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ORIGINAL ARTICLE

Single Nucleotide Polymorphism-Based Noninvasive Prenatal Testing: Experience in India

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Abstract

Introduction Noninvasive prenatal testing (NIPT) has revolutionized prenatal screening for chromosomal aneuploidies in some countries. Its implementation has been sporadic in developing countries. Given the genetic variation of the people in different countries, we evaluated the performance of the SNP-based NIPT in India .

Materials and Methods The Panorama™ NIPT was performed in 516 pregnancies, which had tested intermediate-to-high risk on conventional first and second trimester screening. Results were confirmed either by invasive diagnostic testing or by clinical evaluation after birth.

Results Of 511 samples analyzed, results were obtained in 499 (97.7%). Of these, 480 (98.2%) were low risk and 19 were high risk. A sensitivity of 100% was obtained for detection of trisomies 21, 18, 13 and sex chromosomal abnormalities. The specificity ranged from 99.3 to 100% for abnormalities tested. Taken together, the positive predictive value for trisomies 21, 18, 13 and monosomy X was 85.7%. The average fetal fraction was 8.2%, which is lower than the average observed elsewhere.

Conclusion This is the first report of detailed experience with NIPT in India and demonstrates comparable performance in all aspects of testing to the results elsewhere.

Keywords Prenatal screening · India · NIPT · SNP · Trisomy 21 · Trisomy 18 · Trisomy 13 · Chromosomal aneuploidies

Introduction

The incidence of chromosomal disorders in India is 1:166 live births [1]. Given the large population, around 35,000 fetuses with Down syndrome alone are conceived every year [1]. Therefore, screening and diagnosis for chromosomal disorders is important in India, as in other countries. The current prenatal screening for chromosomal abnormalities consists of analyzing blood hormone levels and ultrasonography. However, these procedures are limited by low sensitivity and high false positive rate of 2–7% [2, 3]. Also, conventional screening misses over 10% of affected fetuses [4]. Invasive diagnostic methods, such as amniocentesis or chorionic villus sampling (CVS), are highly sensitive but cannot be offered to all pregnant women as they carry a small but significant risk of miscarriage [5, 6].

NIPT, a recently developed advanced technology provides a significant improvement over conventional testing, with detection rate of over 99% and a false positive rate of less than 0.1% by investigation of cell-free fetal (placental) DNA from maternal blood [7–11]. Furthermore, a significant reduction, 50–70%, of invasive procedures has been observed in setups where NIPT has been implemented [12, 13].

Commercialized NIPT technologies follow either of the two approaches: a counting-based method using massively parallel sequencing (MPSS) or the single nucleotide polymorphism-based approach (SNP), used in the Panorama™ NIPT developed by Natera Inc. (San Carlos, USA). The technology and the performance of the SNP-based method has been described elsewhere and validated both in high and low risk pregnant women [14–20]. The advantage of this method is that it does not require a reference chromosome, is able to detect vanishing twin, triploidy, maternal mosaicism and is highly accurate [16]. The limitation has been, rarely, the inability to make a call when there is a high genetic homology between parents (consanguinity) [21]. However, improvement of the algorithm has been implemented, such as quantitative multiple model (QMM) that resolves samples with genetic homology [personal communication] and reduction in no-call threshold for sample calling to 2.8% fetal fraction [22]. Several professional societies worldwide have issued guidelines periodically based on available data on appropriate usage of NIPT [13, 23–27].

The Indian population is socioculturally, ethnically and biologically diverse [28] as reflected in the studies of mutations detection that reveal many novel ones. Additionally, the Indian population has a relatively high rate of consanguinity (20–39%) among certain communities [29, 30]. It is not known how these biological factors would influence the SNP-based NIPT test. The collection and

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handling of samples in a tropical country might also affect the test performance. To study the feasibility of performing NIPT in an Indian population given the above considerations, we conducted an Indian study. The main objective was to evaluate the performance of NIPT for trisomies 21, 18, 13, sex chromosome abnormalities and triploidy in a cohort with intermediate-to-high risk on conventional screening.

