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Screening for fetal chromosomal anomalies using cell free DNA at a tertiary care centre

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Abstract

Aim of the study: To Evaluate the usefulness and reliability of cell free DNA as a screening method to identify the mothers at risk of fetus with trisomy 21, 18, 13, sex chromosomal anomalies and other chromosomal abnormalities.

Method: This is a prospective observational study conducted at Chalmeda Anand Rao Institute of Medical Sciences, Karimnagar from a period of October 2020 to October 2022. Patients were included with at least one atypical biochemical marker of first trimester screening test or with abnormal NT scan. The cell free DNA was analysed by VeriSeq NIPT Solution v2 method. The accuracy of cell free DNA assay was evaluated by calculation of sensitivity and specificity. Screen positive cases were validated by comparing with karyotyping analysis.

Results: Among the 250 patients with atypical biochemical markers who underwent a cell free DNA analysis, fourteen (5.6%) were excluded because of loss to follow-up, mis-carriage and affordability constraints. Out of 236, Cell free DNA was positive for Trisomy 21 in 3 pregnant women (n = 3), Trisomy 18 in 1 pregnant woman (n = 1) and Sex Chromosomal Aneuploidy in 1 pregnant woman (n = 1) and negative in 227 pregnant women. Test failure occurred for two pregnant women (0.8%).

Conclusion: Cell free DNA assay is an effective and reliable tool in screening for fetal chromosomal anomalies.

Keywords: Trisomy, Aneuploidy, Karyotyping, Cell free DNA (cfDNA)

Introduction

Cell free fetal DNA (cfDNA) test has widely proved its performance in prenatal screening for trisomy 21, 18 and 13 in both high risk and general population [1]. In recent years, its use has been increasing constantly, due to its excellent sensitivity and specificity, added to its non-invasive character.

Lo (1997) first described the presence of Y chromosome in the plasma of women with male fetus, using the analysis of cell-free DNA (cfDNA) in maternal circulation [2].

cfDNA originates from maternal and placental cell lysis [3]. Starting from week 5 of amenorrhea, the placental cytotrophoblast anchors to the uterine decidua parietalis, decidual spiral arteries supply blood into lacunae between decidua and placenta, and the cytotrophoblast invades, covers and remodels the walls of the uterine spiral arteries. Cytokine-mediated replacement of trophoblast cells, covering spiral-shaped arteries walls, releases DNA [4]. Degraded fetal DNA fragments contain approximately 180 base pairs (bps) and are suspended in the arterial plasma [5].

cfDNA can be early isolated starting from week 10, when its amount is sufficient for a potential clinical use. Its percentage can vary between <4%, which is not useful for diagnosis, and 40%, with an average of 10%, at week 12, when about 90% of plasma circulating cell-free DNA fragments originate from apoptosis in the maternal epithelium, creating a mix of cfDNA and maternal cfDNA. The amount of cfDNA is called "fetal fraction" (FF). A few hours after birth cfDNA can no longer be found in the maternal circulation and it is probably eliminated by renal excretion [5].

The main objective of this study was to evaluate the usefulness and reliability of cfDNA assay in screening for trisomy 21, 18, 13 and sex chromosomal anomalies.

Methods

Study design

According to ACOG recent guidelines [6], prenatal genetic screening (serum screening with or without nuchal translucency [NT] ultrasound or cell-free DNA screening) and diagnostic testing (chorionic villus sampling [CVS] or amniocentesis) options should be discussed and offered to all pregnant patients regardless of maternal age or risk of chromosomal abnormality. After review and discussion, every patient has the right to pursue or decline prenatal genetic screening and diagnostic testing.

This is a prospective observational study conducted at Chalmeda Anand Rao Institute of Medical Sciences, Karimnagar.

