

Non-Invasive Prenatal Testing

Ashutosh Halder*

Department of Reproductive Biology, AIIMS, India

***Corresponding Author:** Ashutosh Halder, Department of Reproductive Biology, All India Institute of Medical Sciences, New Delhi 110029, India.

Received: October 04, 2016; **Published:** October 05, 2016

Non-invasive prenatal testing (NIPT; also known as non-invasive prenatal screening/NIPS) is a laboratory based test using maternal peripheral venous blood from 10th weeks of pregnancy to assess fetal chromosomal abnormalities without risking fetus and mother. It is based on the fact that maternal blood during pregnancy also contains genetic material of the fetus and/or placenta. Chromosomal abnormality targeted by NIPT is mainly chromosomal aneuploidy. Chromosomal disorders are due to lack or excess of chromosome numbers (aneuploidies) or abnormal arrangement (deletion, translocation, inversion, ring, iso-chromosome, etc). Common aneuploidies are trisomy 21, trisomy 18, trisomy 13, monosomy X, trisomy sex chromosomes (47, XXY or 47, XXX), etc. In translocation a portion of one chromosome is transferred to another chromosome. Translocations can be balanced (no gain or loss of genetic material) or unbalanced. In inversion a portion of the chromosome breaks, flips around and rejoins, resulting in change of orientation (physical position) of the genetic material whereas in ring chromosomal ends break (near the tip) and rejoin each other to form a ring. Iso-chromosome is an abnormal chromosome with two identical arms, i.e. either two short (p), or two long (q) arms. Other rare chromosomal abnormalities are chromosomal breakage/instability/fragility, uni-parental disomy, etc. Rarely a conceptus may have an additional set of haploid (triploidy; 69) or diploid (tetraploidy; 92) chromosomes. NIPT is capable of genome-wide aneuploidy detection of fetal genome (23 pairs of chromosomes) with an accuracy of up to 99% but unable to detect all chromosomal abnormalities like small microdeletions/ micro duplications, chromosome rearrangements, translocations, small unbalanced translocations, inversions, uniparental disomy, ring, low mosaicism, etc. Chromosomal abnormalities are very common in early pregnancy and leading cause for pregnancy failure and hence importance in investing in detecting chromosomal abnormalities. About 1 in 13 conceptions and about 50% of first trimester abortions are associated with chromosomal abnormality. About 3 - 4% of all births are associated with a major congenital malformation mainly secondary to chromosomal or sub-chromosomal abnormalities. The advent of noninvasive prenatal testing has allowed these couple the option of having unaffected offspring without risking and this has immensely increased the scope of newer technology in reproductive practice.

NIPT presently analyses cell free DNA (cfDNA) that circulates in maternal plasma using next generation DNA sequencing. Fetal DNA in maternal blood comes from either intact fetal cells or from circulating cell-free fetal DNA (from breakdown of fetal cells, mostly placental). Intact fetal cells may persist for years after a pregnancy whereas cell-free fetal DNA clears from the maternal blood very quickly. Hence, cell-free fetal DNA is originated from current fetoplacental unit. The majority of cell-free DNA in maternal blood is maternal origin and only 5 - 15% fetal origin, depending upon gestational age (lower the gestational age lesser the fetal fraction). This test can also provide information about fetal sex, rhesus (Rh) blood type, some microdeletion/ microduplication syndromes and some monogenic disorders. Microdeletion/microduplication are characterized by small (< 5Mb) chromosomal deletions/duplications in which one or more genes are involved and frequently associated with multiple congenital anomalies. The examples of microdeletion syndromes are DiGeorge or velocardiofacial syndrome (22q11.2), Prader-Willi/ Angelman syndrome (15q11-13), William syndrome (7q11.23), etc.

Present form of NIPT has developed after several decades of research work, initially using fetal cells (fetal erythroblast and chorionic trophoblast) in maternal blood, followed by exfoliated trophoblastic cells in cervical mucus or transcervical cells [1-3] and finally cfDNA in maternal blood [4]. Advances in molecular technology, in particular genome sequencing and bioinformatics have led to the development of NIPT with detection rates approaching with invasive prenatal diagnostic techniques (amniocentesis and CVS; [5]). This cfDNA is exam-

ined using next generation sequencer and bioinformatics analysis tools. It uses massively parallel whole genome sequencing (Sequenom, Verinata, BGI, Berry, Ion Proton, S5, etc platforms) or targeted sequencing (Ariosa platform; selected chromosomal regions of interest) or SNP based targeted sequencing (Natera platform) of cfDNA in maternal circulation. Massively parallel sequencing covers whole genome and has lowest assay failure (about 0.1%) besides gives information on all chromosomes & sub-chromosomal regions. Targeted sequencing covers few chromosomal loci and hence associated with high assay failure besides less informative. SNP sequencing is highly sensitive & specific; can also detect triploidy, uni-parental disomy, maternal contamination (maternal chromosomal abnormality, including mosaicism and maternal malignancy), vanishing twin besides microdeletion syndromes. However, it is associated with high assay failure rate (over 5%), expensive (requires both maternal DNA from leucocytes as well as feto-maternal cfDNA genotyping/sequencing) and difficulties in analysis if pregnancy resulted from egg donation, surrogacy or in case of consanguinity, maternal transplant and multiple gestations. It is also important to know that assay failure cases are at higher risk of aneuploidy and hence all cases with no call should have high-resolution ultrasound and/or invasive prenatal diagnostic test. Various other methods are under exploration to overcome limitations of present form of NIPT. These are DNA microarray NIPT, digital PCR (evaluating methylated DNA and epigenetic differences between fetal and maternal DNA), combinatorial probe-anchor ligation based sequencing, cfRNA sequencing or using intact fetal cells (after enrichment: flow sorted or MAC sorting) and DNA sequencing.