Materials and Methods

Ten leading institutes collaborated in this study, after review and approval from their respective institutional review boards. The flowchart of the study protocol is shown in Fig. 1. Pregnant women were enrolled based on the specified criteria. Singleton pregnancies, between 11 and 18 weeks gestation (CRL 45–84 mm), intermediate-to-high risk (risk > 1:1000 for trisomy 21 and > 1:400 for trisomy 18, Nuchal Translucency (NT) measure < 95th centile) on biochemical, combined, triple or quadruple screening were included. Score of > 1:250 was defined as high risk and between 1:250 and 1:1000 as intermediate risk. While pregnancies with egg/sperm donor, surrogacy, twin or multiple gestation, with known parental chromosomal abnormalities (including known balanced translocations), where invasive testing was planned, NT > 95th percentile or nuchal fold thickness > 6 mm, ≥ 1 malformation in fetus, bone marrow transplant or malignancy patients were excluded.

Twenty milliliters of peripheral blood was collected in Streck^{TM} tubes after genetic counseling and informed consent. A paternal buccal swab was obtained when available. Samples were transported to Medgenome Laboratory in Bangalore. Laboratory testing was performed using validated protocol from Natera Inc., using SNPs on five chromosomes and analyzed by the cloud-based proprietary NATUS algorithm [10, 14, 15, 22], the enhanced version of which became available during the course of the study. Fetal fraction was reported in each case. In accordance with the legal requirements in India, gender was not revealed to any study personnel [31]. Pertinent details were collected from the pregnant women. For women who underwent invasive test, fluorescence in situ hybridization and karyotyping was done on fetal material. Redraws were requested for these reasons: lysis, low sample volume, fetal fraction below threshold (2.8%), failed quality matrices, and algorithm-based outcomes such as low confidence results. Follow-up information was obtained from the participants till the delivery. Information on false negative outcomes was keenly sought.

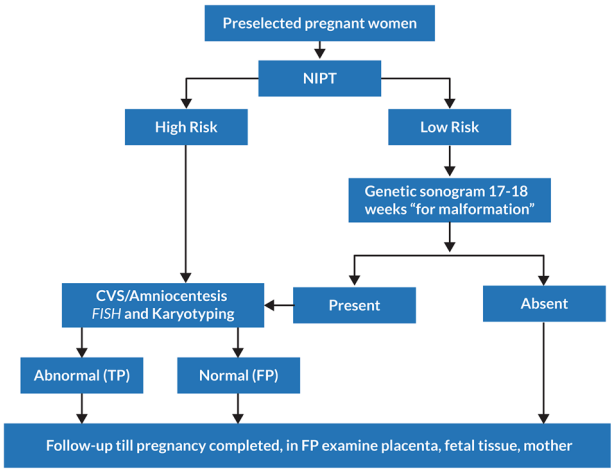

Fig. 1 Study protocol. Samples were selected based on pre-determined criteria; all women were provided genetic counseling pre- and post-NIPT, invasive and ultrasound tests. TP—true positive, FP—false positive

Data Analysis

Sensitivity and specificity for each disorder were calculated separately using only confirmed cases after diagnostic tests or clinical evaluation after birth. Samples that received a no-call were not included. Descriptive data analysis was performed. Where applicable, the *t* test was used for statistical analysis, and $p < 0.05$ was accepted as significant.

Results

The demographic data did not differ significantly among the various groups, except; in the no-call group, the maternal weight and BMI were higher (though statistically not significant), while the fetal fraction was significantly lower as compared with the other two groups ($p < 0.05$) (Table 1). The overall results are depicted in Fig. 2, while details of each high-risk result are shown in Table 2. Fourteen (73.7%) of 19 high-risk NIPT calls and 323 (67.3%) of 480 of low-risk NIPT calls had a prior high risk on conventional screening, while 5 of 19 (26.3%) high risk and 157 of 480 (32.7%) low risk were in the intermediate-risk range ($p > 0.05$). No significant association was found between a high- or low-risk call with advanced maternal age ($p > 0.05$). Table 3 lists the test performance for each chromosomal abnormality, based on the cytogenetic results; combined positive predictive value (PPV) for all chromosomal abnormalities was 81.25%.