The study was approved by institutional ethics committee, and all the participants provided written consent. The inclusion criteria are the women with an age of 18 or more with singleton pregnancy having at least one atypical biochemical marker of first trimester screening test, or with an abnormal nuchal translucency scan of >3mm.The first trimester serum assay include ranges with (free beta HCG <0.5MoM multiple of median, HCG>2.0MoM) or (PAPP-A<0.25MOM, PAPP-A>2.5MoM). The cut off chosen for these values was decided in accordance with the most frequent and clinically relevant values found in literature.

Exclusion criteria includes women if they had a history of conception using ovum donation, had a vanishing twin and higher order gestation.

Sample collection and Analysis

A sample of 7-10 ml maternal peripheral whole blood is collected in a Streck cell-free DNA Blood Collection Tube (BCT), which prevents cell lysis and genomic contamination and stabilizes whole blood, plasma is isolated from maternal peripheral whole blood using standard centrifugation techniques. VeriSeq NIPT Solution v2 is an integrated platform that uses paired-end whole-genome sequencing to detect fetal anomalies [7]. There are 2 screening options: basic and genome wide. Basic screening provides aneuploidy status information for chromosomes 21, 18, 13, X, and Y only. Genome-wide screening provides information on partial deletions/duplications. Both options allow Sex chromosomal anomalies reporting if requested, with or with- out fetal sex reporting for singleton samples. In this study, all samples were run using the genome-wide mode; VeriSeq NIPT Solution v2 incorporates automated sample preparation and sequencing data analysis [7].

CfDNA was extracted from 1 mL of plasma by adsorption onto a binding plate to remove contaminants before eluting. For library preparation, the purified cfDNA fragments underwent an end repair process to convert 5' and 3'overhangs to blunt ends. Next, a deoxyadenosine nucleotide was added to the 3' ends to create a single base overhang.

DNA Analysis

Identification of library fragments by index sequence and alignment of the paired end reads to a human reference genome. Estimation of the fetal fraction of the library by combining information from the distribution of both the lengths and genomic coordinates of the library fragments. After accounting for known biases, a statistical model detects regions of the genome which are under or overrepresented in the library in a manner consistent with an anomaly at the estimated level of fetal fraction. The NIPT report provides summary results for the selected test menu where anomaly detected or no anomaly detected is listed along with a fetal fraction estimate for samples passing QC. The Supplementary Report provides quantitative metrics which characterize each detected anomaly.

Report delivery and clinical follow-up/patient management

NIPT results were delivered and explained within 1-2 working days of receiving the blood sample in the laboratory. All patients were recommended to have confirmatory invasive prenatal testing. The recommendation for the type of follow-up test (amniocentesis [AC] or chorionic villus sampling [CVS]) was based on the type of chromosomal anomaly, gestational age, and patient preference.

Results

Table 1: Patient demographics for the evaluable study cohort for genome-wide analysis

Demographic	Study Cohort
Maternal age (years)	
Mean ± SD	30.08 ± 4.04
Median	29.95
Range	18.2-37.4
Gestational age (weeks)	
Mean ± SD	11.5 ± 0.8
Median	11.8
Range	11.5_12.4

The demographic characteristics of maternal age, gestational week, gravidity are shown in Table 1. The pregnant women were 18.2–37.4 years old (mean age 30.08 years). The range for gestational weeks at NIPT was 11.5-12.4 weeks.

