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Evaluation of Amniocentesis Results in Patients With High-Risk Cell-Free Fetal DNA

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Abstract

Objectives: The present research was conducted to evaluate the accuracy of cell-free fetal DNA (cffDNA) in diagnosing chromosomal abnormalities and measure consistency in diagnostic results between this assay and amniocentesis.

Materials and Methods: This retrospective observational study recruited pregnant women presenting for Down's syndrome screening. These women underwent the nuchal translucency ultrasound and their serum levels of pregnancy-associated plasma protein A and free beta-human chorionic gonadotropin were measured based on the aim of the study. Amniocentesis was administered in subjects with high or uncertain risk for fetal chromosomal abnormalities and cffDNA testing or the quadruple screen test in those with moderate risk. The women diagnosed as high-risk cases in cffDNA testing also underwent amniocentesis to confirm their diagnosis.

Results: Amniocentesis showed trisomy 21 as the most prevalent chromosomal abnormality in 66 (67.3%) cases, followed by trisomy 18 in 12 (12.2%) and trisomy 13 in 7 (7.1%). False-positive cffDNA results were obtained in 8 (8.2%) cases. The coefficient of agreement between these two tests was, however, obtained as 0.845 (P<0.0001), and their results were significantly correlated with each other (χ2=369, P<0.0001). Moreover, cffDNA was found to diagnose prenatal chromosomal abnormalities with a sensitivity of 100%, a specificity of 50%, a positive predictive value of 91.8%, and a negative predictive value of 100%.

Conclusion: Given the high sensitivity of cffDNA observed in diagnosing chromosomal abnormalities, this assay can play a key role as a non-invasive procedure in prenatal diagnoses.

Keywords: Amniocentesis, Prenatal Diagnosis, cffDNA

Introduction

Nowadays, all pregnant women are required to undergo the nuchal translucency ultrasound and maternal serum triple or the quad test for the prenatal diagnosis of chromosomal abnormalities. However, taking these measures does not ensure the detection of 100% of fetal malformations (1-3). Therefore, positive screening test results should be confirmed with further examinations based on chorionic villus sampling (CVS) or amniocentesis (4). Prenatal screening and diagnostic tests are divided into two main groups, namely, non-invasive screening techniques including ultrasound and magnetic resonance imaging, and minimally-invasive tests including cffDNA and preimplantation genetic diagnosis (5). Invasive prenatal screening methods such as CVS, amniocentesis, and cordocentesis have also a long history of application as the gold standard for diagnosing prenatal genetic disorders. Conventional genetic amniocentesis is commonly recommended for gestational ages of 15-20 weeks. Many multicenter studies have demonstrated its diagnostic accuracy in chromosomal abnormalities. Despite its high accuracy, this test is associated with numerous severe complications including procedure-related fetal loss. According to the American College of Obstetricians and Gynecologists, the mortality associated with this method was 1300-1500 in 2007 (6).

With a placental origin, cffDNA detected in maternal peripheral blood from the fourth week of gestation is rapidly cleared from the maternal circulation after childbirth (7). The cffDNA circulating in maternal plasma originates from the trophoblast-derived embryonic DNA (3%-6%) and cell-free maternal DNA (8). Moreover, cffDNA is 200-300 bp in size and thus significantly smaller than cell-free maternal DNA, which lays the foundations for many cffDNA detection techniques. In recent years, this screening test has developed prenatal care and provided new perspectives for traditional fetal medicine. After examining both high-risk and low-risk populations, numerous clinical trials have validated cffDNA as a screening test for common autosomal aneuploidies such as trisomy 21 or Down's syndrome, trisomy 13, and trisomy 18 (9).

Given the above-mentioned explanations, this study aimed at evaluating the accuracy of cffDNA in diagnosing genetic disorders and measuring consistency in diagnostic results between cffDNA and amniocentesis.

