their utilization in the medical field, during the visit before the 11th week of pregnancy. Some opted for FTST, while others voluntarily requested NIPT. Pregnant females were advised that if increased nuchal translucency (more than the 99th percentile) was observed in the 11th and 13th-week ultrasound, invasive tests could be considered instead of NIPT. Risk assessments for trisomy 21, 18 and 13 were included in the NIPT result reports. In accordance with legal procedures in our country, reporting fetal gender is permissible only in cases where abnormalities are detected. Since no abnormalities were identified in the gender chromosome, results regarding fetal gender were not disclosed. In case of positive results, as previously explained, for karyotyping, amniocentesis or chorionic-villus sampling was recommended. Retrospective queries were used to get data regarding maternal age, first trimester body mass index (BMI) value, parity, history of preterm birth, history of miscarriage, presence of pregestational diabetes mellitus, presence of pregestational hypertension, detection of fetal anomalies on ultrasound, and additional pertinent data from patient records and the hospital database.

Statistical analysis

The analyses were conducted with the SPSSx26.0 (IBM-Inc. Chicago, IL, USA). Normality analysis was conducted using the Kolmogorov-Smirnov-test. The quantitative data of the patients were reported as mean ± Standard-Deviation (SD). The Chi-Square test was employed to assess the categorical data and results were presented as counts and percentages (%). Pearson correlation test was used to determine correlations between variables. There was a 95% Confidence Interval (CI) used to analyze the results. The p-value, which was less than 0.05, was accepted as statistically significant.

RESULTS

During the examined four-year period, 406 females in total had either FTST or NIPT. Among these 406 females, while 269 females (66.3%) have chosen FTST as the primary serum screening technique, 137 females (33.7%) opted for NIPT. Among the females subjected to FTST, negative results were obtained in 92.9% (250/269), with 19 females identified as having a high risk of fetal aneuploidies.

Females at high risk established with FTST, 47.3% chose NIPT as the second screening method, while 36.8% underwent amniocentesis. 15.7% of females with high risk either refused further testing or discontinued follow-up. Of the patients who underwent amniocentesis, it was noted that 85.7% had a normal karyotype, while trisomy 21 was detected in 14.3% of cases. For the 9 females who had NIPT as the 2nd screening technique, all NIPT tests came back negative. Among the 137 females who chose NIPT as the main screening technique, in 130 patients (94.9%) negative results were obtained, positive results in 5 patients (3.7%), and in 2 patients (1.4%), results were deferred due to insufficient cfDNA fetal fractions despite repeated testing. Of the 5 patients with positive NIPT results, 4 (80%) were determined to have high-risk for trisomy 21, and 1 patient was determined to have high-risk due to sex chromosome abnormalities. Amniocentesis was recommended for the 4 patients with a high-risk of trisomy 21 based on NIPT results. Three patients accepted amniocentesis, and trisomy 21 was confirmed in all cases. The one patient who declined amniocentesis was found to have trisomy 21 in the newborn following giving birth. In a case where NIPT yielded a positive result for sex chromosome abnormalities, amniocentesis revealed a normal karyotype. For pregnancies in which NIPT was performed during the prenatal period and negative results were obtained, chromosomal analysis conducted due to suspected physical findings in two infants after birth revealed trisomy 21 in both cases, resulting in a false-negative rate of 1.4%. In the 137 females who underwent NIPT, the observed average cfDNA fetal fraction was 10.60 ± 3.85 (range 2% – 20%). During the four-year period, out of 137 NIPT tests conducted, results were determined as deferred in 8 cases because of cfDNA fetal fraction being under 5%. After repeat sampling in these 8 cases, negative results were obtained in 6 patients, while in 2 cases, results were deferred again because of insufficient cfDNA fetal fraction even following further samples. No abnormalities were detected in

pregnancies with insufficient cfDNA fetal fraction (Figure-1).

The mean age of females choosing first trimester screening test was 31.17±4.00, while those choosing NIPT was 32.84±5.09, and age was significantly higher in the NIPT group (p<0.01). The primigravida rate of females who chose the 1st trimester-screening-test was 62.5%, and the primigravida rate of those who opted NIPT was 51.9%, and the rate was seen to be significantly lower in the NIPT group (p=0.032). The miscarriage rate of females who chose the 1st trimester-screening test was 23.8%, and the miscarriage rate of those who chose NIPT was 35%, and the rate was seen to be significantly higher in the NIPT group (p=0.027). The presence of pregestational diabetes mellitus and pregestational hypertension in patients who underwent NIPT was seen to be significantly higher than the other group (p=0.016, p=0.024, respectively) (Table-1).