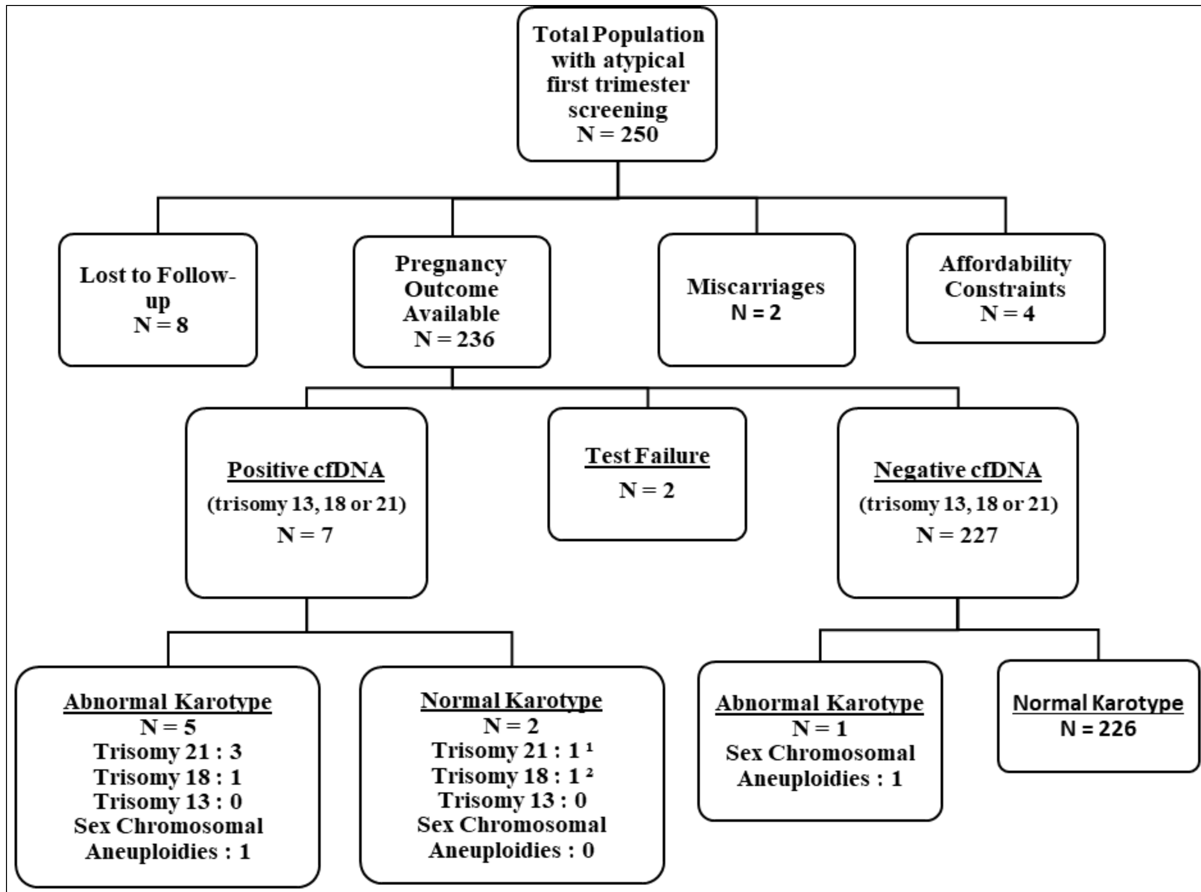

Fig 1: Flowchart of Results

1. False positive due to Placental Mosaicism
2. False positive due to Technical Failure

Among the 250 patients with at least one atypical biochemical markers of first trimester screening, outcome was available for 236 patients in which 2 patients lost to follow up, 2 pregnancies were lost due to miscarriages, 4 were eliminated due to affordability constraints.

Out of 236 patients: Cell free DNA was positive for 7 of which abnormal karyotype for Trisomy 21 (n = 3), Trisomy 18 (n = 1) and Sex Chromosomal Aneuploidy (n = 1) and 2 false positive due to placental mosaicism and technical failure. Test failure occurred for two patients.

On karyotyping 1 sex chromosomal aneuploidy and 226 normal karyotypes are noticed among 227 patients with negative cfDNA.