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Key Messages

- cffDNA screening test has developed prenatal care and provided new perspectives for traditional fetal medicine. After examining both high-risk and low-risk populations, numerous clinical trials have validated cffDNA as a screening test for common autosomal aneuploidies such as trisomy 21.
- Research findings have recommended that cffDNA is adequate for prenatal diagnosis given its noninvasive nature and the detection of chromosomal abnormalities with high diagnostic sensitivity. Also, this procedure should be performed along with confirmatory techniques such as amniocentesis.

Materials and Methods

Study Design

The present retrospective observational study recruited pregnant women presenting to Imam Khomeini Hospital for prenatal diagnosis in 2016-2019. Moreover, cffDNA was employed to identify the subjects at high risk for fetal chromosomal abnormalities. The subjects were excluded from the study if they were unwilling to participate in the procedure or undergo amniocentesis.

Research Protocol

The pregnant women presenting for Down's syndrome screening underwent the nuchal translucency ultrasound and their serum levels of pregnancy-associated plasma protein A and free beta-human chorionic gonadotropin were measured as well. The results were interpreted and the risks were calculated in a software package developed by the Fetal Medicine Foundation.

After the abdominal preparation of pregnant women, amniocentesis was performed under direct ultrasound guidance by inserting amniocentesis needles (i.e., spinal needles No. 1-3) into the uterine space through the abdominal layer and sampling the amniotic fluid. The first 0.5 mL of the aspirated fluid was discarded owing to its possible contamination with native cells. Fetal karyotyping was then performed before removing the needle by collecting 1-2 mL of the amniotic fluid.

Amniocentesis was administered in the subjects with a high or uncertain risk for fetal chromosomal abnormalities and cffDNA testing or the quadruple screen test in those with moderate risk. The women diagnosed as high-risk in cffDNA testing also underwent amniocentesis to confirm their diagnosis. After the abdominal preparation, a sample of the amniotic fluid was collected by inserting an amniocentesis needle into the uterine cavity. The sample volume in mL equaled gestational age in weeks.

Statistical Analyses

All data were expressed using descriptive statistics. The agreement level was evaluated by calculating the Kappa coefficient. The proportions were compared using the Chi-square. Furthermore, sensitivity, specificity, and negative and positive predictive values were calculated, and all statistical analyses were conducted in SPSS. The level of statistical significance was set at $P < 0.05$.

Results

The present descriptive-analytical study evaluated 98 pregnant women aged 19-46 years with a mean age of 35.46 years. Their gestational age and mean gestational age were 10-23 and 14.35 weeks, respectively. The fetal fraction of the samples and its mean value were obtained as 5-25 and 11.48, respectively. The results (Table 1) of amniocentesis showed trisomy 21 as the most prevalent chromosomal abnormality in 66 (67.3%) cases, followed by trisomy 18 in 12 (12.2%) and trisomy 13 in 7 (7.1%).

Table 2 presents the level of agreement between the results of cffDNA and amniocentesis.

False cffDNA results were obtained in 8 (8.2%) cases. However, the coefficient of agreement between these two tests was obtained as 0.845 ($P < 0.0001$) and their results were significantly correlated with each other ($\chi^2 = 369$, $P < 0.0001$).

Based on univariate regression, maternal age, gestational age, and the fetal fraction did not affect the diagnostic accuracy of cffDNA (Table 3).

Moreover, cffDNA was found to diagnose prenatal chromosomal abnormalities with a sensitivity of 100%, a specificity of 50%, a positive predictive value of 91.8%, and a negative predictive value of 100% (Table 4).

Discussion

The prenatal diagnosis of fetal chromosomal abnormalities requires invasive methods, including amniocentesis and CVS, which carry several maternal and fetal risks such as abortion and maternal Rh sensitization (10). In this regard, identifying circulating fetal nucleated cells in the maternal bloodstream was found to be a non-invasive prenatal diagnostic technique. Despite its advantages, clearing these cells from the maternal circulation is complicated due to their extremely low number, namely, 1-2 per million cells (11). Therefore, the researchers have

Table 1. Descriptive Analysis of the Participants

Characteristic	Mean ± Standard Deviation (Range)
Age	35.46±5.41 (19-46)
Gestational age	14.35±2.44 (10-23)
Fetal fraction	11.48 (5-25)
Amniocentesis	**No. (%)**
45XO	2 (2%)
47XXX	3 (3.1%)
T18	12 (12.2%)
T21	66 (67.3%)
NL	8 (8.2%)
T13	7 (7.1%)