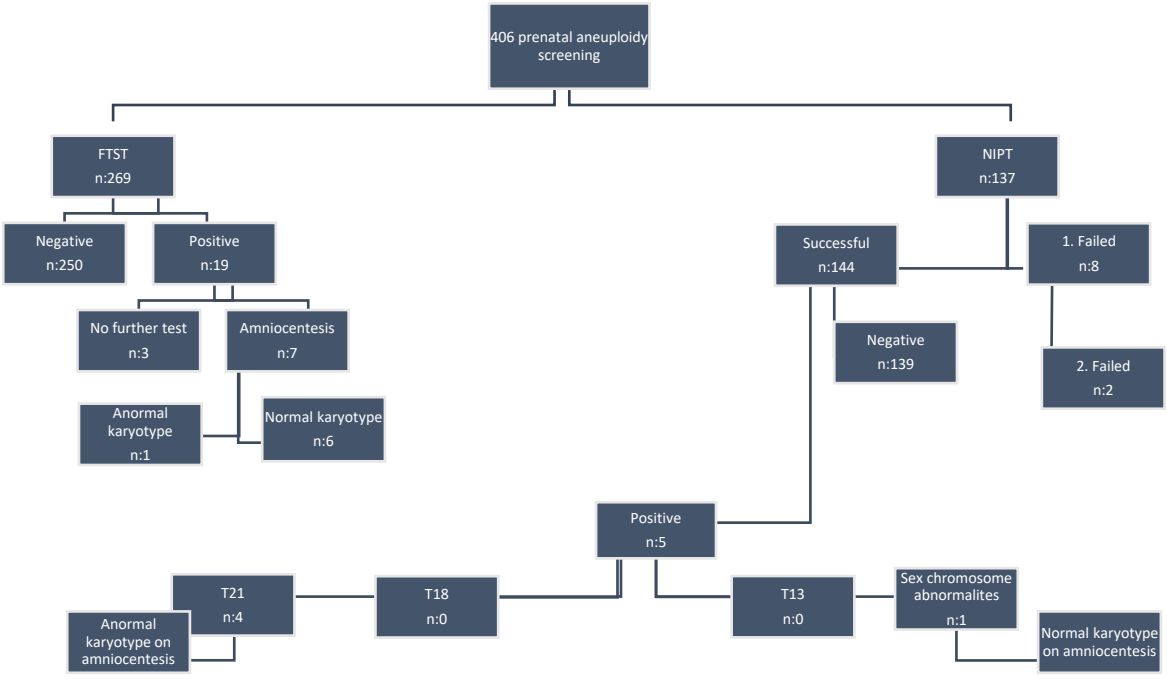

Figure-1. Outcomes from the diagnostic and Maternal serum screening tests

* NIPT: Non-invasive-prenatal test, FTST: First trimester screening test

Table-1. Descriptive statistics of demographics and pregnancy related variables between two groups.

		FTST n-% 269 (66.3%)	**NIPT n-% 137 (33.7%)**	***p*-value**
Maternal Age (year)	<30	66 (24.5%)	23 (16,8%)	<0.01*
	30-35	157 (58.4%)	66 (48,2%)	
	36-40	41 (15.2%)	31 (22.6%)	
	>40	5 (1.9%)	17 (12.4%)	
	Mean±SD	31.17±4.00	32.84±5.09	<0.01**
Primigravid		168 (62.5%)	71 (51.9%)	0.032*
Multi-fetal Pregnancy		15 (5.6%)	4 (2.9%)	0.322*
History of preterm birth		25 (25.8%)	14 (13.2%)	0.859*
History of miscarriage		64 (23.8%)	48 (35%)	0.027*
Pregestational Diabetes mellitus		3 (1.1%)	10 (7.3%)	0.016*
Pregestational Hypertension		2 (0.7%)	5 (3.6%)	0.024*
Fetal structural abnormality		4 (1.5%)	5 (3.6%)	0.172*