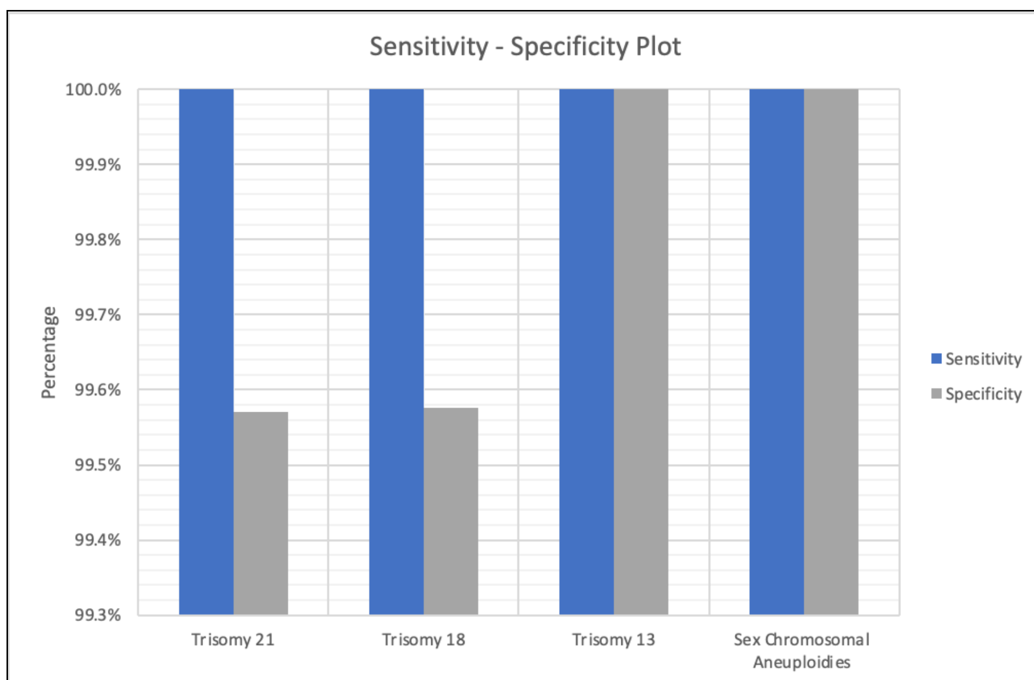

Fig 2: Sensitivity and Specificity plot

Table 2: Sensitivity and Specificity calculation

	Sensitivity	Specificity
Trisomy 21	3/3 = 100%	232/233 = 99.6%
Trisomy 18	1/1 = 100%	235/236 = 99.6%
Trisomy 13	0/0	237/237 = 100%
Sex Chromosomal Aneuploidies	1/1 = 100%	236/236 = 100%

The sensitivity for trisomy 21 is 100% whereas the specificity is 99.6%. The sensitivity for trisomy 18 is 100% and specificity is 99.6%. The specificity for trisomy 13 is 100%. The sensitivity and specificity for sex chromosomal aneuploidies is 100%. Our results on the cfDNA test performance are in agreement with previous studies illustrating the best performance of the cfDNA test for the major trisomies.

Discussion

Fetal genetic and chromosomal aberrations are quite common and approximately 1 in 150 live births is afflicted with some form of chromosomal abnormality which can cause abnormal phenotypes in the fetus [8, 9].

60% of occult spontaneous abortions are secondary to chromosomal aberrations; also, cytogenetic abnormalities are responsible for approximately half of the recognized first trimester abortions and approximately 5% of still births [8].

These chromosomal abnormalities include alterations in number or function, with aneuploidy being the most common abnormality in number of chromosomes secondary to either an extra or a missing chromosome. Among aneuploidies, trisomy 21 (T 21) accounts for >50% of cases, trisomy 18 (T 18) for about 15% cases, and trisomy 13 (T 13) for about 5% cases [9]. Early detection of aneuploidies during pregnancy can have an overwhelming social as well as economic impact on the family. Thus, screening for aneuploidies has become an integral part of obstetric care. Screening identifies high risk population who will benefit from prenatal invasive procedures which are required for diagnosis.

Non-invasive prenatal screening that uses cell-free DNA from the plasma of pregnant women offers incredible possibility as a screening method for fetal aneuploidy.