Table 2. Level of Agreement Between Amniocentesis and cffDNA

Amniocentesis	cffDNA					Kappa	Chi-square
	45XO	47XXX	T13	T18	T21		
45XO	2 (100%)	0	0		0		
47XXX	0	3 (100%)	0	0	0		
T13	0	0	7 (100%)	0	0	Kappa= 0.845, $P < 0.0001$	χ^2=369, *df*=20, $P < 0.0001$
T18	0	0	0	14 (100%)	0		
T21	0	0	0	0	64 (100%)		
NL	0	0	0	0			

Note. cffDNA: Cell-free fetal DNA.

Table 3. Univariate Regression Model of the cffDNA Accuracy

Variable	Univariate	
	OR (95% CI)	*P*
Age	0.97 (0.85-1.10)	0.644
Gestational age	1.09 (0.83-1.42)	0.53
Fetal fraction	0.95 (0.79-1.16)	0.66

Note. OR: Odds ratio; CI: Confidence interval; cffDNA: Cell-free fetal DNA.

Table 4. The Accuracy of cffDNA

Test Accuracy	Level	95% CI
Sensitivity	1	0.95-1.0
Specificity	0.5000	0.24-0.75
Positive predictive value	0.9184	0.84-0.96
Negative predictive value	1.000	0.63-1.0

Note. CI: Confidence interval; cffDNA: Cell-free fetal DNA.

focused on finding non-cellular fetal genomic markers. In 1977, cffDNA was detected in the maternal serum and identified as a more potent genomic marker for prenatal diagnosis (12). Further research found trophoblast apoptosis to release embryonic cell-free DNA into the maternal plasma. This finding turned cffDNA into a viable fetal genetic source with potential applications as a non-invasive prenatal diagnostic test with the least errors and false-positive results. The present study evaluated the agreement in results between cffDNA and amniocentesis and calculated the diagnostic accuracy of cffDNA for fetal chromosomal abnormalities. The results demonstrated a statistically significant coefficient of agreement between the results of amniocentesis and cffDNA in diagnosing chromosomal abnormalities. cffDNA detected all cases with chromosomal abnormalities except for eight false-positives. This relatively-high false-positive rate reduced the specificity of the method to 50%. Univariate regression represented that confounding variables such as maternal age, gestational age, and fetal DNA fraction did not affect the diagnostic accuracy of cffDNA. Using massively parallel sequencing for evaluating the diagnostic accuracy of cffDNA in twin pregnancies in China, Du et al successfully diagnosed fetuses with trisomy 21 and trisomy 15 although they reported one false-positive case for trisomy 13 (13) despite using a different methodology from that of the present research. A unicenter study by Dugo et al revealed six false-positive cffDNA cases (14). A meta-analysis of 37 different articles by Gill et al yielded a total false-positive rate of 0.09% for cffDNA and different false-positive rates for different chromosomal abnormalities. They also found cffDNA to more accurately diagnose trisomy 21 than trisomy 18, trisomy 13, and sex chromosome aneuploidies (15). Similarly, the present study reported trisomy 18 (26%) and trisomy 13 (12.5%) as the most prevalent false-positive results. The biological causes of this error include confined placental mosaicism, vanishing twins, maternal mosaic, copy number variations, and maternal subclinical cancer (16,17). Despite the contribution of cffDNA to the prenatal diagnosis of chromosomal abnormalities, the relatively-high false-positive results of this assay limit the generalizability of its results.

Conclusions

According to the present findings, cffDNA is recommended for prenatal diagnosis given its non-invasive nature and the detection of chromosomal abnormalities with a high diagnostic sensitivity. On the other hand, given its false positives, this procedure should be performed along with confirmatory techniques such as amniocentesis. The present study limitation comprised its small sample associated with its unicenter type. Therefore, it is recommended that further studies be conducted with larger samples in this regard.