Table 1 Demographics of the 516 pregnant women included in the study

		All	Low risk[a]	High risk[b]	No-call[c]
Maternal age (yr)	*n*	516	480	19	12
	Mean (± SD)	31.8 ± 4.8	31.8 ± 4.8	31.7 ± 5.8	32.6 ± 4.0
	Median	32	32	32	32.5
	Range	20–44	20–44	23–42	27–40
Gestational age (weeks and days)	*n*	516	480	19	12
	Mean (± SD)	14w 6d ± 1w 6d	14w 6d ± 1w 6d	14w 3d ± 1w4d	15w 2d ± 1w3d
	Median	14w 4d	14w 4d	14 w 3d	15w 1d
	Range	11w–20 w	11w–20d	12w2d–17w2d	13w4d–17w5d
Maternal weight (kg)	*n*	445	416	19	10
	Mean (± SD)	63.3 ± 11.3	63.1 ± 11.2	63.3 ± 13	67.6 ± 11.8
	Median	62	62	62.9	65.2
	Range	38.5–115	38.5–115	43.1–103	51.5–93
Maternal height (cm)	*n*	283	264	13	6
	Mean (± SD)	160 ± 10	160 ± 10	160 ± 4	160 ± 10
	Median	158	160	160	160
	Range	141.8–187	141.8–187	151–165	151–164
Maternal BMI (ht/m^2)	*n*	281	262	13	6
	Mean (± SD)	25.5 ± 4.2	25.5 ± 4.2	24.2 ± 3.7	27.3
	Median	25.2	25.2	24.5	27.4
	Range	17.1–46.1	17.1–46.1	17.3–32	21–27
Fetal fraction (%)	*n*	516	480	19	12
	Mean (± SD)	8.3 ± 3.4	8.3 ± 3.4	9.2 ± 3.3	3.9 ± 2.5
	Median	7.6	7.6	9.1	3.1
	Range	2.1–18.7	3–18.7	4.4–15.5	2.1–11.5

[a]Low Risk on the NIPT test

[b]High risk on NIPT test (includes the two samples reported as high risk on for sex chromosome abnormalities)

[c]No-call

Follow-Up

Follow-up information was received from 477 of 511 (93.3%) cases. The uptake for invasive testing was 16 (84.2%) of 19 women (Table 2). Two women terminated the pregnancy without confirmatory testing (2/19, 10.5%), and one woman (1/19, 5.3%) refused invasive test. These three unconfirmed cases of trisomy 21 were suggestive of abnormality as intrauterine death was reported in one case and ultrasound abnormalities in the other two. Follow-up information was available in 452 (94.2%) of 480 low-risk cases, eleven of which were confirmed normal after invasive test (performed for other reasons) and the rest were clinically normal at birth. Follow-up information was received in 9 (81.8%) of 11 no-call cases (Table 4). No follow-up was obtained in 28 (5.8%) of 480 cases, which were excluded from statistical analysis where applicable. No false negatives were reported till the time of publication.

Redraws

Of 511 cases, 30 (5.9%) redraws were requested due to a combination of pre-analytic and post-analytic factors. The causes of redraw were: low sample volume/moderate to severe lysis (7), low confidence result (10), fetal fraction below threshold (5), failed libraries (4), failed quality control (2), ambient genotype contamination and an uninformative DNA pattern one each. Pre-analytical factors accounted for 23% of the redraws, while the laboratory processing and algorithm-based redraws were 76.7%. Redraws were received in 22 (73.3%) of 30 samples, and a definitive result was obtained in 19 (86.3%) of 22 redraws.

Turnaround Time

Most samples (97.2%) were received within 48 h. Most (88.8%) samples were reported within 12 days (Fig. 3). The average turnaround time for reporting was 7 calendar days.

