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Research Article,

Non-Invasive Prenatal Genetic Screening to Detect Trisomies In Singleton and Twin Pregnancies

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Abstract:

Background
Screening for fetal anomalies is one of the most critical parts of prenatal testing to reduce the burden of chromosomal aberrations and improve clinical outcomes in pregnancy. The available literature suggests that India carries a strong economic, emotional, and health care burden of new-born genetic diseases.

Material and Methods:
We present a single-center retrospective study of 200 singletons and 6 dichorionic diamniotic twin gestations screened for chromosomal abnormalities and trisomies using NIPT. The patients were presented with a gestation period of 10-12 (plus a few days) weeks, clinically confirmed with singleton or diamniotic dizygotic twins, who underwent NIPT between the years 2021-2022 (mid) at our center. All the patients underwent sonographic examination and biochemical investigations with regular follow-up and genetic counseling. The clinical history and family history of the presence of any genetic disease or any other disease were previously taken by the clinician in all cases. The main clinical indications were increased risk of trisomies found in markers test, advanced maternal age, or presented with some mild complications in pregnancy.

Results;
Out of the total (206) pregnancies, only 2 singleton cases were found at high risk in NIPT screen, prospectively followed up, and showed normal twins in six cases of twin pregnancy growing successfully. The high-risk cases were genetically counselled and further tests using amniotic or chorionic villi samples were recommended by the clinician with regular clinical follow-up.

Conclusion:
This retrospective study clearly indicates that non-invasive prenatal testing in pregnancy is a safe and effective screening test to rule out certain chromosomal abnormalities and to omit the need of further invasive tests among low-risk cases.

Key Words: Non-invasive Prenatal Genetic Test (NIPT), Singleton, dichorionic diamniotic twin gestations, chromosomal aberrations, Trisomies, High Risk, family history,

Introduction:
Non-invasive prenatal screening (NIPT) has been in practice since 2011 and has enabled a paradigm shift in the area of reprogenetics medicine. NIPT screening test is based on the next-generation sequencing (NGS) method and uses circulating cell-free fetal DNA (ccf) present in maternal plasma for detecting fetal chromosomal

aneuploidies [1]. NIPT allows safe and earlier detection of chromosomal aberrations helping in clinical decision-making. This screening test is used in case of pregnancy at high risk. The ccf DNA appears in maternal circulation via fetomaternal traffic due to the lysis of fetal cells within the circulation by the physiological process of apoptosis which occurs in various organs during the developmental phase [2]. However, the rate of apoptosis is twice in pregnant females carrying chromosomally abnormal foetuses as compared to normal ones [3]. This is also directly related to an increase in the gestation period. This fraction of fetal DNA is also known as the fetal fraction. In order to remove this DNA from the circulation, there is an extensive scavenging mechanism [4,5]. The circulating cell-free fetal DNA (cff DNA) can be detected in the mother's blood in early 5-7 weeks of pregnancy but after 10 weeks the test results are more reliable [6]. There are many factors such as confined fetal mosaicism or aneuploidy, vanishing twins, maternal weight and cancerous conditions that can influence fetal fraction levels [7]. We present a retrospective study to rule out 1. the efficacy of non-invasive prenatal screening in singleton and twin pregnancy cases from North India and 2. to rule out certain chromosomal abnormalities including trisomies to identify the high-risk cases.

Material and methods:

10ml maternal blood was collected in a cell-free DNA BCT™ streck tube from each patient with their written informed consent. Plasma was extracted within a few hours (minimum 1-2 hours) after blood collection using a multi-step centrifugation process and stored at -80^{0}C. Plasma DNA extraction (using Qiagen circulating cell-free DNA kit) was done, DNA concentration was measured using g Qubit™ dsDNA HS (high sensitivity) Assay Kit (Thermo Fisher Scientific and the minimum required concentration is 100ng. NIPT protocol using NGS was performed as per the manufacturer's instructions using Ion Plus Fragment Library Kit (by ThermoFisher Scientific). Sequencing data was analysed using Sage^{TM} (Yourgene Bioscience Co.Ltd.) which allows a menu-based chromosome analysis to estimate the risk of a fetus having Down's syndrome and other aneuploidies with a sensitivity of more than 99%. Fetal aneuploidy risk was evaluated using Sage^{TM} software, using an algorithm for detecting aneuploidies. The Z score in NIPT results displays the number of standard deviations the proportion of reads from a particular chromosome (in relation to the proportion of reads from all other chromosomes) is above the mean. A Z-score of more than 3 standard deviations away from the expected value for the DNA fragments derived from a specific chromosome is considered a high-risk result for trisomy [5,8,9]. The sensitivity of NIPT for trisomy 13 is >99%, and specificity is 99.97; for trisomy 18, sensitivity is >99% and specificity is 99.98% and for trisomy 13, >99%% with a specificity of 99.98%. The reference range for chromosomes 21, 18, and 13 was -6 the z-score is between -6<Z score<2.8 then it shows low risk. For sex chromosome aneuploidies it was -3>Z score <3. For chromosome 1-22, low risk lies in the reference range of -6<Z score<6. Gender determination has not been in practice and was not done in compliance with PCPNDT.[10]