Conflict of Interests

Authors declare that they have no conflict of interests.

Ethical Issues

The Ethics Committee of Ahvaz Jundishapur University of Medical Sciences approved this study. All participants were also briefed on the study objectives and procedures.

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References

1. Wieacker P, Steinhard J. The prenatal diagnosis of genetic diseases. Dtsch Arztebl Int. 2010;107(48):857-862. doi:10.3238/arztebl.2010.0857
2. Rizos D. Prenatal Screening for chromosomal abnormalities: where do we stand today in Mediterranean countries? EJIFCC. 2018;29(4):274-279.

3. Hixson L, Goel S, Schuber P, et al. An overview on prenatal screening for chromosomal ab-errations. J Lab Autom. 2015;20(5):562-573. doi:10.1177/2211068214564595
4. Carlson LM, Vora NL. Prenatal diagnosis: screening and diagnostic tools. Obstet Gynecol Clin North Am. 2017;44(2):245-256. doi:10.1016/j.ogc.2017.02.004
5. Fox CE, Kilby MD. Prenatal diagnosis in the modern era. Obstet Gynaecol. 2016;18(3):213-9. doi:10.1111/tog.12278
6. ACOG Practice Bulletin No. 88, December 2007. Invasive prenatal testing for aneuploidy. Obstet Gynecol. 2007;110(6):1459-1467. doi:10.1097/01.aog.0000291570.63450.44
7. Motavaf M, Sadeghizadeh M. Noninvasive Prenatal Test by Cell-Free Fetal DNA in Mater-nal Plasma: Current Progress and Prospective Clinical Applications. J Compr Ped. 2014;5(3):e23254. doi:10.17795/compreped-23254
8. Petersen AK, Cheung SW, Smith JL, et al. Positive predictive value estimates for cell-free noninvasive prenatal screening from data of a large referral genetic diagnostic laboratory. Am J Obstet Gynecol. 2017;217(6):691.e691-691.e696. doi:10.1016/j.ajog.2017.10.005
9. Gil MM, Nicolaides KH. Implementation of maternal blood cell-free DNA testing in early screening for aneuploidies. Maternal-Fetal Medicine. 2019;1(1):2-6. doi:10.1097/fm9.0000000000000009
10. Cunniff C. Prenatal screening and diagnosis for pediatricians. Pediatrics. 2004;114(3):889-894. doi:10.1542/peds.2004-1368
11. Singh R, Hatt L, Ravn K, et al. Fetal cells in maternal blood for prenatal diagnosis: a love story rekindled. Biomark Med. 2017;11(9):705-710. doi:10.2217/bmm-2017-0055
12. Smith JF, Blumenfeld Y. Cell-free fetal DNA in maternal plasma: progress and potential. NeoReviews. 2008;9(8):e332-7. doi:10.1542/neo.9-8-e332
13. Du E, Feng C, Cao Y, Yao Y, Lu J, Zhang Y. Massively parallel sequencing (MPS) of cell-free fetal DNA (cffDNA) for trisomies 21, 18, and 13 in twin pregnancies. Twin Res Hum Genet. 2017;20(3):242-249. doi:10.1017/thg.2017.23
14. Dugo N, Padula F, Mobili L, et al. Six consecutive false positive cases from cell-free fetal DNA testing in a single referring centre. J Prenat Med. 2014;8(1-2):31-35.
15. Gil MM, Quezada MS, Revello R, Akolekar R, Nicolaides KH. Analysis of cell-free DNA in maternal blood in screening for fetal aneuploidies: updated meta-analysis. Ultrasound Ob-stet Gynecol. 2015;45(3):249-266. doi:10.1002/uog.14791
16. Zhang H, Gao Y, Jiang F, et al. Non-invasive prenatal testing for trisomies 21, 18 and 13: clinical experience from 146,958 pregnancies. Ultrasound Obstet Gynecol. 2015;45(5):530-538. doi:10.1002/uog.14792
17. Romero R, Mahoney MJ. Noninvasive prenatal testing and detection of maternal cancer. JAMA. 2015;314(2):131-3. doi:10.1001/jama.2015.7523