At present NIPT represents combination of screening as well as diagnostic tests, however almost all professional societies recommended as screening test. The sensitivity and specificity of NIPT for sexing, RhD genotyping and trisomy 21 detection matches with diagnostic test (over 99.5%) however for other chromosomal abnormalities, sub-chromosomal abnormalities (microdeletions or microduplications syndromes), etc sensitivity or specificity at present is far behind diagnostic test. NIPT seems a better alternative to maternal serum biochemical screening methods currently in use due to its higher sensitivity, specificity, low false positive rate and needs only one-time blood sample (currently detection rate in first trimester screening i.e., maternal serum dual marker and ultrasound or second trimester quadruple screening is just over 80% and with integrated screening is about 95% with false positive rate over 5%). NIPT cannot provide information on fetal structural defect and hence need for additional high-resolution malformation scan in second trimester. Antenatal ultrasound screening at 16 - 18 weeks permits detection of structural malformations, including soft markers for aneuploidy. If malformations are known to be commonly associated with chromosomal abnormalities, fetal karyotyping or DNA microarray from amniocytes should preferably be advised and in case couple refuses or only soft markers for aneuploidy are evident then NIPT may be recommended as alternative before giving definitive counseling. NIPT should not be recommended for the genetic evaluation of the etiology of ultrasound detected gross fetal anomalies, as both resolution and sensitivity, or negative predictive value, are inferior to those of conventional karyotyping and microarray analysis.

NIPT is now part of prenatal screening test in many developed country and selectively in developing countries, including in India. This test needs strict regulation like all prenatal diagnostic tests to ensure not to be misused, with attention to fetal sexing and selective abortions. In India all laboratories doing NIPT need to be registered under prenatal diagnosis act. Before NIPT, pretest and post-test reproductive genetic counselling is mandatory regarding benefits, limits, interpretation, options, etc. Counsellor should provide accurate information regarding screening test and should inform that this is not a diagnostic test at present. Counsellor should also convey that this test cannot detect all chromosomal abnormalities or malformations besides information that test interpretation is based on placental trophoblasts and not directly from fetus. Most importantly, information must be conveyed that normal NIPT does not ensure unaffected pregnancy, although risk is extremely low.

NIPT may be used as primary screening from 10 weeks onwards (preferably around 15 - 16 weeks of pregnancy when test failure rate is at minimum) in place of first & second trimester screening. A positive NIPT result should be confirmed by invasive prenatal diagnostic test. In case of normal result an ultrasound examination should be advised to exclude open fetal defect or other fetal malformations. Alternatively, NIPT can be used selectively in high risk cases (advanced maternal age, soft ultrasound markers in fetus, previous baby with chromosomal abnormality, family history of chromosomal abnormality, balanced translocations in either parent, etc or in case of positive

biochemical screening) to decrease number of invasive prenatal diagnostic procedure thus fetal losses. NIPT seems going to change the landscape of fetal aneuploidy screening and will not necessarily be limited to few trisomy and sex-chromosome abnormality rather going to be used as comprehensive non-targeted genomic approach (all chromosomes) including microdeletion syndromes in near future. However, in very rare occasions low fetoplacental chromosomal mosaicism or cases with fetal chromosomal aberration only (absence of the chromosomal aberration in the placenta) potentially will be missed with NIPT. In conclusion, NIPT has the potential of helping the prenatal screening practice better and going to facilitate reproductive choices, provided balanced pretest information and non-directive counseling are available as part of the screening.

Bibliography

1. Sherlock J., *et al.* "Prenatal detection of fetal aneuploidies using transcervical cell samples". *Journal of Medical Genetics* 34(1997): 302-305.
2. Adinolfi M., *et al.* "Detection of Fetal Cells in Transcervical Sample & Prenatal Diagnosis of Chromosomal abnormalities". *Prenatal Diagnosis* 15 (1995): 943-950.
3. Tutschek B., *et al.* "Isolation of Fetal Cells from transcervical samples by Micromanipulation: Molecular confirmation of their fetal origin and diagnosis of fetal aneuploidies". *Prenatal Diagnosis* 15 (1995): 951-960.
4. Lo YM., *et al.* "Presence of fetal DNA in maternal plasma and serum". *Lancet* 350(1997): 485-487.
5. Mackie FL., *et al.* "The accuracy of cell-free fetal DNA-based non-invasive prenatal testing in singleton pregnancies: a systematic review and bivariate meta-analysis". *BJOG* (2016).

Volume 3 Issue 5 October 2016

© All rights reserved by Ashutosh Halder.