** :Two-sample t-test, * :Chi-squared test

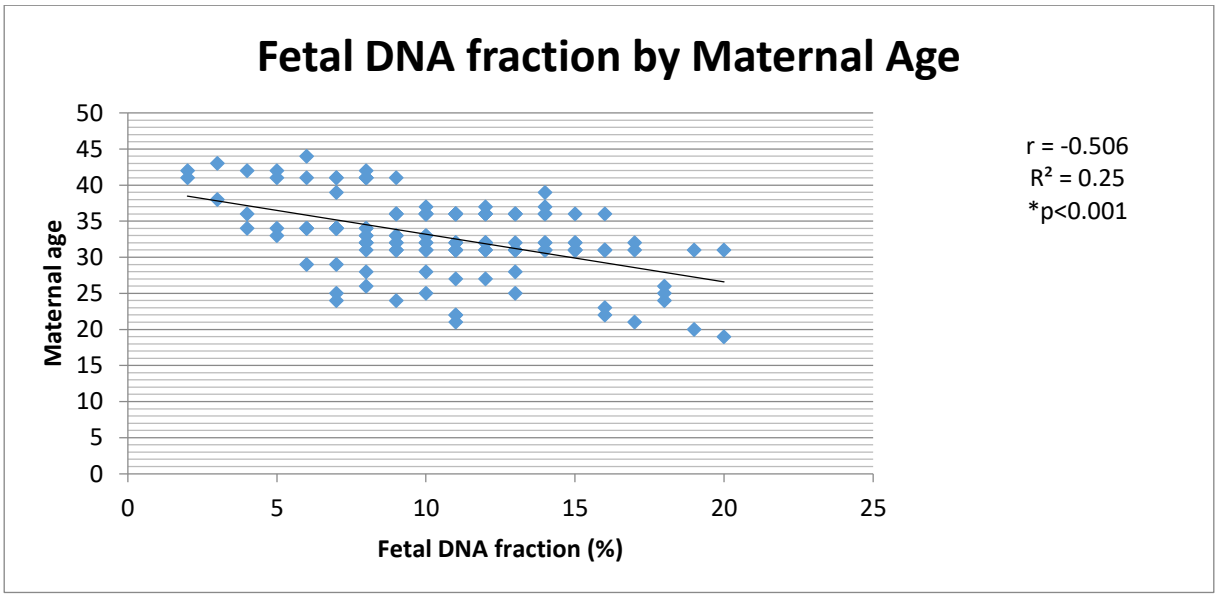

Figure-2. Fetal DNA fraction by maternal age.

*: Pearson correlation test

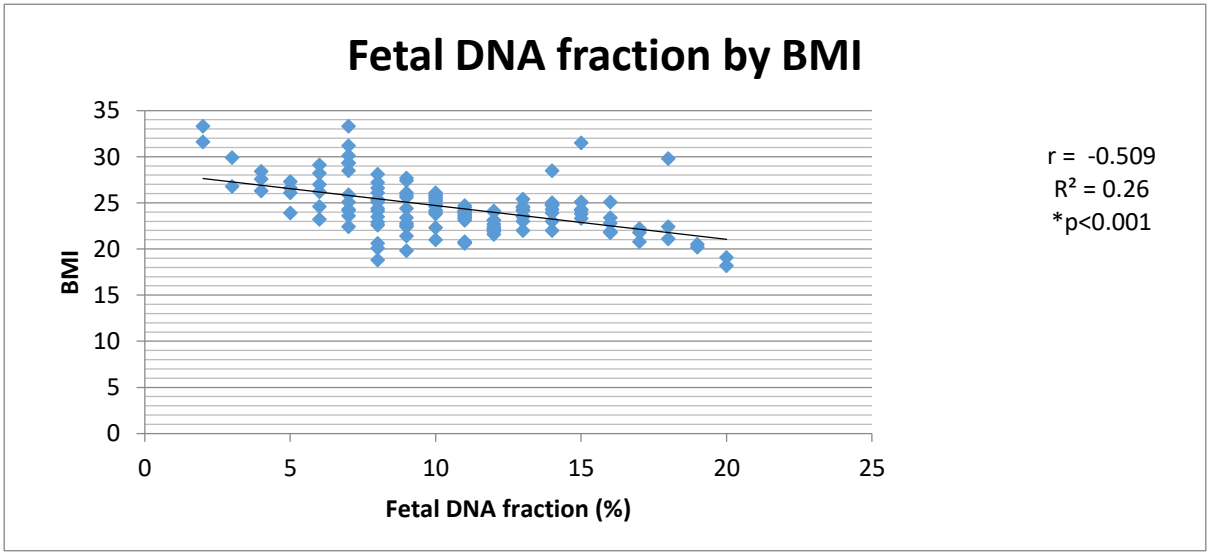

Figure-3. Fetal DNA fraction by BMI.

*: Pearson correlation test

A negative and statistically significant correlation was seen across age and cfDNA fetal-fraction ($p<0.01$, $r=-0.506$). According to regression analysis, the R2 value is 0.25. Therefore, 25% of the variance in fetal DNA fraction percentage is explained by age ($p<0.01$) (Figure 2).