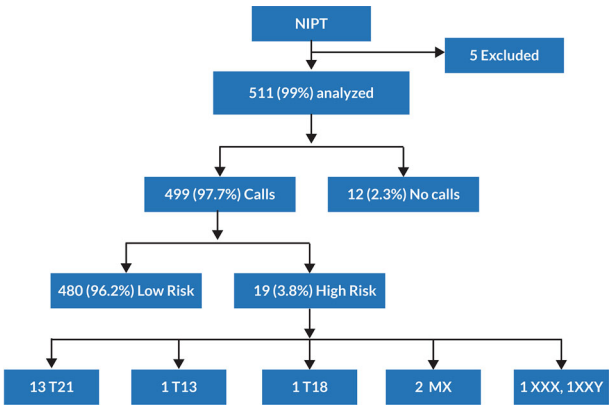

Fig. 2 Summary of Panorama™ results of the five excluded cases: two were out of specification for testing, one was twin gestation with fetal demise (one twin), two samples did not meet the pre-analytical criteria. T21—trisomy 21, T13—trisomy 13, T18—trisomy 18, MX—monosomy X

Fetal Fraction (FF)

The average FF in the no-call cases (3.9%) was significantly lower than in samples that received a call (8.7%) (Welch two-sample t test, $p < 0.001$) (Table 1). The lowest FF for which a call was made was 3%. Five (1%) of 511 cases were found to have a FF below threshold (2.8%). Redraws were received in 3 of 5 cases with low FF; however, a call on redraw could be made only in one (Table 4). The FF was directly proportional to gestational age and inversely proportional to maternal weight (Fig. 4).

No-call

The overall no-call rate was 2.3% (12/511). This was defined as no result on the original sample (when no redraw is received) or on a redraw. Redraws were received in only 3 of the 11 samples, which were no-calls again (Table 4).

Ethnicity

Information was available in 453 (88.6%) of 511 samples confirming that the sample cohort consisted of pregnant women of Indian origin.

Consanguinity

Of 511 samples, consanguinity information was available in 479 (93.7%). Of these 17 (3.5%) were consanguineous, and all 17 samples received a call either the regular algorithm or QMM. Only 3 (17.6%) of 17 pregnant women who reported consanguinity yielded an uninformative DNA pattern on initial analysis. All three samples received a definite call by QMM algorithm. No-call due to uninformative DNA pattern was observed in 7 (1.3%) of 511 (all chromosome no-call—5 cases, and no-call on chromosome X—2 cases) samples. Of these, only one (partial no-call) had history of consanguinity and interestingly also screened high risk of trisomy 21. Of the samples with total uninformative DNA pattern, 80% (4/5) could be resolved on QMM algorithm.

Obesity

Obesity (defined as BMI > 30) was observed in 39 (13.9%) of 281 pregnant women. Calls were available in 38 (97.4%) cases on the first draw, of which one was high risk of trisomy 21 (confirmed on invasive testing). Only 2.7% (1/37) did not receive a call on the first run, and a redraw was requested but was not received.

Discussion

Overall, 97.9% samples received a result within 12 days. The good performance in this study is consistent with the overall performance of NIPT [8, 19, 20, 32]. The overall positive predictive value of trisomy 21, trisomy 18, trisomy 13 and monosomy X was 85.7%, which was comparable to previous publication—82.9% for the four aneuploidies [23]. However, the PPV for trisomy 21 was 80%, which was slightly lower than 90.9% in the clinical validation study [20]. If the three unconfirmed high-risk trisomy 21 cases were true positives, the PPV would increase to 84.6%. Nevertheless, this PPV is much higher than the conventional screening methods [8, 33]. The PPV for sex chromosome abnormalities including monosomy X was 75%, which is higher than that published previously—48.4% [34]. It is important to note here that PPV is not intrinsic to the test but depends on the prevalence of the condition in the tested population [35]. An overwhelming number of women (96.6%), intermediate-to-high risk on conventional screening, were low risk on NIPT. Therefore, potentially, these women avoided invasive procedures for aneuploidy confirmation [13]. The high negative predictive value is a significant benefit in terms of reassurance to pregnant women.

