ABSTRACT Aim: Non-Invasive Prenatal Test is the testing for cell-free fetal DNA from maternal blood during pregnancy for Trisomy 21, 18, 13 and Monosomy 45XO. Our study aims to review our institution’s experience in the use of a non-invasive prenatal test for aneuploidy screening. Methods: It is a retrospective study of pregnant women who had undergone a non-invasive prenatal test between January 2015 and December 2018 at Fernandez Hospital. A detailed Ultrasonogram is done as a mandatory prerequisite before NIPT to rule out significant structural anomalies; pre and post-test counselling was done to all pregnant women who underwent a non-invasive prenatal test. Results: 322 women had undergone a non-invasive prenatal test during the study period. 32% of the study population was of advanced maternal age. The low fetal fraction was seen in 2.1% of the study population. An abnormal non-invasive prenatal result (aneuploidy detected or inconclusive result) was reported in 36/322 (11%). NIPT had a sensitivity of 100% and specificity of 99.6% in our study. Ever since NIPT has been introduced in our clinical practice the rate of invasive tests performed declined, and the number of non-invasive prenatal test performed increased yearly. Conclusion: NIPT is an excellent method for screening for aneuploidy. There is growing uptake for NIPT and a decline in the rate of invasive tests performed. The occurrence of false-positive results emphasises that NIPT is a screening test and not to be considered as a replacement for diagnostic tests.

KEYWORDS Aneuploidy screening, non-invasive prenatal test, invasive tests
data supporting the utility of this technology, NIPT was offered in our institution as an option for prenatal aneuploidy screening in high-risk pregnant woman[6]. The purpose of our study was to review our institution’s experience with the use of NIPT for aneuploidy screening.

Material and Methods

A descriptive study of patients who received NIPT from January 2015 to December 2018 was performed. In January 2015, the Department of Fetal Medicine at Fernandez Hospital, Hyderabad started offering NIPT to pregnant women at ≥ten weeks gestational age. The inclusion criteria were: Advanced maternal age (AMA; ≥35-years-old at the estimated date of delivery (EDD) with singleton pregnancy), Ultrasound markers suggestive of aneuploidy, Family history of Down syndrome, or First- or second-trimester screen positive mothers. Exclusion criteria are-Mothers who did not deliver at our, institution, structural malformations in ultrasound, increased nuchal translucency on ultrasound, Multiple pregnancies, IVF conceptions, Mothers with renal transplant and autoimmune diseases. All pregnant woman who meets the above criteria is offered pre-test counselling, explaining that NIPT is a high-efficiency screening test, but not a diagnostic test. It provides information regarding the presence of only trisomy 21, 18, 13, or sex chromosomes abnormalities in the fetus. The turnaround time was explained, along with 1–3% possibility of re-sampling risk, if there was inadequate concentration of fetal DNA in the maternal blood sample. All pregnant woman electing NIPT underwent ultrasound to confirm viability and gestational age and to evaluate for the significant structural anomalies (figure 1). Maternal blood samples were collected and sent to the laboratory for testing. The samples were analyzed based on single nucleotide polymorphisms (SNPs). Results of the test were communicated to the couple with post-test counselling. In case of a positive result (high risk for aneuploidy), confirmation through amniocentesis or CVS was recommended. In case of negative results (low risk for aneuploidy), couple reassured, and pregnancy was followed with standard obstetric care till term. Maternal demographics, NIPT results and pregnancy outcomes were obtained from the electronic medical records. The study was approved by the Institutional Review Board and Ethical Committee of Fernandez Hospital.