The cfDNA fetal-fraction and BMI have a statistically significant negative connection ($p<0.01$, $r=-0.509$). According to regression analysis, the R2 value is 0.26. Therefore, 26% of the variance in fetal DNA fraction percentage is explained by BMI ($p<0.01$) (Figure 3).

DISCUSSION

Comparative analyses revealed that the Patients in the NIPT group were older and more had multiple fetal pregnancies. Additionally, the initial screening negative rate for fetal aneuploidy was 92.9% in the FTST group and 94.9% in the NIPT group, indicating similar rates. In the FTST group, the screening positive rate was 7.1%, while in the NIPT group, it was 3.7%, demonstrating a higher positivity rate in the group undergoing FTST. In NIPT, insufficient cfDNA fetal-fraction rates were found to be 5.6% in the first screening and 1.4%

in the second screening. It was observed that females who had previously had abortions preferred to select NIPT as the primary serum screening method instead of the combined test. Perhaps the most significant advantage of NIPT, compared to traditional serum screening methods, is its capacity to cut down on the quantity of invasive diagnostic procedures. The study's comparative analysis of demographic characteristics between the NIPT group and the combined test group revealed differences in terms of maternal age, history of pregnancy and abortion, presence of diabetes mellitus, and presence of hypertension. However, parameters related to fetal structural anomalies and multi-fetal pregnancies were similar between the groups. It is noteworthy that patients with a history of previous miscarriage opt for NIPT over the combined test. Further research is needed to investigate the precise reasons for choosing NIPT as the primary serum screening method. However, the great efficacy of NIPT, which is distinguished by a decreased false-positive rate in comparison to conventional screening tests, is thought to confer upon them the opportunity to alleviate concerns associated with false positives. The potential of negative results from NIPT because of inadequate cfDNA fetal fraction is one of the primary fears. In our study, the failure rate due to insufficient cfDNA fetal fraction was found to be high at 5.6%, compared to previously published series. This rate was higher than what is reported in the literature. In routine practice, both the ACOG and the ACMG suggest evaluating invasive diagnostic tests for individuals with low cfDNA fetal fraction in NIPT tests (18,19). It is known that in approximately half of the patients with test failures, the issue can be fixed by obtaining a 2nd sample afterwards. In this current study, interpretable results were obtained in 75% of females with insufficient cfDNA fetal fraction in the initial sampling after repeat sampling. There is limited knowledge about clinical and biological elements affecting this parameter, aside from gestational age and maternal weight (20). In this present research, a negative relation is detected between cfDNA fetal-fraction and weight and Maternal age. Cases with low cfDNA fetal fraction have been mostly omitted from several prior research looking at NIPT, and unable to obtain results. However, studies have reported an association between low cfDNA fetal fraction and increased aneuploidy risk (21,22). Therefore, When NIPT performance is analyzed, excluding a low cfDNA fetal percentage may lead to an overestimation of the fetal aneuploidy detection rate. To resolve the issue of the relation between low cfDNA fetal-fraction and fetal aneuploidy risk, further advanced studies with larger sample sizes are needed. In our study, 33.7% of all patients chose NIPT as the primary screening method. This rate is higher compared to the NIPT application rates reported in other studies in the literature (23). The high preference for NIPT over the combined test observed in this study may be attributed to the characteristics of tertiary care clinics where there is a higher prevalence of high-risk pregnancies. Additionally, this may be influenced by the patients' comparatively high socioeconomic class at our facility, which is situated in one of the most urbanized areas. The undeniable great performance and efficacy of NIPT notwithstanding, the procedure of integrating this test into real-world clinical settings requires further investigation and should be determined with cautious evaluation. At the moment, a number of recommendations, such as the ACOG's December 2012 Guidelines, state that low-risk ladies should not be provided NIPT (24). It is important to carefully choose the target group for this new screening technique while considering strong evidence from recently created guidelines. Generalizing the results of this study to the overall population is challenging. Firstly, the obtained results were derived from a tertiary medical center where the prevalence of high-risk-pregnancies is high. Secondly, a retrospective research design was employed, limiting our capability to identify the specific elements that influenced individual decisions regarding a particular test. As various elements, such as clinical conditions, economic status, and previous awareness of NIPT can influence the choices made by patients, and previous awareness of NIPT may affect the choices made by patients, future research should evaluate these aspects.

CONCLUSION

The present study outcomes indicate that the implementation of NIPT significantly impacts the field of prenatal aneuploidy screening by potentially swapping out combined tests and reducing invasive tests. Our research may provide practical insights to clinics and hospitals in the procedure of integrating NIPT into prenatal screening workflows and contribute valuable reference information to prenatal care.

Conflict of interest: The author(s) declare that there is no conflict of interest.

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