The average fetal fraction in Indian pregnant women was 8.3%, which is lower than the average fetal fraction range published in the Western population (10.69%) [11, 19]. These results are, however, consistent with lower fetal fractions in women of South Asian origin between 11 and 14 weeks [36]. This study did not observe negative impact of fetal fractions on NIPT calls in obese women, probably because the degree of obesity observed among Indian women is lower than in the women in other studied population, and mean BMI observed in the no-call cohort

Table 2 Summary of abnormal NIPT results, confirmation and outcome

	MA (years)	GA	Prior risk	NIPT	FF (%)	NIPT risk score	Confirmatory invasive test	TP/FP	Outcome
1	23	14w4d	1:68 risk on combined screening	T21	9.2	>99/100	Amniocentesis	TP	Terminated
2	34	17w	1:50 risk on quadruple screening	T21	6.4	>99/100	Amniocentesis	TP	Terminated
3	30	12w3d	1:157 risk on combined screening	T21	12.9	>99/100	Not performed	NA	IUD at 24 weeks
4	34	13w	1:50 risk on combined screening	T21	14.7	>99/100	CVS	TP	Terminated
5	37	13w3d	1:48 risk on combined screening.	T21	6.9	>99/100	Not performed	NA	Terminated
6	35	12w3d	1:160 risk on combined screening	T21	10.1	>99/100	Amniocentesis	TP	Terminated
7	24	15w1d	1:8 risk on combined screening	T21	14.4	>99/100	CVS	TP	Terminated
8	38	17w2d	1:55 risk on triple screening	T21	6.9	>99/100	Amniocentesis	FP	Term delivery, normal baby
9	24	14w3d	1:115 risk on combined screening	MX	8.0	>99/100	Amniocentesis	*TP	Terminated
10	30	13w4d	1:90 risk on combined screening	MX	9.1	>99/100	Amniocentesis	TP	Terminated
11	28	17w	1:318 risk on quadruple screening	T21	15.5	>99/100	Amniocentesis	TP	Terminated
12	38	15w4d	1:1250 risk on combined screening	Suggestive XXY	10.7	–	Amniocentesis	FP	Term delivery, normal baby
13	36	15w1d	1:82 Biochemical risk (T13/18–1:371)	T21	7.8	>99/100	Amniocentesis	TP	Terminated
14	32	12w2d	1:1065 on combined screening	T21	4.7	>83/100	Amniocentesis	FP	Term delivery, normal baby
15	42	14w6d	1:199 risk on combined screening	Suggestive XXX	9.0	–	Amniocentesis	TP	Term delivery
16	38	14w2d	1:729 risk on combined screening	T21	4.6	>99/100	Amniocentesis	TP	Terminated
17	31	13w5d	1/329 risk on combined screening	T13	9.4	>99/100	Amniocentesis	TP	Terminated
18	24	12w3d	1:50 risk on combined screening	T21	9.9	>99/100	Not performed	NA	Terminated
19	25	16w5d	>1:50 risk on combined screening	T18	4.4	>99/100	Amniocentesis	TP	Terminated

MA—maternal age at expected date of delivery; GA—gestational age represented in weeks and days; prior risk refers to the risk on combined screening, triple screening or quadruple screening; IUD—intrauterine death, MX—monosomy X, *Isochromosome X with deletion of p arm was noted on karyotyping, which is a variant of Turner syndrome. Confirmation of the NIPT result was performed by fluorescence in situ hybridization followed by karyotyping or Karyolite BOBS (sample 12) after either amniocentesis or chorionic villus sampling (CVS) (sample 4 and 7). TP—true positive, FP—false positive, N.A—not available. The aneuploidy risk score of Sample 12 and 14 was lower than < 1000, but the samples were still included in the study

was only 27.3 [37]. The no-call rate observed was identical to the no-call rate in the validation study [20]. The no-call rates in other studies ranged between 2.9 and 8.1% [8, 19, 35]. Previous publications and guidelines have indicated that no-calls are likely to be aneuploid [11, 19]. From our no-call cases, a suspected T18 could not be confirmed. In practice, therefore, the decision to resample or proceed to invasive testing, given a no-call result, should be made based on reason for the no-call, the original fetal fraction, the probability of receiving a result on a redraw, established guidelines and the applicable regulations of prenatal testing.