Results

During the study period, 393 pregnant women underwent NIPT, of which 322 patients were included in the study based on the criteria mentioned in methodology. The mean maternal age of mothers who experienced NIPT in the study was 31 years. The mean gestational age of performing NIPT was 15 weeks, range of performing NIPT was between 11-24 weeks in our study (Table 1). Advanced Maternal age (32%) is the most common indication for which NIPT was performed. (Table 2). Over the study period, the number of NIPT performed increased yearly (34 in 2015 to 182 in 2018). The rate of utilizing amniocentesis declined annually after the availability of NIPT (344 in 2015 to 255 in 2018 (figure 3). Aneuploidy was detected using NIPT in 2.8% (9/322), Inconclusive result was reported in 8.3% patients (27/322). Of the 36 patients with aneuploidy detected or inconclusive result 33% (12/36) underwent invasive prenatal testing. No pregnant women with normal NIPT underwent invasive prenatal tests. The sensitivity and specificity were 100% and 99.6% respectively, for the detection of aneuploidy in chromosomes 21. Of the nine high risks for aneuploidy, diagnostic testing was done in six patients with two false positives for Trisomy 21 and one false positive for XXX. Three pregnant women with abnormal NIPT two high risk for Trisomy 21 and the other high risk for XXX declined diagnostic testing. Out of the 27 pregnant women with the inconclusive result, 12 underwent repeat sampling, six pregnant women underwent invasive diagnostic testing, and remaining 9 declined both repeat sampling and invasive testing. NIPT report was inconclusive because of the low fetal fraction (<4%) and high BMI in 2.1% (7/322). Among these, four underwent repeat sampling, and one case underwent amniocentesis and revealed normal karyotype. Two pregnant women didn’t opt for either amniocentesis or repeat sampling. Of the 322 patients 286 (88.8%) had normal NIPT of which 281 had normal newborn examination and remaining five were intrauterine fetal demise, the cause was due to maternal factors (pre-eclampsia, PPROM). The remaining 36 pregnant women had abnormal NIPT, of which 1.86% (6/322) opted for termination
of pregnancy in which two underwent TOP based on NIPT result and the other four karyotype revealed Trisomy 21. One pregnant woman (1/322) had NIPT result high risk for XXX; however, postnatal karyotype was normal. In two pregnant women (2/322) one NIPT high risk for Trisomy 21 and the other NIPT high risk for XXX, Amniocentesis done revealed normal karyotype, delivered a healthy live baby with normal newborn examination. Among remaining 27 cases who were inconclusive on, NIPT, 3.72% (12/322) opted for repeat sampling of which all had normal newborn examination. Six pregnant women with inconclusive results opted for amniocentesis, which revealed normal karyotype and of which five had normal newborn examination, and one was intrauterine fetal demise (figure 2). The other nine declined both repeat sampling and invasive tests, of which eight had normal newborn examination, and one was intrauterine fetal demise.

**Discussion**

Essential factors in the decision to undergo NIPT include safety of the pregnancy & accuracy[7-9]. The integration of NIPT in current obstetric care at the centre has proven to be extremely useful. The most typical indication was advanced maternal age followed by high-risk results on biochemical screening (first or second trimester), presence of soft markers for aneuploidy in obstetric ultrasound, previous child with Down’s Syndrome which is like the study done by Prathima et al.[1] The significant

| INDICATION | ALL(322) | NORMAL NIPT(N=286) | ABNORMAL NIPT(N=36) |
|------------|----------|--------------------|---------------------|
| ADVANCED MATERNAL AGE | 112(25%) | 93 (22%) | 19 (6%) |
| FAMILY HISTORY OF DOWNS | 4 (1.2%) | 4 (1.2%) | 0 |
| PREVIOUS CHILD WITH DOWNS | 13 (4%) | 11 (3.4%) | 2 (0.6%) |
| FT5 SCREEN POSITIVE | 97 (30%) | 88 (27%) | 9 (3%) |
| UNDISCLOSED NASAL BONE | 55 (16%) | 50 (15%) | 8 (1%) |
| SECOND TRIMESTER SCREEN POSITIVE | 22 (10%) | 20 (9%) | 2 (1%) |

Table 1: Cohort demographics.

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Table 2: Indication for NIPT in the study.

![Figure 3: Changing trends in Invasive tests and NIPT yearly.](image)
Table 4

|                  | ANEUPLOIDY CONFIRMED | NO ANEUPLOIDY |
|------------------|----------------------|---------------|
| NIPT HIGH RISK   | 4 (TRUE POSITIVE)    | 1 (FALSE POSITIVE) |
| NIPT LOW RISK    | 0 (FALSE NEGATIVE)   | 286 (TRUE NEGATIVE) |

Table 5

|                | Beamon et al | Gujjie et al | Taylor et al | FHF Study |
|----------------|--------------|--------------|--------------|-----------|
| SENSITIVITY(%) | 87.5         | 100          | 99.3         | 100       |
| SPECIFICITY(%) | 99.5         | 99.9         | 99.9         | 99.6      |

benefit was that 286 (88%) of 322 cases with negative results did not have to undergo invasive procedures.