Results:

NIPT results in all the cases for screening for trisomy 21 (T21), 18 (T18), and 13 (T13), sex chromosomes, and other chromosomes among singleton and twin pregnancy cases were analysed. The test was performed with the written informed consent of the patient. The pre-test and post-test genetic counseling was performed extensively. The average age was 34.5 years and the mean fetal fraction was 11.12%. The patients were not having any infection or allergic condition and there was no family history of any inherited disease. The method of conception was natural in 10% of cases and was using other methods including IVF (In vitro fertilization) in rest of the cases. Few patients <4% had a family history of hypertension and diabetes. Most of the patients were in the age group of (22-46years) and were singleton with normal phenotypes except for 2 high-risk pregnancies. There were 15% of patients in the age group of 22-30 years and 85% of the patients were in the age group of 31-46 years. Most of the patients were of advanced maternal age (more than 70%). In our study, there were six cases of twin pregnancies conceived by IVF (In vitro fertilization), IUI (Intrauterine Insemination), and ICSI (Intracytoplasmic sperm Injection) methods and most of the (72%) singleton cases were IVF conceived. During follow-up, clinical findings showed normal fetal growth and normal amniotic fluid volume. (Displayed with patient's consent)

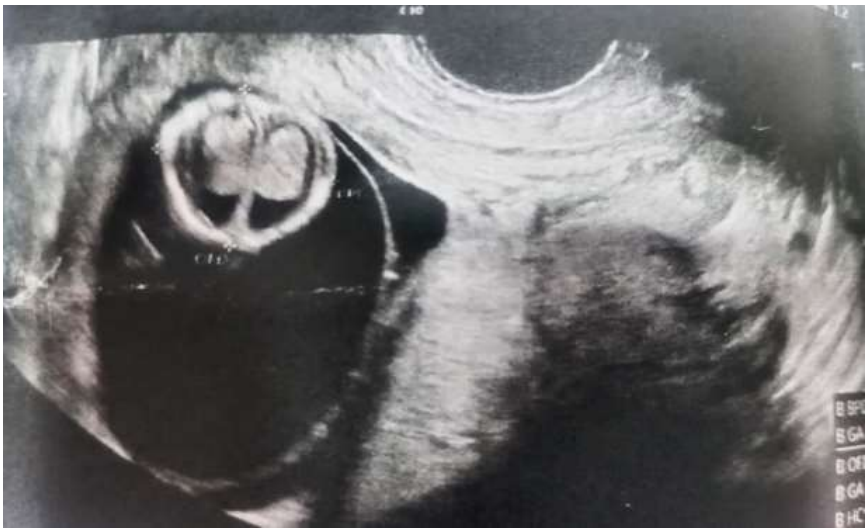

Fig :1 Normal live twin fetus attached to the normal placenta in one of the cases

NIPT results found no chromosomal aneuploidy or trisomy and all markers were normal except in two cases found to be at higher risk for chromosome 21 (Z score 4.79) fetal fraction of 11.6% and another case was at higher risk for chromosome 17 (Z score -7.15) and chromosome 22 (Z score 7.32) with a fetal fraction of 20.34%. Among six cases with twin pregnancies, results showed normal at low risk with an average fetal fraction of 11.5% (Table 1). The clinician was informed for making a decision based on NIPT results after clinically correlating. All the results were discussed during post-test counseling with patients. We didn't observe any adverse outcomes in all the pregnancy cases and patients are being followed-up. No false negative or positive results were found.

All these cases underwent genetic counseling, each couple was counselled to undergo amniocentesis and the samples were recommended to be analyzed using cytogenetics in cases at high risk for confirmation.

Discussion:

NIPT screening test is integrated into clinical practice as a prenatal screening test as per the American College of Medical Genetics and Genomics (ACMG) statement, 2013, and as a second-tier test for high-risk pregnancy in India[11]. NIPT has been recommended as an adjunct to serum aneuploidy detected in the first or second trimester [12]. The incidences of autosomal trisomies and chromosomal aneuploidies accelerate with an increase in maternal age[13]. The risk of Down syndrome has been estimated to be 1:173 at 38 years old and 1:136 at 39 years old[14,15]. However, there are studies concluding that AMA had no significant correlation with chromosomal aneuploidies.