Genetic counseling is mandatory, as recommended by several professional societies [27]. In the present study, a fetus with confirmed 47, XXX, was continued to term after counseling, and the baby is doing well postnatally. All

Table 3 Performance of noninvasive prenatal test in detecting Trisomies 21, 18,13, monosomy X and sex chromosome abnormalities (SCA)

	N	TP n	FP n	FN n	UC	Sensitivity (%, C.I. 95%)	Specificity (%, C.I. 95%)	PPV (%, C.I. 95%)	NPV (%, C.I. 95%)
Overall	19	13	3	0	3	100 (73.5–100)	99.3 (98.1.9–99.9)	81.25 (58.4–93.5)	100 (98.9–100)
Trisomy 21	13	8	2	0	3	100 (63.1–100)	99.4 (98.4–99.9)	80 (50.1–94.1)	100 (98.9–100)
Trisomy 18	1	1	0	0	0	100 (2.5–100)	100 (99.1–100)	100 (2.5–100)	100 (98.9–100)
Trisomy 13	1	1	0	0	0	100 (2.5–100)	100 (99.1–100)	100 (2.5–100)	100 (98.9–100)
Monosomy X	2	2	0	0	0	100 (15.8–100)	100 (98.97–100)	100 (15.8–100)	100 (98.9–100)
Other SCA (XXX and XXY)	2	1	1	0	0	100 (2.5–100)	99.7 (98.3–99.9)	50 (12.4–87.6)	100
Theoretical PPV values						Trisomy 21		All four abnormalities	
Lower boundary (all unconfirmed cases considered false positives)						61.5% (8/13)		70.6% (12/17)	
Upper boundary (all unconfirmed cases considered true positives)						84.6% (11/13)		88.2% (15/17)	

PPV—Positive predictive value, NPV—negative predictive value (NPV), UC—unconfirmed, TP—true positives, when high-risk samples were confirmed by invasive testing, FP—false positive, high-risk samples that tested normal on confirmatory testing

Table 4 Summary of no-call cases

Sl no	MA	GA	Original sample	FF (%)	Repeat sample (FF %)	Outcome
1	29	13w4d	Low confidence	3.2	N.R.	Term normal baby
2	33	16w	QC failure	2.9	N.R.	Term normal baby
3	28	16w2d	Low FF	2.1	N.R.	Severe oligohydramnios, other abnormalities, terminated
4	32	14w2d	Low FF	2.3	Low FF (2.3)	IUGR baby at term
5	36	16w3d	QC failure/ambient and genotype contamination	11.5	N.R.	Term normal baby
6	37	13w4d	Low confidence	4	N.R	Amniocentesis performed, FISH and karyotype normal
7	36	17w5d	Low confidence	2.8	Low confidence (2.8)	LTFU
8	30	17w2d	Low FF	2.6	N.R	LTFU
9	33	13w6d	Low confidence	3.1	N.R	T18 suspected, unconfirmed, terminated
10	27	16w5d	Low FF	2.3	Low confidence (3.1)	Preterm normal baby
11	40	14w	Fetal Haploblock	4.5	Not requested	LTFU
12	31	14w1d	Fetal Haploblock	4.5	N.R	Amniocentesis performed, FISH and karyotype normal

MA—maternal age at term, GA—gestational age at first sample, IUGR—intrauterine growth retardation, LTFU—lost to follow-up, FF—fetal fraction, NR—requested but not received, Low FF—fetal fraction below threshold < 2.8%. Redraw was not requested for a no-call sample with an uninformative result due to fetal haploblock (Sample 11). However, with the newer version of the NATUS algorithm available during the study, a redraw was requested for a subsequent sample with a no-call due to fetal haploblock (Sample 12)

other confirmed high-risk pregnancies were terminated. Unfortunately, two cases high risk on NIPT were terminated without confirmation. This undesirable outcome observed in other studies too remains a challenge [20].

Although consanguinity is cited as one of the reasons for inability to make a call in the SNP-based NIPT [21], it did not impact the current study. Limitations of the study were the small sample size, a biased cohort, as samples were preselected and a lack of complete follow-up (follow-up information was received in 93.3% subjects) and the inability to determine the cause of false positives.