Based on our experience, a steady increase in the number of pregnant women undergoing NIPT, with a significant decline in the rates of invasive tests was observed. These findings are comparable to those obtained from the study of Chetty et al. [10] and Beamon et al.[6] in examining the uptake of NIPT. Within the study population, NIPT appeared to have high sensitivity, specificity in our study 100% and 99.6% respectively for the detection of aneuploidy in chromosomes 21, 18 and 13 with one false positive case for Trisomy 21. (Table 4)

Discordant NIPT was found in 2 cases of 322 cases in our study. Beamon et al. published a single centre experience with NIPT, where discordant NIPT was found in 2 cases of 208 cases studied [6].Mennuiti et al. published 8 cases with results between NIPT and cytogenetic testing of the pregnancy [11]. Our combined experiences highlight the importance of confirming all abnormal results with invasive testing. This further reiterates that validation of positive NIPT results is essential, and it is appropriate to classify this as a screening test only.

Two of our pregnant woman high risk for NIPT underwent termination without confirmation from diagnostic tests which emphasises the need for proper pre and post-test counselling, explaining that NIPT is an advanced screening test and not a diagnostic test.

In our study the overall inconclusive results were 27/322(8.35%), which is in comparison with the study published by Beamon et al. (11.1%) [6]. However, this inconclusive rate is significantly higher than the cumulative inconclusive rate reported by Yaron in the review article (2.06%) [13]. Although the exact reason for this higher unclassified rate is unknown in our cohort, poor pregnancy outcomes (two intrauterine fetal demise due to severe maternal preeclampsia) occurred within this subset of patients with inconclusive results. More information regarding inconclusive results and adverse pregnancy outcomes like intrauterine fetal demise, stillbirth, pre-eclampsia, abortion placenta is needed. Postnatal karyotypes should be obtained when the NIPT results are inconclusive 6. In our study low fetal fraction with high BMI was detected in 2.1% (7/322). In the MELISSA trial, the rate of insufficient fetal DNA detected was 3% [12]. Maternal obesity is associated with an increased amount of total cell-free DNA, resulting from adipocyte necrosis[14]. The fetal fraction is the percentage of cell-free fetal DNA as compared with total cell-free DNA; thus, an increase in total cell-free DNA would result in a decreased fetal fraction. These data suggest proper pretest counselling before offering NIPT in obese women as there is an increased need for invasive tests and greater chance of inconclusive result.

SENSITIVITY= TRUE POSITIVE/TRUE POSITIVE+FALSE NEGATIVE=4/4X100=100%

SPECIFICITY=TRUE NEGATIVE/TRUE NEGATIVE+FALSE POSITIVE=286/287 X100=99.6%

The study has few potential limitations. First, we employed a retrospective study design, which limits our ability to determine the precise factors influencing a given testing decision. Second, it is difficult to generalise the results of present study to general population of India as the study was done in a tertiary referral centre with high proportion of high-risk pregnant women. Since various factors, including individuals clinical conditions, economic consideration, and prior NIPT knowledge, may affect patients decisions, these factors should be evaluated in future prospective studies.

Conclusion

Based on our initial experience, NIPT has had increasing uptake among patients who otherwise may have opted for other screening or diagnostic tests and thus qualifies as an ‘advanced’ screening test. The utility of NIPT is very high if done for correct indications. The pre- and post-test counselling plays a vital role in explaining the limitations, benefits, and the interpretation of the result to the couple. It is still a screening test and high-risk cases mandate invasive test but not the termination of pregnancy based on the result obtained by the NIPT High cost of the test which is a crucial factor in decision making by the couple is the limiting factor for its universal use in a developing country like India.

Disclosure Statement

There were no financial support or relationships between the authors and any organization or professional bodies that could pose any conflict of interests.

Competing Interests

Written informed consent obtained from the patient for publication of this case report and any accompanying images.

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