The current retrospective study demonstrates the effectiveness of NIPT among singleton and twin pregnancies from North India. In our study, we screened 6 twin and 200 singleton pregnancy cases using NIPT. The biochemical markers and maternal advanced age were the major factors to opt for NIPT to predict the best possible clinical outcome in case of susceptibility to trisomies and other chromosomal aneuploidies.

We didn't find any correlation between advanced maternal age with chromosomal aneuploidies. We report only two cases of singleton pregnancy at high risk out of 206 cases. Advanced maternal age 35 years or more is an exclusive factor that is always taken into account during prenatal screening. Our results are in parallel with previous reports, although these findings are not similar across the globe. The AMA has been found to contribute to only 6.2% of 1265 patients (with AMA) towards chromosomal aneuploidies in a large study of prenatal diagnosis with clinical indications of amniocentesis[16]. The biochemical markers using serum are not accurately reliable as compared to the NIPT test, because of hormonal changes during pregnancy.

We report two cases of chromosomal aneuploidies one with chromosome 21 and another case at higher risk of chromosome 17 and chromosome 22. Since IVF-induced singleton and twin pregnancies after the implantation have a higher rate of susceptibility to developing chromosomal aneuploidies, therefore, NIPT screening is the best

alternative. In an Indian cohort including 513 (465 cases) the frequency of high risk reported was 3%, of which trisomy 21 was the highest (43%). The fetal fraction was reported at 11.12 and 11.5% in singleton and twin cases respectively which is similar to the results reported in a Pilot Pan Indian study; 11.3% +/- 1.1 in the first and 11.3% +/- 1.5 in the second trimester respectively [12] .

In our study 6 cases of twin pregnancies underwent NIPT due to a high risk of chromosomal abnormalities considering AMA. The first-trimester screening was done using NIPT and was found normal at low risk. Previous studies have already established the role of NIPT in twin as well as in singleton and twin pregnancies [17]. In a dizygotic pregnancy, the minimum threshold fetal fraction should be 8%, to detect chromosomal aneuploidy. NIPT is offered in the first or second-trimester screening (minimum 10 weeks gestation), followed by the nuchal translucency scan at 12 weeks or as a part of a contingent strategy[18,19]. The major indications for the test were positive biochemical markers and advanced maternal age in IVF twin pregnancy, and in certain cases, ultrasound findings suggested the susceptibility of the presence of aneuploidy.

Early, accurate and timely investigation using the NIPT method helps clinicians in the process of decision-making. This test benefits the patients by omitting the need of undergoing painful invasive procedures. These invasive procedures include amniocentesis and chorionic villus sampling which cause emotional anxiety as well as the associated risk of miscarriage. The NIPT results with a high-risk pregnancy should be counselled using a multidimensional and supportive approach and offering genetic counseling so that the related psychological distress and anxiety can be reduced.

In our cases, all the patients and their partners underwent pre and post-test counseling with regular follow-up during their visit to the clinic and telephonic to assess the health of the mother and fetus.

Table1: Patient characteristics of Twin pregnancies:

Age(years)	Gestatin	Ultrasound findings and Biochemical Investigations	Obs History	Clinical Indications	Infection	Family-History-of Inherited-Genetic Disorder/any-other disease	Mode-of conception
34.8	12 weeks	Normal	Gravida 1	AMA	Nil	Nil	IVF
35	12 weeks 4 days	Normal	Gravida 2 previous miscarriages and 1 ectopic pregnancy	AMA,	Nil	Diabetes	IUI
38	12 weeks	Normal	Previous risk of preeclampsia	A mild complication of bleeding, AMA	Nil	Nil	ICSI
36	12 weeks	Normal		AMA, fetal nuchal translucency (NT) thickness, nasal bone fetal heart rate (FHR) and maternal serum-free beta-hCG and pregnancy-associated plasma protein-A (PAPP-A), and risk from the previous history	Nil	Nil	IVF
36	11 weeks 1 day	Normal		AMA	Nil	Nil	ICSI
38	12 weeks and 4 days	Normal		AMA,	Nil	Chronic hypertension	ICSI

Conclusion:

Our study clearly indicates that NIPT holds the promise of being an effective and safe method of prenatal screening in a minimum of 10 weeks of singleton and twin pregnancy. NIPT helps to avoid the invasive tests of amniocentesis and chronic villi sample for further cytogenetic analysis, which are painful and have limitations of longer turnover time, however, strongly recommended in high-risk NIPT results with follow-up.

Conflict of Interest
The authors declare no conflict of interest.

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