Indian Council of Medical Research recommends genetic screening services to all pregnant women in the National and Family Welfare Program [38]. NIPT, in view

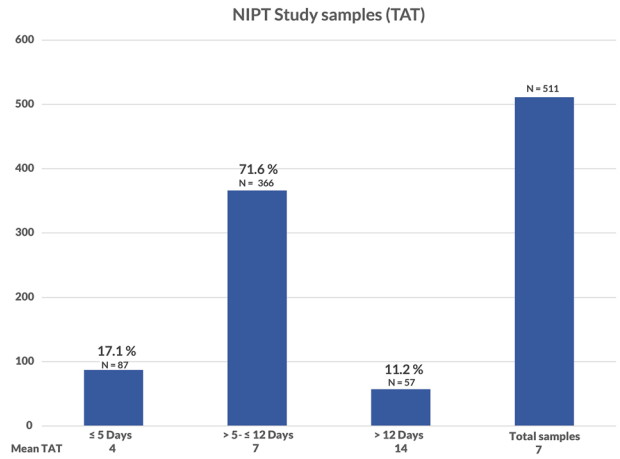

Fig. 3 The turnaround time (TAT) for reporting NIPT samples

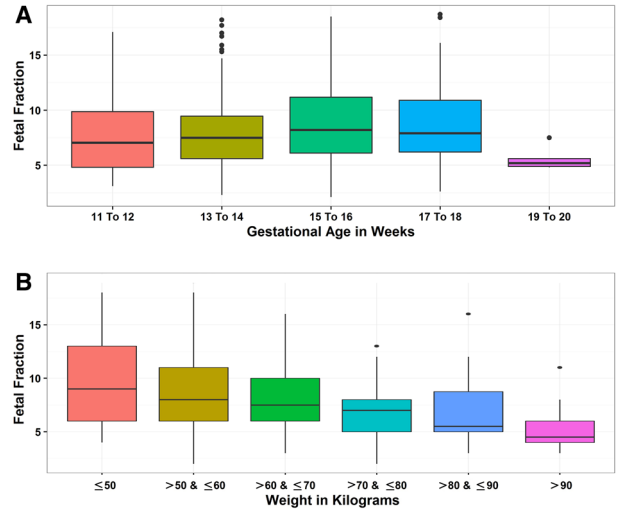

Fig. 4 Comparison of fetal fraction with gestational age and maternal weight. Fetal fraction of cfDNA is plotted against gestational age (weeks) and maternal weight ($n = 445$) in kilograms A: Thick line with in box plot represents mean fetal fraction of each bin. Box plot representing quartile 2 and quartile 4, i.e., with in 25th and 75th percentile and outer hash marks representing lower and upper data. Total number of NIPT study subjects, 511. **a** Small dip was observed at weeks 19–20 which could be explained by the smaller sample size in this cohort. **b** Percentile of subjects with respect to each bin mentioned above the outer hash mark. Solid black line in each box is the average fetal fraction of that group. Outliers are represented as dark color dots next to hash marks of each bin

of its strong performance in the Indian scenario, safety, accuracy and its easy extension to peripheral areas would be a valuable addition to the prenatal screening program, once the cost comes down. The experience gained in this study has enabled us to consider the implications and suggest modifications to international guidelines before applying these to India [39]. Currently, NIPT may be used in cases with high-risk results on conventional screening to avoid unnecessary invasive tests. Further reduction in cost and greater awareness would provide the benefits of this remarkable technology more widely.

Compliance with Ethical Standards

Conflict of interest Authors Dr. Ramprasad VL, Dr. Priya Kadam, Dr. Venkataswamy E, Shruti Lingaiah, Riyaz Akhtar, Francis Kidangan, Chandran R., Kiran C., and Ravi Kumar G. R. were/are employed with Medgenome Laboratories Private Limited during the course of the project.

Ethical approval The study was reviewed and approved by ethical review board of the institutions that participated in the study. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent Informed consent was obtained from all individual participants included in the study.

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