Goals of NIPT

The goals of performing NIPT include

- Offer a non-invasive test which is easily available to all pregnant women
- Reduce the risk of miscarriage associated with invasive procedures
- Enable a high detection rate
- Reduce the number of false positive results, hence invasive procedures

Technical Basis of NIPT

NIPT is a relatively new method of prenatal screening to detect aneuploidy using cell free fetal DNA (cff DNA) in maternal blood. Cff DNA originates from the placenta resulting from the apoptosis of the syncytiotrophoblasts and is released as small DNA fragments of 150-200 bp [10]. It increases in conditions of abnormal placentation. It is not detectable within hours postpartum [5].

Traditional aneuploidy screening consists of maternal serum screening and ultrasound. These methods have an overall false positive rate of 5% [11, 12] Follow-up diagnostic tests for a positive screening result may include an invasive procedure such as chorionic villus sampling or amniocentesis for karyotyping. These follow-up invasive tests have more integrated risks compared to initial screens.

As compared to traditional serum screening for common aneuploidies, NIPT has consistently demonstrated low false positive rates (higher specificity), and higher detection rate (higher sensitivity) [13] In addition, NIPT can lead to 89% fewer unnecessary invasive tests as compared to traditional serum screening [13].

Implication for clinical practice

CfDNA was commenced initially as a second-line test for pregnancies at high risk of major trisomies based on conventional screening tests. CfDNA is now considered as an acceptable first-line screening test in few countries to all pregnant women at any background risk. Our study dismissed the concerns of using traditional biochemical screening as first tier by the reducing the detection of false positive results, also by reducing the performance of unnecessary diagnostic testing for false positive atypical biochemistry, hence these inferences show the possibility of not using any more PAPP-A and HCG in the first trimester screening for major trisomies.

Conclusions

- NIPT is highly accurate in detecting common trisomies, it also enables the detection of other aneuploidies and structural chromosomal abnormalities with high positive predictive value.
- Its high specificity and efficiency can effectively improve the detection rate of chromosomal abnormalities, thereby reducing the birth defects. Therefore the use of NIPT in both high risk and low risk groups should be promoted.

Conflict of Interest

Not available

Financial Support

Not available

References

1. Pe'er Dar BJ. Cell-free DNA screening for trisomies 21, 18, and 13 in pregnancies at low and high risk for aneuploidy with genetic confirmation, American Journal of Obstetrics and Gynaecology. American Journal of Obstetrics and Gynaecology; c2022, 4.
2. Lo YM. Presence of fetal DNA in maternal plasma and serum. London, England: National Library of Medicine; c1997.
3. Lo YM NC. Presence of fetal DNA in maternal plasma and serum. National Library of Medicine; c1997.
4. Tjoa ML, CDTB. Trophoblastic oxidative stress and the release of cell-free feto-placental DNA. National Library of Medicine; c2006.
5. Council MO. Higher Health Council of Italy. Retrieved from; 2015 May. https://www.salute.gov.it/imgs/C_17_pubblicazioni_2438_allegato.pdf
6. Committee AC. Screening for Fetal Chromosomal Abnormalities: ACOG Practice Bulletin, Number 226. National Library of Medicine; c2020.
7. Illumina. (2022, October 19). Illumina. Retrieved from Illumina: https://sapac.support.illumina.com/downloads/veriseq-nipt-solution-v2-package-insert-1000000078751.html
8. Bianchi DWHM. Noninvasive Prenatal Testing and Incidental Detection of Occult Maternal Malignancies.

JAMA, National Library of Medicine. 2015;314(2):162-169.
9. AODG. Recent Advances in Obstetrics and Gynecology. AOGD Belletin; c2020, 19.
10. MT Laurie JB. Simultaneous digital quantification and fluorescence-based size characterization of massively parallel sequencing libraries. Notional Library of Medicine; c2013. p. 61-67.
11. PB. Screening for Fetal Aneuploidy. National Library of Medicine; c2016. p. 979-981.
12. Benn PBA. Position statement from the Aneuploidy Screening Committee on behalf of the Board of the International Society for Prenatal Diagnosis. National Library of Medicine; c2013. p. 622-629.
13. Bianchi DWPR. DNA sequencing versus standard prenatal aneuploidy screening. National Library of Medicine; c2014. p. 799-808.

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